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P078 Activity of cell-free culture supernatants of Lacticasebacillus casei against Candida albicans biofilms

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P079 Synergistic activity of amphotericin B with posaconazole against Trichosporon asahii biofilms

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P080 Luliconazole shows a signifficant effect against planktonic growth and biofilm formation in Lomentospora prolificans and Scedosporium spp.

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P081 Dynamic of Secondary Metabolites production during Aspergillus fumigatus Biofilm Development

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P082 Investigation of the Relationship Between HOG1 Gene and Biofilm Formation in the Emerging Pathogen Candida auris

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P083 Using FTIR Spectroscopy to investigate the formation of a mixed species biofilm
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P085 Candidemia and Therapeutic Success in ICUs: A 3-Year Sectional Look
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P086 Post-tuberculosis pulmonary aspergillosis - Three distinct cases from a single center
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P087 Differences in Candidemia Management Between Hub and Spoke Hospitals in a Northeastern Region of Italy (Friuli-Venezia Giulia).
Doctor Monica Geminiani[1], Denise D'Elia[1], Dellai Fabiana[1], Alberto Pagotto[1], Sarah Flammini[1], Marco Garzitto[1], Carlo Tascini[1]
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P088 Ocular Pythiosis in a Sri Lankan Patient
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P089 Successful treatment of invasive pulmonary trichosporonosis with isavuconazole in a COVID-19 positive patient with hematologic malignancies
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P090
The cost of oral anti-fungal treatment for chronic pulmonary aspergillosis in Uganda

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P091 Emulated trials of liposomal amphotericin B treatment duration in patients with pulmonary mucormycosis
Anne Coste[1], Raphaël Porcher[2], Anne Conrad, Sylvain Poirée, Pierre Peterlin, Claire Defrance, Valérie Letscher-Bru, Florent Morio, Thomas Gastinne, Marie-Elisabeth Bougnoux, Felipe Suarez, Gilles Nevez, Damien Dupont, Florence Ader, Carine Halfon-Domenech, Sophie Ducastelle-Leprêtre, Françoise Botterel, Laurence Millon, Gaelle Guillerm, Séverine Ansart, David Boutoille, Marie-Pierre Ledoux, Jean-Etienne Herbrecht, Christine Robin, Giovanna Melica, François Danion, Elodie Blanchard, Olivier Paccoud, Dea Garcia-Hermoso, Olivier Lortholary, Raoul Herbrecht[3], Fanny Lanternier[4]
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P092 Clinical and demographic factors affecting trough levels of isavuconazole in critically ill patients with or without COVID-19
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P093 Clinical characteristics, outcome and factors associated with mortality of mucormycosis: A retrospective study at a tertiary care hospital in Pakistan
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P094 The clinical characteristics and outcomes of invasive mucormycosis after allogeneic hematopoietic stem cell transplantation
Chuan Li[1], Rui Ma[1], Yun He[1], Xueyi Luo[1], Danping Zhu[1], Jingrui Zhou[1], Na Li[1], Yu Wang[1], Lanping Xu[1], Xiaohui Zhang[1], Kaiyan Liu[1], Xiaojun Huang[1], Yuqian Sun[1]
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P095 The first six patients treated with olorofim for refractory fungal infection at our institution: clinical and microbiological perspectives
Professor Deborah Marriott[1], Quoc Nguyen[1], Ms Michelle Le[1]
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P096 MORTALITY IN ELDERLY PATIENTS WITH ASPERGILLOSIS
Elena Valle Calonge[1,2], Sara Fueyo Álvarez[1], Julieth Caballero Velasquez[1], José Gutiérrez Rodríguez[1,2], María Teresa Peláez García[2,3], Eva Maria Lopez Álvarez[1,2]
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P097 Clinical characteristics of patients with biopsy confirmed fungal pulmonary nodules
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P098 Clinico-radio-biological description of 54 mucormycosis of the central nervous system: a french national cohort study
Clémentine De La Porte Des Vaux[1], Alexandra Serris[1,2], Corentin Provost[3], Anne Coste[4], Blandine Denis[5], Raoul Herbrecht[6], Romain Sonneville[7], Olivier Lortholary[1], Olivier Naggara[3], Fanny Lanternier[1]
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P099 Exploring Management Strategies and Complications in Genitourinary Mucormycosis in Immunocompetent Hosts - A prospective study from Western India
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P100 Epidemiology and Outcome of mucormycosis
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P101 Safety profile after exposure to different amphotericin B formulations in 1879 patients with invasive fungal infection: a Brazilian observational study

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P103 Histoplasmosis: frequent invasive fungal infection in a tertiary level hospital in Medellín, Colombia.

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P104 Fusarium keratitis in Northeast Brazil: a 10-years prospective study

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P105 A case of rhino-orbital mucormycosis: the role of molecular diagnostic on formaldehyde-fixed and paraffin-embedded (FFPE) tissue samples

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P106 Comparison of various microbiological methods for diagnosis of invasive pulmonary aspergillosis in hematological patients

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P107 Alternative method for identifying candida species in clinical practice

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P108 Naso-oropharyngeal colonization by Candida species and associated factors in laboratory-confirmed COVID-19 patients in Semnan, Iran

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P109 Current state of diagnostic and antifungal treatment opportunities of clinical mycology in Hungary

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P110 Development of an ELISA assay for Scedosporium/Lomentospora serodiagnosis.

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P111 A simple PCR-RFLP method for rapid and accurate identification of Candida auris

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P112 Fungal infections during Covid-19 pandemic in a tertiary care hospital

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P113 Clinical mycology capacity and access to antifungal treatment in Portugal

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P114 Using Aspergillus IgG to predict and avoid invasive Aspergillosis pneumonia

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P115 Evaluation of Candida colonization of oral cavity, anal area, ear canal and urine samples of hospitalized infants and children

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P116 Standardization of Gold Nanoparticles with thiolated DNA for the detection of Candida in blood

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P117 A new machine learning-based approach to classifying patients with invasive fungal disease

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P118 Development of a low-cost molecular assay for the diagnosis of Sporothrix brasiliensis

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P119 Development of digital droplet PCR assay to quantify Aspergillus species - Is it useful?

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P120 Phenotypic characterisation and rapid identification of Candida auris by semi-nested colony PCR

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P121 Multicenter Evaluation of the VirClia Galactomannan Assay on Serum from Patients with Hematological Malignancies

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P122 Factors influencing the probability for positive beta-D-glucan in patients with candidemia

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P123 Establishment of a novel qPCR based on mitochondrial markers for the detection of eukaryotic pathogens

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P125 STANDARDISATION OF MULTIPLEX PCR FOR IDENTIFICATION OF DERMATOPHYTES

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P126 Performance of single sample β-(1→3)-D-glucan assays: the Fungus (1-3)-β-D-Glucan lateral flow assay, the Fungitell® STAT, and the Fujifilm β-glucan test

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P127 Reporting on the diagnostic accuracy of a rapid Aspergillus-specific lateral flow device in patients with fungal keratitis.

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P128 Comparison of MALDI-ToF MS instruments and databases for identification of uncommon yeasts, Aspergillus and rare filamentous fungi

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P130 Prospective service evaluation of T2Candida for the diagnosis of Invasive Candidiasis in the ICU: including an audit of antifungal stewardship.

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P131 Mucorales extracellular polysaccharides: potential targets for the development of new biomarkers?

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P132 The current state of laboratory mycology and access to antifungal treatment in Argentina

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P133 Direct detection of Candida auris from blood and urine samples and from surveillance swabs using a laboratory-developed real-time PCR Method

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P134 Invasive bone cryptococcosis - A case report

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P135 Rapid Identification of Clinically Relevant Candida Species from Positive Blood Cultures Using a New Molecular Assay

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P136 Diagnostic performance of the T2Candida panel at Karolinska University Laboratory

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P137 Development of a multiplex pan-Aspergillus and section Terrei specific qPCR-assay targeting the mitochondrial genome

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P138 Proposal of a strategy using ELISA for the serological diagnosis of farmer lung disease.

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P139 An evaluation to assess the analytical and clinical performance of a Candida auris Real-Time PCR Kit

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P140 Positive lateral flow assay in suspected cryptococcosis. Retrospective study.

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P141 Interdigital Candidiasis in patients with type 2 diabetes and molecular identification of species

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P142 Serological response to Candida albicans Hyr1 protein for diagnosis of Invasive Candidiasis

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P143 Comparison of Germ Tube Testing in Different Media: In Search of “Fast and Furious”

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P144 Optimization of the recovery of Aspergillus fumigatus from self-collected expectorated sputa of Cystic Fibrosis patients

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P145 EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF SERUM (1,3)-B-D-GLUCANE ASSAY FOR DIFFERENTIATION BETWEEN PNEUMOCYSTIS PNEUMONIA AND PNEUMOCYSTIS JIROVECII COLONIZATION

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P146 Development and validation of an LC-MS for the measurement of a new antifungal drug olorofim (F901318)

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P147 Utility of chest x-ray scoring in screening chronic pulmonary aspergillosis in patients with history of pulmonary tuberculosis

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P148 Diagnostic Performance of the Commercial Wantai Mp1p Enzyme Immunoassay for Diagnosis of Talaromycosis

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P149 Development of Interferon-Gamma Release Assays for Diagnosing Latent Talaromycosis
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P150 Chorus Aspergillus galactomannan Ag assay evaluation for the diagnosis of aspergillosis
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P151 Pneumocystis jirovecii qPCR Ct-values during PJP treatment: A prospective longitudinal follow up study
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P152 1-3-β-D-glucan and qPCR for the diagnosis of Pneumocystis Pneumonia (PCP): a retrospective audit with optimisation of qPCR and 1-3-β-D-glucan thresholds.
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P153 Candida albicans germ tube antibodies in invasive candidiasis: a comparison of the manual Vircell-IgG-immunofluorescence assay with the fully automated VIRCLIA-IgG-MONOTEST
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P154 Real-world use of serum (1,3)-β-D-glucan in Candidemia: ECMM Candida III multinational European Observational Cohort Study
Lisa Kriegl[1,2,3], Benedict Rogers[4], Lewis White[5], Riina Rautemaa-Richardson[6], Juergen Prattes[1,2,3], Tadeja Matos[7], Malgorzata Mikulska[8,9], Matteo Bassetti[8,9], Ola Blennow[10], Guillaume Desoubeaux[11], Helena Hammarström[12], Tihana Bicanic[13], Thomas Longval[14], Jon Salmanton-García[15,16], Maiken C. Arendrup[17,18,19], Philipp Koehler[15,16], Oliver A. Cornely[15,16,20,21], Martin Hoenigl[1,2,3]
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P155 Searching for biomarkers of Invasive Candidiasis: characterization of Candida albicans Hyr1 and potential use for diagnostics

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P156 Noninvasive sampling method and PCR for the diagnosis of feline sporotrichosis by Sporothrix brasiliensis

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P158 The current state of laboratory mycology and treatmetn in the Balkans

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P159 Clinico-microbiological profile of Chronic Pulmonary Aspergillosis: A descriptive study from a tertiary care hospital in Southern India.

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P160 The current state of laboratory mycology in Nordic countries

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P161 An evaluation to assess the clinical performance of a Mucorales IVD Real-Time PCR Kit

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P162 Analytical and Clinical Validation of a Pan Fungal PCR Kit

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P163 Identification of the most immunoreactive Candida auris antigens through the study of the humoral response to systemic infections in mice

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P164 Histoplasmosis and cryptococcosis: histopathological pitfalls and relevance of a histomolecular diagnosis based on massive parallel sequencing

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P165 EVALUATION OF PCR-HIGH-RESOLUTION MELT (HRM) ANALYSIS FOR THE DETECTION AND IDENTIFICATION OF MUCORALES FROM CULTURE ISOLATES USING PAN-MUCORALES-SPECIFIC PRIMERS

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P166 Laboratory capacities to diagnose and treat invasive fungal infections in Italy: local results from an ECMM survey

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P167 Effect of different aliquot volumes on bronchoalveolar lavage galactomannan as a biomarker for diagnosis of pulmonary aspergillosis: a proof-of-concept study

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P168 EVALUATION OF NEW TOOLS FOR THE DIAGNOSIS OF HISTOPLASMOSIS

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P169 Cross-reactivity of Aspergillus galactomannan antigen test among emerging non-fumigatus Aspergillus species

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P170 Mycoflora of a University Hospital; fungal contamination in air, water, and surface samples

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P171 ISOLATION AND IDENTIFICATION OF FUNGI ON MOBILES PHONES AND HANDS OF HEALTHCARE WORKERS AND MEDICAL STUDENTS IN TERTIARY CARE CENTRE

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P172 Of yeasts and birds: identification and antifungal susceptibility of Candida spp. from the digestive tract of wild birds in France
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P173 Culturomics analysis of gut mycobiota in patients with ulcerative colitis and characterization of Candia albicans isolates
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P174 Clinically Relevant Yeasts in Sand: A Multiannual Evaluation of the Romanian Black Sea Coast
Mr. Mihai Mares[1], Andra-Cristina Bostanaru-Iliescu[1], Oana-Raluca Rusu[1], Valentin Nastasa[1], Ferry Hagen[2], Dr. Joao Brandao[3]
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P175 Surveillance for airborne and linen-associated fungi within hospitals in a U.S. city

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P176 Pan-Malassezia qPCR: a tool to quantify Malassezia burden in human mycobiota
Victor Euzen[1], Théo Ghelfenstein-Ferreira[1], Yasmine Benhadid-Brahmi[1], Alexandra Teboul[1], Sarah Dellière[1,2], Mazouz Benderdouche[1], Veronique Charlier[1], Marie Desnos-Ollivier[2], Samia Hamane[1], Pr Alexandre Alanio[1,2]
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P177 Effect of pH on lipase production by clinical Candida isolates
Mireya Flores[1], Asier Ramos Pardo[1], Elena Sevillano[1], Guillermo Quindós[1], Elena Eraso[1], Vladimir Kaberdin[1,2]
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P178 A citizen science project to investigate environmental yeasts in urban soils (FungiSol)
Raquel Supo-Santillan[1], Isabelle Ourliac-Garnier[1], Manon Cadeau[2], Marjorie Albassier[1], Estelle Robert[1], Julien Auclaire[1], Patrice Le Pape[2], Florent Morio[2]
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P180 Environmental surveys for the risk assessment of invasive fungal infections in Spanish hospitals
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P183 Ochratoxins and deoxynivalenol contamination of cereals in kibra

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P184 Invasive fungal infections caused by rare yeast-like fungi. Results of a prospective study

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P185 Chaetomium spp: a rare case of severe fungal keratitis and endophthalmitis

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P186 Necrotizing pneumonia caused by Curvularia hawaiiensis and Mycobacterium tuberculosis coinfection in a patient with ascariasis: a case report

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P187 Candida auris fungus spreading in a Greek hospital

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P188 An imported case of histoplasmosis in an immunocompetent patient

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P189 Isolation of resistant yeast species including Candida auris from soil samples-emergence of resistant yeast strains from soil to clinical settings.

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P190 Antifungal resistance profile and azole resistance dynamics of Candida auris in a 13 year collection of clinical isolates across India

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P191 A disseminated Arhtrocladium infection associated with tuberculosis in a pregnant woman

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P192 In vitro whole leukocyte infection model and detection of hydrophobic surface-binding protein A (HsbA) in L. corymbifera

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P193 An emerging Aspergillus granulosus disseminated infection following a long-term course of azole antifungal therapy: a case report

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P194 In-silico chromosome-level assembly of Sporothrix pathogenic genomes using a hybrid long- and short-read sequencing approach

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P195 CLINICOMYCOLOGICAL PROFILE OF DEMATIACEOUS FUNGI AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN- A STUDY FROM SOUTH INDIA

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P196 A case of Millerozyma farinosa (Pichia Farinosa) fungemia: Recognition and management of a rare fungal infection

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P197 Profile of genes encoding efflux pumps in Candida auris clade V; no relationship with resistance to fluconazole

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P198 Characterisation of key amino acid residues in CYP51 that confert intrinsic short-tail azole resistance in Mucor circinelloides

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P199 Invasive and subcutaneous infections due to rare species of filamentous fungi, Parengyodontium album and Microascus cirrosus

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P200 The first domestic isolation of terbinafine- and itraconazole-resistant Trichophyton indotineae in Chinese mainland

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P201 Genetic characterization of emerging multidrug resistant Candida auris isolates in Malaysia

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P202 Invasive mycoses caused by rare mold fungi. Results of a prospective study

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P203 Romania on the Candida auris Roadmap – First Outbreak in a Tertiary Hospital

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P204 Candida haemulonii complex, an emerging threat from tropical regions?

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P205 Genotyping analysis of a Candidemia Outbreak Caused by Candida parapsilosis isolates in a Northern Italy hospital.

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P206 Development and evaluation of a rapid molecular diagnostic strategy for the detection of T. indotineae from patient samples

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P208 First report of a Candida auris outbreak in a tertiary hospital in Northern Greece

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P209 Scedosporium and Lomentospora Emerging Ocular Infections in India: Case series

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P210 Development of a microsatellite typing panel for the pathogenic yeast Trichosporon asahii

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P211 Clinico-epidemiological features and Mycological Profile of Keratitis from a tertiary care teaching hospital in central India

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P212 Kodamaea ohmeri: an emerging pathogen of fungemia in Pakistan

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P213 First case report of Talaromycosis in an HIV patient living in Pakistan

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P214 INCIDENCE OF CANDIDA AURIS CANDIDEMIA IN COLONIZED PATIENTS IN A TERTIARY HOSPITAL OF ATHENS

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P215 Antifungal susceptibility, biofilm forming and genetic features among Candida auris isolated from blood in patients with candidemia

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P216 An emerging species of Cryptococcus gattii sensu lato in the American Southwest: C. decagattii (VGVI)

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P217 Invasive Infection due to Trichosporon asahii, Associated Risk Factors And Outcomes In Tertiary Care Center

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P218 Emergence of phaeohyphomycosis due to Cladophialophora bantiana in France

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P219 Oral histoplasmosis in an elderly immunocompetent male patient - A commonly missed out diagnosis by dental surgeon.

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P220 CNS blastomycosis: The first reported case in a Kuwaiti graduate student from America.

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P221 Multiple forms of Histoplasmosis in Immunocompetent patients from Delhi and around Delhi

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P222 Disseminated Histoplasmosis in an immunocompetent lady. The first reported case in Kuwait.

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P224 In vitro synergy of amphotericin B and flucytosine against Talaromyces marneffei: Implications for combination therapy against an endemic mycosis

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P225 Improving the laboratorial diagnosis of endemic mycoses: a new RT-qPCR assay for the diagnosis of human sporotrichosis.

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P226 Evolution of Histoplasma fungal load by qPCR under treatment in HIV-patients with disseminated histoplasmosis under treatment with liposomal amphotericin B

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P227 Atovaquone exposure, Pneumocystis jirovecii cytochrome b mutations and genotypes: French data and review of the literature

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P228 Healthcare burden of fungal infections in hospitalized patients

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P229 Malassezia infection in a scalp condition: About three zoonotic cases

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P230 Prevalence of Bacterial Vaginosis and Candida Among Pregnant Women in Palestine

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P231 Hazardous Indoor Exposures to Mycotoxins and Microfungal Contamination Correlate with Different Immunodeficiencies

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P232 Recurrent vulvovaginal candidiasis in Colombian women:
Main clinical and microbiological characteristics

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P234 Epidemiological Trend, Species Distribution and Clinical Outcome of Invasive Candidiasis in a North-Eastern Italian Hospital: A Five Years Monocentric Experience

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P235 Experience of Diagnosis of Chromoblastoma (2016- 2022): report from a clinical laboratory of Pakistan

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P237 Epidemiological trends of vulvovaginal candidiasis among symptomatic women in Greece

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P238 Invasive Aspergillus flavus rhino-sinusitis in an immunocompetent patient using intranasal heroin

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P240 Epidemiological trends of fungemia due to rare moulds in a Greek tertiary care academic hospital

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P241 Histoplasma seropositivity at the human-animal-environment interface in Upper River Region, The Gambia: A cross-sectional study using a household sampling approach

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P242 Predominance of Trichophyton tonsurans causing tinea capitis: A 12- years retrospective study in the north of Iran

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P243 Invasive infections by non-albicans Candida in a fourth level hospital in Colombia: an approach to their epidemiology and antifungal susceptibility

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P244 Monitoring of airborne fungi during the second wave of COVID-19 in the referral university hospital in southeastern Iran

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P245 Rapid assessment and containment of Candida auris transmission in a tertary-care hospital in Northern Greece.
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P246 Recurrent candidemia: clinical and genetic analysis
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P247 Gender differences and outcomes of allergic bronchopulmonary aspergillosis
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P249 Candidemia in COVID-19 pandemic: incidence and characteristics in COVID-19 versus non-COVID-19 patients in Northern Greece
Paraskevi Mantzana[1], Efthymia Protonotariou[1], George Meletis[1], Areti Tychala[1], Angeliki Kassomenaki[1], Nikoletta Vlachodimou[1], Triantafyllia Chatziantoniou, Olga Vasilaki[1], Georgia Kagkalou[1], Lemonia Skoura[1]
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P250 Epidemiological study of clinically human dermatophytosis and characterizing the causative agents using PCR-RFLP typing, in Golestan province, north of Iran
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P251 Increasing number of cases of Candida auris in Greek healthcare facilities, 2019-2023
Georgia Vrioni[1], Mamali Vassiliki[2], Maria Siopi[3], Joseph Meletiadis[3], Nikolaos Kesesidis[4], Ioannis Karakasiliotis[4], Theodora Koliou[1], Helen Karahaliou[1], Stavroula Kritikou[1], Afroditi Milioni[1], Stefanos Charpantidis[5], Christina Anthoulaki[6], Vassiliki Baka[7], Theodora Biniari[8], Nikoletta Charalampaki[9],

Stella-Faidra Chatzi[10], Dimitra Chatzidaki[11], Efrosini Chinou[12], Maria Damala[13], Konstantina Daskalopoulou[14], Ioannis Deliolanis[15], Ioannis Dendrinos[16], Vasileios Dimitriou[17], Panagiota Giannopoulou[9], Stamatina Golegou[8], Helen Kafkoula[7], Simona Karabela[18], Stefanos Karachalios[8], Paraskevi Karagiannidou[19], Anna Katsiaflaka[20], Eirini Lamprou[21], Olga Legga[22], Maria Martsoukou[23], Aggeliki Mavroidi[24], Georgios Mouratis[25], Aleksia Mkakosi[26], Evaggelia Oikonomopoulou[27], Maria Orfanidou[28], Fotini Palliogianni[29], Eleftheria Palla[24], Aggeliki Pantazatou[15], Kalliopi Panteli[30], Konstantina Papaefstathiou[31], Helen Papadogeorgakis[32], Vassiliki Papaioannou[33], Polykarpos Papanikolaou[6], Helen Prifti[13], Chrysoula Silleli[34], Tilemachos Skalidis[35], Nikoletta Skarmoutsou[23], Anastasia Spiliopoulou[29], Nantia Statiri[11], Sofia Tsiplakou[33], Anastasia Tsiriga[34], Christina Vossou[35], Ioanna Voulgaridi[20], Anna Xanthaki[34], Olympia Zarkotou[2], Athanasios Tsakris[1]
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P252 Distribution of Aspergillus Species and Prevalence of Azole Resistance in clinical and environmental Samples from a Spanish Hospital

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P253 Epidemiological study of onychomycoses in Athens, Greece: a five-year retrospective analysis (2018-2022)

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P254 The current state of laboratory mycology and access to antifungal treatment in the BeNeLux – preliminary results

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P255 Epidemiology and susceptibility of Nakaseomyces (formerly Candida) glabrata bloodstream isolates from hospitalised adults in South Africa

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P256 Prevalence and association between exposure to aspergillus spores in indoor and outdoor air and sensitization among asthmatics-A case-control aero-mycological study

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P257 Candida auris: Outbreak, surveillance and epidemiological monitoring in Northern Greece

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P258 Prevalence and burden of chronic pulmonary aspergillosis in patients with post-tuberculosis lung disease: a community survey

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P259 Frequency of Candida sojae among other Candida species in paediatric haematooncology patients.

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P260 Natural history of allergic bronchopulmonary aspergillosis: a long-term follow-up study of 182 subjects

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P261 Exploring the impact of the introduction of Elexacaftor/Tezacaftor/Ivacaftor for Cystic Fibrosis treatment and its potential impact on Aspergillus-Related Diseases

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P262 A prospective longitudinal study of chronic pulmonary aspergillosis in newly diagnosed pulmonary tuberculosis patients from diagnosis till end-of-treatment

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P263 The ReCap study: preliminary results of a nationwide French multicenter prospective study of Candida parapsilosis resistance to fluconazole

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P264 Species distribution and antifungals susceptibility of clinical isolates of Penicillium and Talaromyces from respiratory samples in a French University Hospital

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P265 Epidemiological landscape of fungemia due to rare opportunistic yeasts in a Greek tertiary care academic hospital
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P266 Epidemiological characteristics of cryptococcosis cases at a tertiary care centre in India
Harsimran Kaur[1], Parakriti Gupta[2], Anwesha Chakraborty[1], K Karthick[1], Harpreet Singh[3], Vikas Suri[3], Aastha Takkar[4], Anup Ghosh[1], Shivaprakash M Rudramurthy[1]
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P267 Comparison between candidaemia episodes caused by Candida albicans vs non-albicans in a 12-year cohort in a tertiary-care Spanish hospital
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P268 Identification and characterization of cryptic species of Candida isolated from ICU patients
Teresa Nascimento[1,2], João Inácio[3], Daniela Guerreiro[1], Cristina Toscano[4], Isabel Faria[4], Patrícia Patrício[5], Priscila Diaz[6], Dr. Agostinho Carvalho[7], Helena Barroso[1]
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P269 Candidaemia Incidence Soared during COVID-19 Pandemic: A Ten-Year Review from a Belgian Tertiary Hospital
Corentin Deckers[1], Mehdi Khourssaji[1], Florian Bélik[1], Pierre-Emmanuel Plum[2], Nathalie Ausselet[2], Pierre Bulpa[3], Anne Sonet[4], Maxime Regnier[5], Olivier Denis[1], Te-Din Huang[1], Isabel Montesinos[1]
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P270 Outbreak of single lineage Aspergillus flavus infections in a Danish hospital ward
Alexander Gewecke[1], Rasmus Hare[1], Christian Salgård[2], Lisbeth Kyndi[2], Marlene Høg[2], Désiré Nahimana[1], Nissrine Abou-Chakra[1], Jenny Knudsen[2], Søren Rosendahl[3], Maiken Arendrup[1,2,4], NH Vissing[5]
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P271 A rare case of cervical hyalohyphomycosis caused by Fusarium species, in a middle-aged immunocompetent female patient.

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P272 Rapid Literature Review on the Epidemiology and Burden of Disease Caused by Non-Aspergillus and Non-Mucor Mould Pathogens in Europe, Asia and Australia

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P273 Insights from a retrospective study at a South -East Rajasthan hospital on hematogenous affinity of Candida species in bloodstream infections

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P274 Increase of Invasive Aspergillosis caused by Aspergillus section Nigri in a General Hospital

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P275 Candidaemia in a Neonatal Intensive Care Unit: a twelve-year period study (2010 - 2022)

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P276 Incidence rate of fungal secondary infections in COVID-19 patients; retrospective data of infection control committee in a Turkish university hospital

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P277 Emergence of fungal colonization and infections in COVID-19 mechanically ventilated ICU patients

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P278 CANDIDA AURIS ISOLATION AMONG CRITICALLY ILL PATIENTS AND RISK FACTORS FOR CORRESPONDING INFECTIONS
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Dr Teena Thomas[1], Dr Jayanthi Savio[2], Dr Priyadarshini Padaki[3], Dr Camila Catherine Dcoutho[4], Dr Cecil Ross[5]
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Lamia Ammari[1], Khadija El Mnif[2], Rim Abdelmalek[3], Boutheina Mahdi[4], Olfa Smaoui[5], Imen Beji[6], Aida Berriche[7], Badreddine Kilani[8]
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P296 Fungal otitis : infectious diseases department experience

Aida Berriche, Mariem Romdhani, Olfa Smaoui, Boutheina Mahdi, Abir Mbarek, Imen Beji, Aicha Kallel, Rim Abdelmalek, Lamia Ammari, Kalthoum Kallel, Badreddine Kilani
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P297 Population genomics of human pathogenic fungus Aspergillus fumigatus isolated from multi patient cohorts and possible link with environmental A. fumigatus

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P298 Invasive Fungal Infections in Patients with Hematological Neoplasia: A Retrospective Multicenter Study in Greece – Preliminary Data

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P299 Emergence of Pathogenic Aspergillus niger with Triazole Resistance: A Cause for Concern or a New Phenomenon?

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P300 What about fungal peritonitis in patients on continuous ambulatory peritoneal dialysis?: Results of a Tunisian study

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P301 Aspergillus flavus Necrotizing Fasciitis Following Doxorubicin Extravasation in A Lymphoma Patient: A Case Report

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P302 Rare presentation of disseminated cryptococcosis due to Cryptococcus deneoformans in an immunosuppressed patient – Look deep and think fungus!

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P303 Antifungal treatment considerations: treatment selection, sequence, and duration in patients with acute myelogenous leukemia and invasive mold infections.

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P304 Aspergillosis in patients with lymphoproliferative malignancy: new population at risk?

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P305 Mucormycosis Associated with COVID-19: Results Of Prospective Multicenter Study

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P307 Impact of COVID-19 on the epidemiology and outcomes of candidemia: A Retrospective Study from a tertiary care center in Lebanon

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P308 Candidalysin activates IL-1β-producing activity in vulvovaginal candidiasis

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P309 A case of a deep mycosis due to a terbinafine resistant Diaporthe

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P310 Hepatosplenic candidiasis in patients with hematological malignancies - a retrospective multicenter register study

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P311 The light and dark side of IFN-g in the immune challenge against fungi

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P312 Clinical Characteristics and Prognosis of Hepatosplenic Candidiasis in Patients with Hematological Malignancies: A 15-Year Single Center Experience Study

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P313 Clinico-epidemiological and microbiological parameters of mucormycoses outbreak during COVID-19 pandemic in and around New Delhi, India

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P314 A case of pulmonary cocidioidomycosis in the Netherlands

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P315 Utility and Pitfalls of β-D-Glucan for Diagnosis and Monitoring of Chronic Disseminated Candidiasis in Pediatric Cancer Patients

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P317 Mucormycosis: A 14-year Retrospective Study from a Tertiary Care Center in Lebanon

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P318 Af-CAR-NK92 cells secreting IL-15 as potential off-the-shelf therapy for invasive pulmonary aspergillosis

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P319 CARD9 deficiency promote immune-suppressive landscape in chronic fungal infections

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P320 Usefulness of broncho-alveolar lavage in classifying invasive fungal disease in paediatric malignancies

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P321 Use of Galactomannan from Bronchoalveolar Lavage to Detect Invasive Aspergillosis Early After Lung Transplantation

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P322 Histopathology of Cutaneous Invasive Fungal Infections in a Tertiary Cancer Center: Causes, Discordance with Culture, and Histopathologic Determinants of Outcome

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P323 Candidemia in patients over 80 years old; for the upcoming aging society

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P324 Paediatric Allergic Bronchopulmonary Aspergillosis an experience from tertiary care centre in southern India emphasizing the need for developing diagnostic guidelines

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P325 Aspergillus-Infection of Giant Neonatal Omphaloceles: Report of two Cases

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P326 Candida Speciation and Antifungal Susceptibility in Pediatric Urine Samples at a Tertiary Care Hospital in Rajasthan, India

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P327 The first case of mucormycosis in a child with rheumatoid arthritis in Russia

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P329 Liposomal amphotericin B als antifungal prophylaxis in children and adolescents undergoing allogeneic hematopoietic cell transplantation.

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P330 Successful Treatment of Mucormycosis with Adjunctive Sargramostim and Hyperbaric Oxygen in Children and Adolescents with Acute Leukemia

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P331 Invasive aspergillosis in children with non-hematological diseases: results of a multicenter study

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P332 Fatal invasive aspergillosis in a child with chronic granulomatous disease

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P333 Unveiling the Emerging of Fluconazole-Resistant Candida Albicans in Preterm Neonate with Acute Kidney Injury: A Growing Challenge at Vajira Hospital

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P334 Co-infection Invasive Pulmonary Aspergillosis and Pneumocystis jiroveci pneumonia in a B-cell Acute Lymphoblastic Leukemia child

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P335 Invasive Aspergillosis in Hospitalized children in a TertiaryCare Hospital of New Delhi

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Mucoral monitoring of the cases with SARS-CoV-2 in Northwest of Iran

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P337 Clinical features and risk factors of invasive mold infections associated with COVID-19: a single center experience

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P338 Is COVID-19-associated-pulmonary aspergillosis (CAPA) a myth? Frequency of Aspergillus detection from respiratory samples in ICU patients with and without COVID-19.

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P339 Mucormycosis, COVID & Diabetes: Triad or a Dyad?

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P340 COVID-19 associated invasive candidiasis: Results of a Multicenter Study

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P341 Invasive aspergillosis in adult patients with COPD

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P343 Does antiviral and immunomodulatory treatment of COVID-19 influence the outcome of patients with COVID-19-associated pulmonary aspergillosis?

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P344 Sino-nasal mycobiome characteristics of COVID-associated mucormycosis and severe COVID-19: a prospective comparative study

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P346 IMMUNE RECONSTITUTION IN PLHIV AND HISTOPLASMOSIS

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P350 Derivation of an immunological biomarker model to predict invasive mould infection more than 10 days before diagnosis

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P351 Analysis of unconventional T-cell response in blood during Pneumocystis pneumonia

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P352 Investigating invasive aspergillosis and neutrophils response against Aspergillus in patients treated with acalabrutinib

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P353 C5a licenses phagocytes for sterilizing anti-fungal immunity during systemic candidiasis

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P354 Vancomycin disrupts macrophage antifungal immunity

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P355 Host ecto-5'-nucleotidase (CD73) suppression impairs neutrophils NET formation response upon Candida albicans infection

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P356 CD56-mediated activation of human natural killer cells is triggered by galactosaminogalactan of Aspergillus fumigatus

Linda Heilig[1], Sarah Wong[2], Nora Trinks[3], Fariha Natasha[3], Sebastian Wurster[4], Vishukumar Aimanianda[2], Ulrich Terpitz[3], Thierry Fontaine[5], Lea Strobel[1], Francois Le Mauff[6,7], Donald Sheppard[6,7,8,9], Sascha Schäuble[10], Oliver Kurzai[11,12], Kerstin Hünniger[12], Esther Weiss[1], Mario Vargas[13], Lynne Howell[13,14], Hermann Einsele[1], Jürgen Löffler[1]

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P362 Analysis of mutations in ERG11 gene of Candida albicans

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P363 Survey the effect of Licorice extract on Gene regulation of aflR and Aflatoxin production in Aspergillus Parasiticus by Real-time PCR

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P364 Analysis of Microsatellite Length Polymorphism for Clinical Isolates of Candida albicans from Animals

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P365 New insights on titanization process in Cryptococcus neoformans/gattii species complex (CNGSC)

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Effect of linoleic acid conjugated with zinc oxide nanoparticles in inhibiting expression MDR and CDR genes Candida albicans by PCR

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P367 Isolation, identification, determination of drug sensitivity and ERG11 gene mutation of Candida species isolated from vulvovaginal candidiasis

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P368 Longitudinal study looking at the performance of Aspergillus molecular diagnostic workflows from 2019 to 2022.

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P369 Diversity of vaginal bacterial and fungal microbiome among women with RVVC in the Southern Nigeria

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P370 Mitochondria complex I deficiency in Candida albicans arrests the cell cycle at S phase through suppressive TOR and PKA pathways

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P371 Differential levels of expression of cyp51 A, B, C genes among azole resistant Aspergillus flavus clinical isolates

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P372 The transcriptional and cellular responses of Candida auris to macrophage phagocytosis: variations on a theme

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P373 Diploid whole-genome MLST of Candida albicans in vulvovaginal candidiasis patients: a tool to Unravel genetic diversity and clinical phenotypes

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P374 Dual RNA-Seq reveals expression signatures beneficial for iron uptake and intracellular long-term interaction of Lichtheimia corymbifera (Mucorales) with macrophages

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P375 Evaluation of the Whole Genome Sequencing (WGS) and Ef-1 alpha/ITS1/ITS2 sequencing for T. indotineae/mentagrophytes/interdigitale typing and identification among Belgian strains.

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P376 A Novel Combination of mutations in Insig and Cyp51A confers multi-azole Resistance to Aspergillus fumigatus
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P377 Assessing the importance of Iff cell wall adhesins for virulence of the pathogenic yeast Candida auris
Katherine Miranda-Cadena[1], María Alvarado[2], Jesús Alberto Gómez[2], María Teresa Blázquez[2], Ana Sáez[1], Iker Dominguez[1], Cristina Marcos-Arias[1], Estibaliz Mateo[1], Elena Sevillano[1], Guillermo Quindós[1], Piet de Groot[2], Elena Eraso[1]
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P379 Pangenome Analysis Predicts Specific Genes in Molecular Identification of Mucorales
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P380 A study on function and localization of Sporothrix globosa Cyclophilin B
Huaiqiu Huang[1], Zixian Liang[1], Ling Hu[1], Rong Wu[1], Tingting Xia[1]
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P381 β-glucan masking to evade the host immunity in the emerging pathogen Candida auris
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P382 Phenotypic response and RNA-seq profile of PIG1- deleted conidia in Scedosporium apiospermum
Hélène Guegan[1], Wilfried Poirier[2], Kevin Ravenel[2], Sarah Dion[3], Aymeric Delabarre[3], Dimitri Desvillechabrol[4], Xavier Pinson[5], Odile Sergent[3], Isabelle Gallais[3], Jean-Pierre Gangneux[1], Sandrine Giraud[2], Amandine Gastebois[2]
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P383 De novo whole genome sequence of Myriodontium keratinophilum, an emerging dermatophyte pathogen
Amtoj Kaur[1], Arijit Pal[2], Sandhyarani Mahanto[2], Priya Nagpal[2], Gulnaz Bashir[3], Ashutosh Singh[1,4], Kusum Jain[1], Vivekanandan Perumal[2], Anuradha Chowdhary[1,4]
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P384 Zataria multiflora-loaded nanostructured lipid carrier topical gel as a new approach in onychomycosis treatment: a randomized double-blind placebo-controlled clinical trial
Dr. Hamidreza Aghili, Maryam Moazeni[1], Yaser Nasirzadeh fard[2], Hamidreza Kelidari[3], Mojtaba Nabili[4]
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P385 Antivirulence drug discovery to disarm Candida albicans with metabolites from myxobacteria.

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P386 Determination of toxicity of compounds with antifungal activity on a fruit fly model

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P387 Design and evaluation of nanoencapsulated oregano essential oil as alternative treatment to Candida albicans infection

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P388 In vitro inhibitory effect of some local medicinal plants from the southern part of Nigeria on the growth of Candida

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P389 Successful Treatment of Refractory Invasive Aspergillosis with a Novel Antifungal Agent Olorofim in a Leukemia Patient: A Case Report

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P390 Brazilian Brown Propolis shows in vitro antifungal activity against Paracoccidioides brasiliensis.

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P391 Antifungal Activity of a New Derivative of 5-Aminoimidazole-4-Carbohydrazonamide

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P392 Effect of subcutaneous or systemic administration of Celecoxib in mice infected with Paracoccidioides brasiliensis

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P393 Determination of antifungal activity of new compounds against agents of cromoblastomycosis

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P394 In vitro antifungal activity of 3-bromopyruvate (3-BP) against etiological factors of pityriasis versicolor

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P395 Revitalizing our antifungal arsenal with natural products isolated from Arctic bacteria

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P396 In vitro study of the activity of cannabidiol in monotherapy and combined with anidulafungin and fluconazole against Candida clinical isolates

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P397 Pandemic Response Box® library as a source of antifungal drugs against Scedosporium and Lomentospora species
Rodrigo Rollin Pinheiro[1], Mariana Ingrid Dutra da Silva Xisto[1], Yuri Castro-Almeida[1], Victor Pereira Rochetti[1], Luana Pereira Borba-Santos[2], Yasmin da Silva Fontes[3], Antonio Ferreira-Pereira[3], Sonia Rozental[2], PhD Eliana Barreto-Bergter[1]
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P398 Brazilian Brown Propolis enhances fungicidal activity of neutrophils against Paracoccidioides brasiliensis and Paracoccidioides lutzii
Enrico Picoli Marinho[1], Lauana Aparecida Santos[1], Julia Castro-Dutra[1], Thayana Dutra-Andrade[1], Masaharu Ikegaki[1], Rinaldo Poncio Mendes[2], Professor Eva Burger[1]
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P399 Celecoxib reduces antibody titers and inflammatory cytokines in mice infected with Paracoccidioides brasiliensis
Lauana Aparecida Santos[1], Julianne Caravita Grisolia[1], Julia Castro-Dutra, Bruno José Nascimento Gomes[1], Nayara Andrade Dias[1], Luiz Cosme Cotta Malaquias[1], Professor Eva Burger[1]
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P400 Impact of the COVID-19 pandemic on ReSTORE: Phase 3 trial of rezafungin and caspofungin to treat invasive candidiasis and candidaemia
George R Thompson III[1], Jose Vazquez[2], Oliver A Cornely[3], Methee Chayakulkeeree[4], Alex Soriano[5], Patrick M Honore[6], Bart J Kullberg[7], Matteo Bassetti[8], Marin Kollef[9], John Pullman[10], Taylor Sandison[11], Anita F Das[11], Nick Manamley[12], Cecilia Dignani[13], Huang Haihui[14], Peter G Pappas[15]
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P401 Miltefosine as a potential repurposing drug that affects cell biology of mucormycosis agents
Victor Rochetti[1], Mariana Ingrid Dutra da Silva Xisto[1], Rodrigo Rollin Pinhero[1], Yuri Castro-Almeida[1], Luana Pereira Borba-Santos[2], Sonia Rozental[2], PhD Eliana Barreto-Bergter[1]
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P402 In vitro repurposing drugs against Sporothrix brasiliensis
Vanice Poester[1], Jessica Hidalgo[1], Lara Jardim[1], Mariana Trápaga[1], Vanessa Rabello[2], Rodrigo Almeida-Paes[2], Rosely Zancopé-Oliveira[2], Melissa Xavier[1]

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P403 Evaluation of the in vitro activity of anidulafungin and tacrolimus in combination against Candida parapsilosis

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P404 A Glimpse into the Treatment of Candida auris: A study of the Combination of Pentamidine and Auranofin.

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P405 In vitro anticandidal effect of amino acid substitutions on the Jelleine-II peptidic sequence

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P406 Effect of Celecoxib on the morphology and expression of cytokines in the granulomas of P. brasiliensis-infected mice

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P408 In vitro and in vivo effect of the Ca37 monoclonal antibody against Candida auris

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P409 Evaluation of the therapeutic potential of CD5-based CAR-NK adoptive cell transfer in experimental models of Aspergillus fumigatus infection

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P410 Real-world observational study of olorofim: data from compassionate use in France in 15 patients

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Anne-Pauline Bellanger[10], Jean-Pierre Gangneux[11], Boualem Sendid[12], Emilie Cardot[13], Cléa Melenotte[1], Claire Rouzaud[1], Agnès Lefort[14], Sylvie Colin de Verdière[15], Olivier Brugière[15], Macha Tetart[16], Emmanuel Eschapasse[17], Ana Berceanu[18], Pierre Tattevin[19], Romain Levy[20], Emmanuel Faure[21], Fanny Lanternier[1,3]
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P411 Activity of Rezafungin Against Non-albicans Candida Isolates Causing Bloodstream Infections in European Medical Centres (2014–2022)

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P412 Outcomes of invasive fungal disease in a subgroup of patients receiving olorofim/azole combination in an open label salvage study.

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P414 Efficacy Assessment of SF001, a Third-Generation Polyene Antifungal, in the Immunosuppressed Mouse Model of Invasive Pulmonary Aspergillosis
Teclegiorgis Gebremariam[1], Yiyou Gu[1], Eman Youssef[1], Sondus Alkhazraji[1], Joshua Quran[1], Nathan Wiederhold[3], Ashraf Ibrahim[1,2]
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P415 In-vitro Antifungal Activity of Five Novel Anti-Fungal Compounds Against Talaromyces marneffei
Joseph Barwatt[1], Thu Nguyen[1], Heera Sambath[1], Thuy Le[1]
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P416 Once-weekly rezafungin versus daily caspofungin to treat candidaemia and invasive candidiasis: pooled analysis of clinical trial participants in Europe
Oliver A Cornely[1], Matteo Bassetti[2], Alex Soriano[3], Patrick M Honore[4], Bart J Kullberg[5], George R Thompson III[6], Marin Kollef[7], Jose Vazquez[8], John Pullman[9], Taylor Sandison[10], Anita F Das[10], Nick Manamley[11], Cecilia Dignani[12], Methee Chayakulkeeree[13], Huang Haihui[14], Peter G Pappas[15]
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P417 Nikkomycin Z for the treatment of sporotrichosis caused by Sporothrix brasiliensis
Vanice Poester[1], Lívia Munhoz[1], David Stevens[2,3], Aryse Melo[4], Mariana Trápaga[1], David Larwood[2,5], Melissa Xavier[1]
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P418 Anti-melanin activity of diphenyl diselenide against Cryptococcus neoformans
Jéssica Benelli[2], Vanice Poester[1], Emília Andrade[1], Diulien Lima[1], Bruna Esperon[1], Melissa Xavier[1]
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P419 Synergistic compounds with azoles kill drug-resistant Candida albicans by accumulation of eburicol resulted from inhibition on Erg251
Jiacun Liu[1], Ruina Wang[1], Zi Ye[1], Quanzhen Lv[1], Yu Liu[1], Fei Xie[1], Tingjunhong Ni[2], Dazhi Zhang[1], Dr. Lan Yan[1]
[1]Navy Medical University, Shanghai, China, [2]Tongji University, Shanghai, China
P420 Antifungal drugs against drug-resistant fungi: new targets, new mechanisms and novel compounds
Fei xie[1], Tingjunhong Ni[2], Guangxin Xia[3], Yumeng Hao[1], Junhe Bao[1], Yuanying Jiang[2], Lan Yan[1], Dazhi Zhang[1]

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P421 Mechanistic insight on the cell membrane and virulence property of thymoquinone against Candida tropicalis

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P422 Peer Communitiy In (PCI), PCI Infections, and Peer Community Journal: diamond open access to publish research on fungal infections

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P423 Evolutionary drivers in the Chaetothyriales (black yeasts and relatives)

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P424 Neutralisation of the Candida albicans toxin, candidalysin, blocks epithelial damage and dampens inflammatory responses associated with vulvovaginal candidiasis immunopathogenesis

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P425 Candida albicans translocation through the intestinal barrier is promoted by fungal zinc acquisition and limited by host NFκB-mediated barrier protection

Jakob Sprague[1], Tim Schille[1], Verena Trümper[1], Adrian Lier[1], Stefanie Allert[1], Peter Großmann[5], Julian Naglik[2], Sascha Schäuble[5], Gianni Panagiotou[5], Duncan Wilson[3], Lydia Kasper[1], Bernhard Hube[1,4]
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P426 An Alpha-Glucan from Lomentospora prolificans Mediates Fungal–Host Interaction Signaling through Dectin-1 and Mincle

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P427 Neutrophil Recruitment Failure and Tendency Toward Maturation as Critical Factors for Sustained Low Immune Response in Candida glabrata Vaginal Infection

Xiaowei Zhang[1,2,3], Jinli Lyu[1,2,3], Chao Fang[4], Yuxia Zhu[1,2,3], Xinyang Liu[1,2,3], Liting Huang[1,2,3], Yingying Shan[1,2,3], Xiaoping Liu[5], Yiheng Liang[1,2,3], Chunfeng Liu[1,2,3], Shangrong Fan[1,2,3]

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P428 Physiological and transcriptional changes exerted by exogenous homoserine-lactone exposure in Candida auris

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P429 Analysis of melanin content of Fonsecae pedrosoi isolates using Fourier transform infrared spectroscopy (FTIR) and chemometric methods

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P433 Potential drug-drug interactions of antifungal prophylaxis and midostaurin in FLT3-mutated acute myeloid leukemia – clinical implications of therapeutic drug monitoring

Carolin Joisten[1,2], Carsten Müller[3], Sibylle Mellinghoff[1,2], Christian Maurer[1], Karl-Anton Kreuzer[1], Martin Wiesen[3], Philipp Köhler[1], Professor Oliver Cornely[1,2,4,5], Jannik Stemler[1,2,4]

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P434 Pharmacodynamic and pharmacokinetic consideration of cinnamaldehyde as an anticandidal agent

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P435 Physiological-based pharmacokinetic analysis of drug–drug interactions between isavuconazole and vincristine in pediatric subjects

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P436 Posaconazole-Induced Excess Mineralocorticoid Syndrome with Hypertension, Hypokalemia, and Inhibition of 11-beta-hydroxylase in Pediatric Patients

Tempe Chen[1,2], Jagmohan Batra[1,2], Rachit Chawla[3], Natalie Quanquin[1], David Michalik[1,2], Kavita Sharma[1], Cristina Farkas-Skiles[1], Bhavita Patel[1,4], Jacqueline Casillas[1,4], Ramesh Patel[1,4], Jong Chung[1,4], Meena Kadapakkam[1,4], Maki Okada[1], Thomas Walsh[5,6,7]
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P437 A Phase I, Single-dose, Parallel Group Study to Assess the Pharmacokinetics of Olorofim in Subjects with Hepatic Impairment

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P438 A new PK/PD target for micafungin and Candida parapsilosis supports current clinical breakpoint

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P439 A Phase I, Single-dose, Parallel Group Study to Assess the Pharmacokinetics of Olorofim in Subjects with Renal Impairment

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P440 Cerebrospinal Fluid Concentrations of Posaconazole in Pediatric Leukemia Patients

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P441 Quality of compounded itraconazole capsules and its possible impact on the treatment of cat-transmitted sporotrichosis (CTE): a Brazilian pilot study

Francelise Bridi Cavassin[1], Isadora Regina Dallazuana[1], Thiago Lopes De Mari[1], Flavio Queiroz-Telles[2]
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P445 Two cases of superficial fungal infection caused by non-albicans Candida species manifest greenish-black discoloration

Leyao Shi[1], Dongmei Shi[2]
[1]Shandong University Of Traditional Chinese Medicine, Jinan, China, [2]Jining No. 1 People's Hospital, Jining, China

P446 Significance of molecular diagnosis in toenail onychomycosis

Eleonora Dubljanin[1], Ivana Colovic Calovski[1], Isidora Vujcic[2], Sandra Sipetic Grujicic[2], Aleksandar Dzamic[1]
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P447 Prevalence of mold-related onychomycosis: feedback from 15 years in a French University hospital

Lorra Monpierre[1,2], Geneviève Cremer[1], Nicolas Louboutin[1,2], Frédérique Boquel[1,2], Cécile Angebault[1,2], Françoise Foulet[1,2], Françoise Botterel[1,2]
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P448 Epidemiology of Candida africana isolates from vagina in French Guyana

Jeanne Bigot[1,2], Yasmine Kalboussi[1], Yannick Bonkoto Nkoy[1], Sandra Vellaissamy[1], Denis Blanchet[3], Magalie Demar[3], Juliette Guitard[1,2], Christophe Hennequin[1,2]
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P449 LABORATORY CAPACITIES TO DIAGNOSE AND TREAT INVASIVE FUNGAL INFECTIONS IN AUSTRIA, GERMANY AND SWITZERLAND

Jon SALMANTON-GARCÍA[1], Martin Hoenigl[2], Birgit Willinger[3], Oliver A. Cornely[1]
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P450 Personalized approach to prediction of recurrence of foot onychomycosis due to the formation of risk classes

Yulia Ivanova[1], Vladislav Khairutdinov
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P451 mycetoma in Turkana County - North-western Kenya

María Francisca Colom Valiente[1,2], Consuelo Ferrer[1,2], John Lochuke Ekai[3], David Ferrández[1], Laura Ramírez[1], Simion K. Leting[3], Carmen Hernández[4]
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P452 Fifteen cases of Trichophyton mentagrophytes genotype VII among men who have sex with men

Arnaud Jabet[1,9], Sarah Delliere[2], Sophie Seang[3], Aziza Chermak[3], Luminita Schneider[3], Thibault Chiarabini[4], Alexandre Teboul[5], Geoffroy Hickman[6], Alizée Bozonnat[6], Cécile Brin[3], Marion Favier[3], Yanis Tamzali[3], François Chasset[7], Ana Canestri[3], Stéphane Barete[5], Samia Hamane[2], Mazouz Benderdouche[2], Alicia Moreno-Sabater[9], Eric Dannaoui[8], Christophe Hennequin[9], Arnaud Fekkar[1], Renaud Piarroux[1], Anne-Cécile Normand[1], Gentiane Monsel[3]
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P453 Genotypic characterization of Trichophyton mentagrophytes isolates from companion animals in France

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P454 Dermatophytes frequency and diversity in dermatomycoses within the area of Zagreb (Croatia)

Ana Čičmak[1], Daniela Jakšić[2], Ida Ćurtović[2], Domagoj Kifer[2], Mario Sviben[1,3], Maja Šegvić Klarić[2]
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P455 Species identification and in vitro anti-fungal susceptibility testing of Aspergillus section Nigri strains isolated from otomycosis patients

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[1]Tehran University of Medical Siences, Tehran, Iran, [2]Iran University of Medical Siences, Tehran, Iran

P456 Human and Zoonotic Dermatophytes in Romania: An Updated on Species and Clinical Aspects

Andra-Cristina Bostănaru-Iliescu[1], Ferry Hagen[2], Bert Gerrits van den Ende[2], Mihai Mareș[1]
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P458 Human adaptation and diversification in the Microsporum canis complex

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P459 A new family of black yeast-like fungi

Meizhu Wang[1,2], Tian Pan[1], Sarah Ahmed[2], Bruno Paulo Rodrigues Lustosa[3], Ricardo Belmonte Lopes[4], Yu Quan[2], Abdullah M.S. Al-Hatmi[5], Maria Eduarda Grisolia[3], Bruna Jacomel F.S. Lima[4], Vania Aparecida Vicente[3,4], Yu Cao[6], Yingqian Kang[1], Sybren de Hoog[2]
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P461 Unveiling the Candida spp. burden: workload and cost considerations in a tertiary hospital.

Ana Soriano-Martín[1,2], Roberto Alonso[1,2,3,4], Marina Machado[1,2], Elena Reigadas[1,2,3,4], Emilio Bouza[1,2,3,4], Patricia Muñoz[1,2,3,4]
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P462 Global incidence and mortality of fungal disease

David Denning[1]
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P463 Whole genome sequencing of Candida auris using nanopore technology. An analysis during the outbreak of the fungus in Northern Greece.
Aikaterina Poulopoulou[1], Antigoni Malousi[2], Styliani Pappa[1], Anna Sidiropoulou[1], Georgios Tzimagiorgis[2], Timoleon-Achilleas Vyzantiadis[1], Elisavet Georgiou[2]
[1]Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Greece, Thessaloniki, Greece, [2]Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Greece, Thessaloniki, Greece
P464 Fungal and bacterial co-infections of the respiratory tract among patients with COVID-19 hospitalized in intensive care units
Hasti Kamali Sarvestani[1], Zahra Rafat[2]
[1]Tehran University of Medical Siences, Tehran, Iran, [2]Giulan University of Medical Siences, Rasht, Iran
P465 Candida colonization and candidemia in intensive care units: Mapping of the Candida invasion
Alba Ruiz[1]
[1]HOSPITAL UNIVERSITARIO Y POLITECNICO LA FE, VALENCIA, Spain
P467 The first warning sign in the Nasal and Oral for Initiating Preemptive Anti-fungal Therapy in Febrile Neutropenic Patients.
Hasti Kamali Sarvestani[1], Mohammadreza Salehi[1]
[1]Tehran University Of Medical Siences, Tehran, Iran
P468 A Two-Year Surveillance of Candida Blood-stream Infections in a Tertiary Cancer Center in Muscat; First Report from Sultanate of Oman
Bassem Awada, George M. Varghese, Manyando Mulipi, Mansour Al-Mandhari, Jorge Abarca
[1]Sultan Qaboos Comprehensive Cancer Care Center, Muscat , Oman
P469 Pneumocystis jirovecii pneumonia in pediatric systemic lupus erythematosus patient: A challenge for diagnosis and prophylaxis
Miss Plobkwon Ungchusak[1], Wanalee Akrachotisakul[2]
[1]Department of pediatrics, Center of Clinical Medicine - Lerdsin Hospital, Bangkok, Thailand, [2]Department of pediatrics, Mukdahan Hospital, Mukdahan, Thailand
P470 An unusual case of Cryptococcal Meningitis in a patient with Chronic Lymphocytic Leukemia
Spyros Orfanopoulos[1], Marina Kardamitsi[1], Evangelia Douka[1]
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P471 Clinical outcome in a US patient with A.ustus complex pulmonary invasive aspergillosis treated with olorofim in a Phase2b study (NCT03583164)
Andrej Spec[1], Mike Birch[2], Corinne Pinder[2], Emma Harvey[2], Daniela Zinzi[2], John Rex[2]
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Accepted Posters

P001

Determination of the fitness cost of the Fusarium solani species complex to voriconazole in the invertebrate model Galleria mellonella

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Objectives:
The genus *Fusarium* spp., mainly known as a plant pathogen, is cosmopolite and present in soil, water, and air. The *Fusarium solani* species complex (FSSC) is the most represented in human pathology among the genus. *Fusarium* can cause skin and ocular infections, but can also lead to severe invasive fungal infections, fusariosis, in immunodeficient patients. The number of fusariosis cases has been steadily increasing over the last two decades.
Therapeutic drugs for fusariosis treatment are limited. Indeed, *Fusarium* spp. presents a low susceptibility to most antifungals. However, ESCMID and ECMM published joint guidelines for managing fusariosis and recommended using voriconazole, associated or not with amphotericin B. Despite these recommendations, many studies have demonstrated a high variability of voriconazole MIC between *Fusarium* strains.
An *in vivo* model of fusariosis is, therefore, necessary to determine the consequences in terms of virulence and response to treatment of the strains with low in vitro susceptibility to voriconazole.
The objectives of this work are to develop a model of invasive fusariosis in the invertebrate *Galleria mellonella* and to identify a fitness cost for isolates with limited susceptibility to voriconazole.

Materials & Methods:
Twelve FSSC isolates were used. These strains present different genotypes and MICs ranging from 2 to 16 µg/ml.
For the virulence study, one hundred thousand conidia are injected into each larva. The larvae are then incubated at 37°C in the dark and daily monitoring is carried out for 5 days. A larva is considered dead in the presence of melanisation and/or in the absence of spontaneous turning.
A pathological examination is carried out after sagittal section and staining with Grocott and HES to validate the model.

Results:
On the pathological examination, we can observe septate filaments and budding spores within a granuloma in larval tissue.
By using an inoculum of one hundred thousand conidia per larva, larvae die quickly, with 50% mortality achieved between 1 and 2 days. This virulence differed according to the MICs of the studied strains (p=0.001) (figure 1). Indeed, on day 1, survival is 70% for strains with a high MIC of 8 or 16, whereas it is 29% for strains with a lower MIC of 2 or 4. These data, therefore, show a positive fitness cost.

Conclusions:
We were able to develop an *in vivo* model of fusariosis *via* the invertebrate model *Galleria mellonella* widely used in mycology.

A positive fitness cost is observed for *Fusarium solani* species complex during early infection. This fitness cost is present in *Aspergillus flavus* but absent in *Aspergillus fumigatus* resistant to azoles.
The prospects of this work will be to evaluate the response to treatment with voriconazole *via* this model, to establish a correlation between the *in vitro* and *in vivo* data.

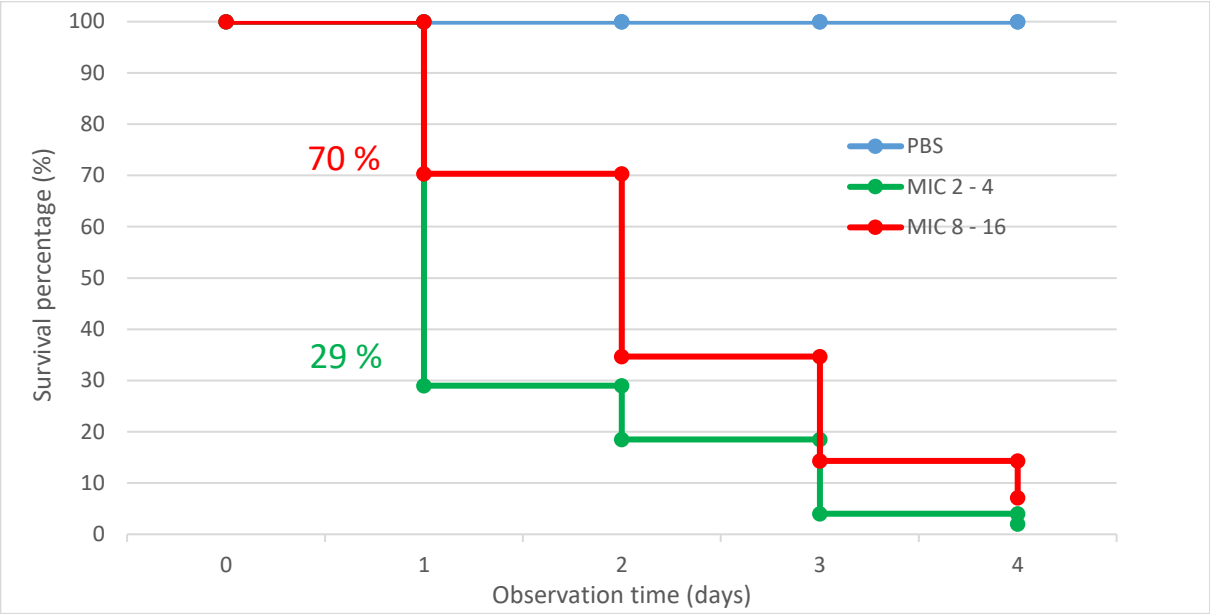

Figure 1: Positive fitness cost of FSSC to voriconazole in the *Galleria mellonella* model

P002

In vivo efficacy of rezafungin, anidulafungin, caspofungin and micafungin against four Candida auris clades in a neutropenic murine infection model

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Objectives: *Candida auris* is a multidrug resistant and critical priority fungus against which echinocandins (anidulafungin, caspofungin and micafungin) are first line drugs. Rezafungin is the first new drug approved to treat candidemia and invasive candidiasis in more than 10 years. Rezafungin is a once-weekly, next-generation echinocandin with excellent *in vitro* and *in vivo* activity against the clinically important *Candida* species. However, data on *in vivo* efficacy of the four approved echinocandins against different *C. auris* clades are absent.

Methods: Ten isolates representing four *C. auris* clades (South Asian n=2; East Asian n=2; South African n=2; South American n=4; two of which were of environmental origin) were used. BALB/c male mice were given cyclophosphamide 4 days before infection (150 mg/kg), followed by administration of 100 mg/kg cyclophosphamide every third day until the end of the experiments. In the lethality (ten mice/group) and fungal tissue burden experiments (five mice/group) mice were infected intravenously (10^7 and $8x10^6$ CFU/mouse, respectively). Twenty mg/kg dose of rezafungin on days 1, 3 and 6; once-daily treatment for 6 days with 3 mg/kg of caspofungin (Cancidas®), 5 mg/kg of micafungin (Mycamine®) and 5 mg/kg of anidulafungin (Eraxis®), were initiated 24 hours post-infection. These doses correspond to the currently used doses of the four echinocandins in clinical practice. After 21 days, survival rates were compared using the Kaplan-Meier logrank test. Fungal tissue burden (kidneys, hearts and brains) on day 7

were analysed with the Kruskal-Wallis test with Dunn's post-test. Histopathological examination on day 7 with haematoxylin-eosin and Periodic Acid Schiff was also performed (two mice/group).

Results:

Echinocandin MICs were not higher than the tentative MIC breakpoints suggested by the Centers for Disease Control and Prevention. Regardless of isolates and clades all echinocandin regimens improved survival (P values were from <0.001 to <0.0001, Fig. 1). At day 7 the survival rates for South Asian, East Asian, South African, and South American clades were 80-100%, 40-100%, 60-100% and 70-100%, respectively with all treatment arms (Fig. 1).

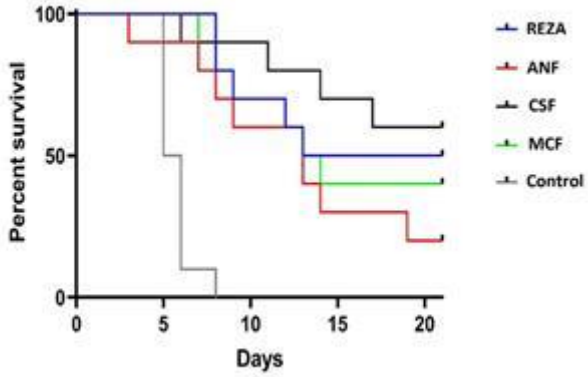
In the fungal tissue burden experiments all echinocandins frequently produced >3-log mean fungal kidney and heart burden decreases some of which were not statistically significant (Fig. 2), Rezafungin, regardless of the clades, produced 3-5 and 2-4 log CFU decreases in the kidneys and hearts, respectively. In contrast, echinocandins did not inhibit fungal growth in the brain (Fig. 2).

The histopathological examination showed that all echinocandins were effective in sterilization of kidneys, with the exception of mice infected with clinical isolate from the South American clade and treated with micafungin. Rezafungin- and to a lesser extent caspofungin- treated mice, regardless of clinical isolates and clades, did not show fungal cells in their hearts. Echinocandins treated mice regardless of the clades always showed medium and/or large foci of fungal cells in their brains.

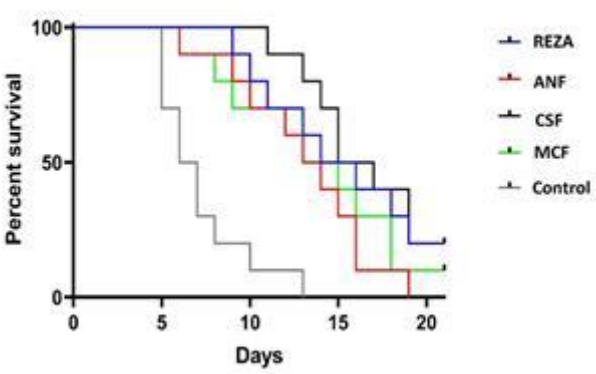
Conclusions: Consistent with prior echinocandin *in vitro* data demonstrating activity against WT strains, this class was highly efficacious *in vivo* against *C. auris*. Rezafungin

activity regardless of clades was comparable to or better than the previously approved three echinocandins.

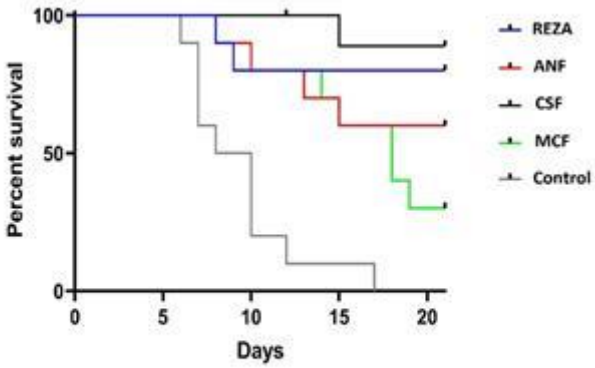
Isolate 27, South Asian clade



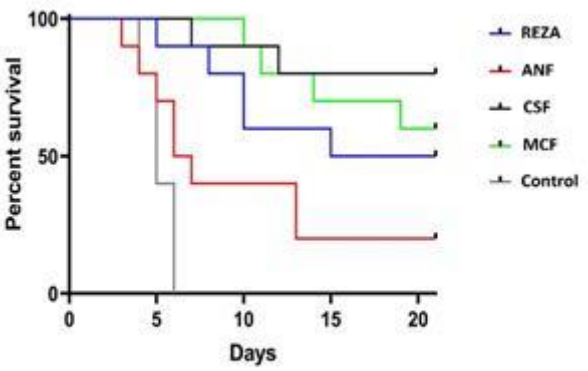
Isolate 196, South Asian clade



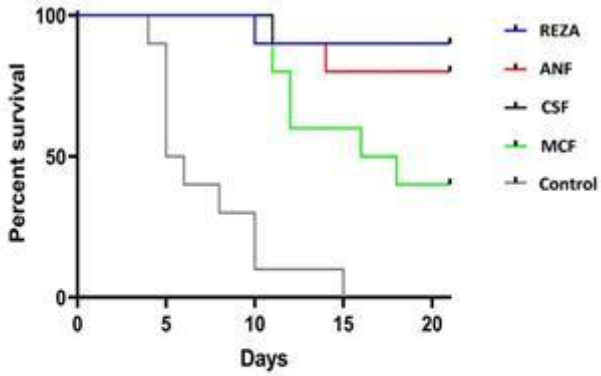
Isolate 12372, East Asian clade



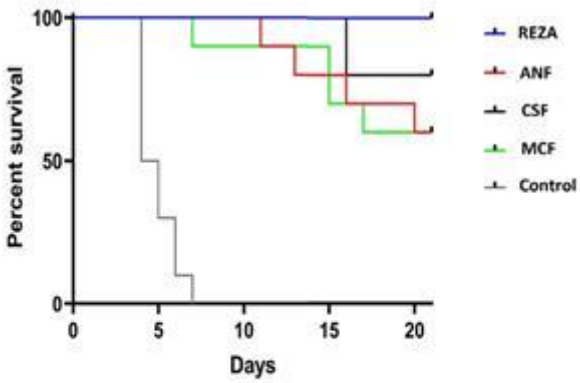
Isolate 12373, East Asian clade



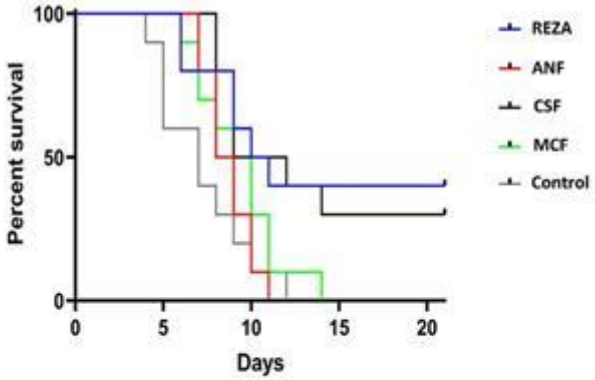
Isolate 2, South African clade



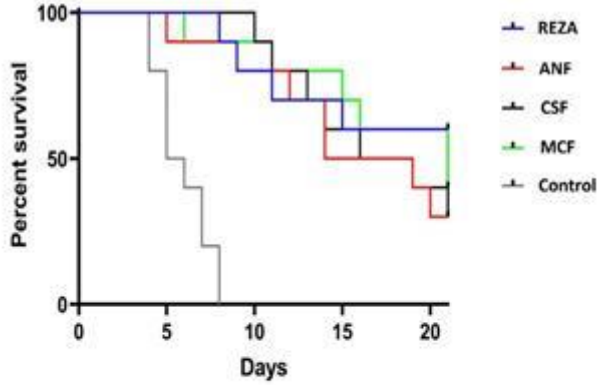
Isolate 204, South African clade



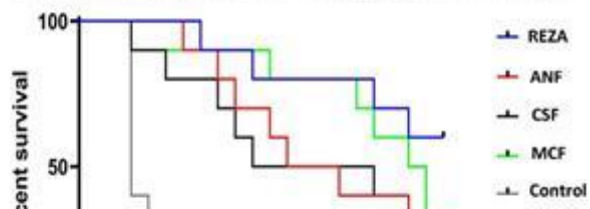
Isolate I-24, South American clade



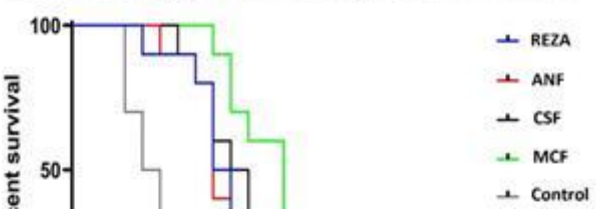
Isolate I-156, South American clade

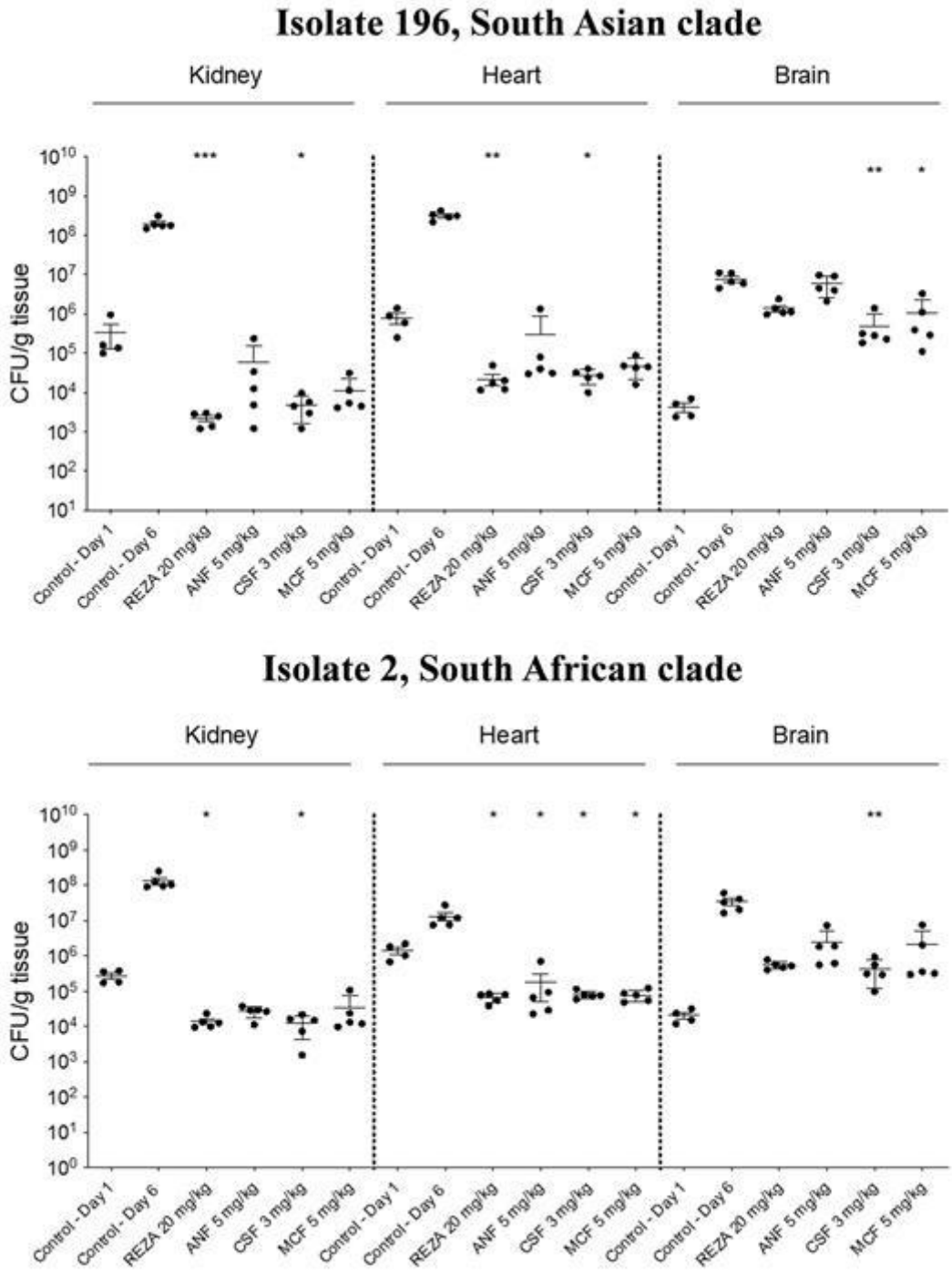


Isolate 13108 (environmental), South American



Isolate 13108 (environmental), South American




Isolate 196, South Asian clade
Kidney
Heart
Brain
CFU/g tissue
Control - Day 1
Control - Day 6
REZA 20 mg/kg
ANF 5 mg/kg
CSF 3 mg/kg
MCF 5 mg/kg
Isolate 2, South African clade
Environmental isolate 13112, South American clade

P003

Pharmacokinetic profiles of isavuconazole and isavuconazonium in larvae of the invertebrate Galleria mellonella evaluated by liquid chromatography tandem mass spectrometry

Theresa Bruchmann[1], Christopher-Nils Mais[2], Svenja Duenschede[1], Eric Dannaoui[3,4], Gert Bange[2,5], Patrick Schwarz[1,6]

[1]Center for Invasive Mycoses and Antifungals, Philipps University Marburg, Marburg, Germany, [2]Center for Synthetic Microbiology (SYNMIKRO), Department of Chemistry, Philipps University Marburg, Marburg, Germany, [3]Université Paris Cité, Faculté de Médecine, AP-HP, Hôpital Necker-Enfants Malades, Unité de Parasitologie-Mycologie, Paris, France, [4]Working Group Dynamyc, Faculté de Médecine, Hôpital Henri Mondor, Créteil, France, [5]Max Planck Fellow Group, Molecular Physiology of Microbes, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, [6]Department of Internal Medicine, Hematology and Oncology, University Hospital Giessen, Giessen, Germany

Objectives:
Larvae of the invertebrate *Galleria mellonella* can be used to evaluate the antifungal efficacy of mono and combination therapies by mortality studies. Isavuconazole is a broad-spectrum azole antifungal drug approved for first-line therapy of mucormycosis in patients. Isavuconazole is administered as a water-soluble prodrug (isavuconazonium), which is transformed into the active form by a plasma esterase in the human body. However, until now no studies could demonstrate the efficacy of isavuconazole in larvae infected with Mucorales species, possibly related to a missing esterase for isavuconazole prodrug transformation. The first aim of the present study was to evaluate the drug levels of isavuconazonium and isavuconazole in larvae of *G. mellonella* after isavuconazonium administration over time. The second aim of the study was, if isavuconazonium is transformed to isavuconazole in the larvae, to evaluate whether the measured concentrations of isavuconazole are potentially high enough to use the *G. mellonella* mortality model to test antifungal combinations including isavuconazole as a partner in experimental mucormycosis.

Materials & Methods:
Volumes of 10 µl PBS containing 16, 32, 48, 64 and 80 µg of isavuconazonium were injected into the larvae. Each group for each concentration and timepoint contained 5 larvae. At time points of 0.5, 1, 2, 5, 8, 16, and 24 hours animals were sacrificed by placing them into freezer for 10 min at -20°C. A small incision was made beside the last pseudopod using a sterile needle and hemolymph was released by application of a light pressure to the animal. The hemolymph of the larvae of each group was pooled in 1.5 ml tubes containing phenylthiourea to prevent melanization. The pooled hemolymph was frozen until further use. After thawing, masses of isavuconazonium and isavuconazole were measured in the samples by liquid chromatography tandem mass spectrometry and quantification peak areas were determined. A calibration curve was prepared using hemolymph samples spiked with isavuconazonium and isavuconazole ranging from 3-20 µg/ml.

Results:
Peak concentrations of isavuconazole and isavuconazonium were reached at 0.5 hours after administration. Higher administered concentrations of isavuconazonium led to higher concentrations of both drugs in the animals. Concentrations of the drugs decreased gradually

over time. High doses of isavuconazonium let to peak isavuconazole levels in the hemolymph of the larvae of >100 µg/ml.

Conclusions:
For the first time, it was possible to measure concentrations of isavuconazonium and isavuconazole in larvae of the wax moth G. mellonella. This study demonstrated that G. mellonella contains an esterase that transforms isavuconazonium to isavuconazole. Measured concentrations in the hemolymph were above the MICs of isavuconazole-susceptible Mucorales species. Therefore, the G. mellonella mortality model is suitable to test antifungal combinations including isavuconazole in experimental mucormycosis in the future.

P004

In vivo Effectiveness of Fluconazole and Posaconazole against Coccidioides posadasii Meningitis Caused by Fluconazole Resistant Isolates

Laura Najvar[1], Nathan Wiederhold[1], Rosie Jaramillo[1], Marcos Olivo[1], Jose Lopez-Ribot[2], Chiung Yu[2], Professor Thomas Patterson[1]
[1]UT Health San Antonio, San Antonio, United States, [2]University of Texas at San Antonio, San Antonio, Unites States

Objectives: *Coccidioides* species are endemic to the desert southwest of the United States, Northern Mexico, and in parts of Central and South America. *Coccidioides* meningitis is associated with significant morbidity and mortality. Treatment generally consists of long-term, high-dose fluconazole. However, reduced fluconazole *in vitro* susceptibility (MIC 16 mg/L) and fluconazole resistance (MIC ≥32 mg/L) has been reported to occur in the U.S. Correlations between *in vitro* susceptibility and *in vivo* outcomes are not well established. Our objective was to evaluate the *in vivo* effectiveness of fluconazole and posaconazole, including supratherapeutic doses, against fluconazole-resistant *C. posadasii* strains in an established murine model of CNS coccidioidomycosis.
Methods & Materials: *C. posadasii* clinical strains DI23-1 (cultured from a patient in Colorado) and DI23-1 (Texas) were used (fluconazole MICs ≥64 mg/L, posaconazole MICs ≤0.125 mg/L). Infection was established in immunocompetent mice via intracranial inoculation with arthroconidia. Oral therapy with vehicle control, fluconazole (25 mg/kg QD or 25 mg/kg BID) or posaconazole (10 mg/kg QD or 25 mg/kg BID) began 48 hours post-inoculation and continued for 7 days in the fungal burden arm and 14 days in the survival arm. Fungal burden was assessed by colony-forming unit (CFU) enumeration. In the survival arm, mice were followed for two weeks off therapy until day 30.
Results: Against infections caused by either *C. posadasii* strain, both median survival (>30 days) and percent survival to day 30 (range 80% - 100%) were significantly improved with either posaconazole dose compared to control (median survival 9 - 10.5 days, 0% percent survival; $p<0.001$ for all comparisons). Both fluconazole doses also enhanced median survival (13.5 - 25.5 days; $p<0.001$) compared to control, but percent survival (range 0-30%) was not improved. Fungal burden demonstrated greater variability between the strains and different doses of each antifungal. Against DI23-1, CFU counts on day 9 in mice treated with either fluconazole dose (range 3.87 - 4.40 $\log_{10}$ CFU/g) were not significantly different compared to the vehicle control group (4.91 $\log_{10}$ CFU/g) but were significantly lower with either posaconazole dose (0.0 - 1.09 $\log_{10}$ CFU/g; $p<0.01$). Against DI23-2 reductions in fungal burden were similar with both fluconazole doses and posaconazole 10 mg/kg QD (range 2.16-3.25 $\log_{10}$ CFU/g) and were lower than vehicle control (5.10 $\log_{10}$ CFU/g; $p<0.01$). The supratherapeutic dose of posaconazole had the lowest fungal burden (0.66 $\log_{10}$ CFU/g). In the survival arm, rebounds in fungal burden were observed for each azole against each isolate once therapy was stopped.
Conclusion: Survival was moderately enhanced with fluconazole as were reductions in fungal burden. However, the majority of mice succumbed to infection once therapy was stopped and rebounds in fungal burden were observed. Survival was markedly enhanced with posaconazole, which resulted in greater reductions in fungal burden with the supratherapeutic dose. Further studies are needed to determine if posaconazole therapy may be effective against coccidioidomycosis caused by fluconazole-resistant strains.

P005

Evaluation of the toxicological aspects of Aureobasidium pullulans var. pullulans-derived mycotoxin Orcinotriol and its prophylaxis with Cinnamaldehyde

Ankita Kumari[1], Karuna Singh[1], Gunjan Uttam[1]
[1]Animal Mycology Lab, Department of Zoology, MMV, Banaras Hindu University, Varanasi, India

Objective
Though mycotoxins are well documented indoors, their effects on human physiology are not yet completely understood. In this regard, orcinotriol (Orc), a mycotoxin derived from an indoor fungal contaminant, *Aureobasidium pullulans* var. *pullulans,* has been assessed for its toxicological potential, and a complete organ study has been presented. Due to the multivariate exposure and association of mycotoxins, prevention is always better; therefore, the prophylactic role of cinnamaldehyde (Cin) has also been evaluated.

Methods
SwissADME, PreADMET, and QikProp were used for *in-silico* toxicokinetic evaluations.
The LD_{50} of Orc was measured using an MTT assay. Orc concentrations at 12.5, 25, and 50 µg/ml were investigated *in vitro using splenocytes*. Further, immune functions were studied using leukocytes, subjected to *in vitro* exposure to 0 and 50 µg/ml Orc and flow cytometric measurement of ROS.

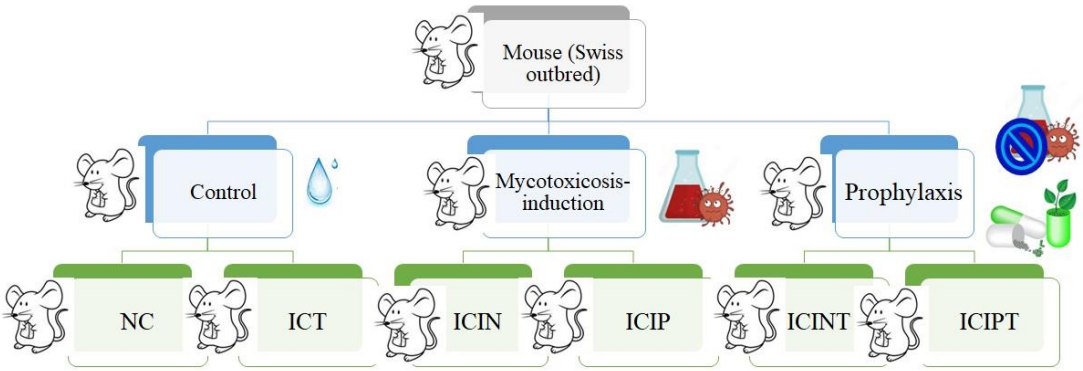


Figure 1. Flowchart for grouping of mice

Serial no	Dose	Dosage	Route	Frequency	Overall duration (weeks)	Group (n)
1	DW	20 muL	IN	OD	12	NC 1 (5)
2	DW	50 muL	IP	OD	12	NC 2 (5)
3	Cin	210 mg/kg BW	PO	OD	10	ICT (5)
4	Orc	50 mg/kg BW	IN	OD	12	ICIN (5)
5	Orc	25 mg/kg BW	IP	OD	12	ICIP (5)
6	Orc + Cin	Orc – 50 mug/kg BW, Cin – 210 mg/kg BW	Orc – IN, Cin – PO	Orc – OD, Cin – PO	Orc – 12, Cin – 10	ICINT
7	Orc + Cin	Orc – 25 mug/kg BW, Cin – 210 mg/kg BW	Orc – IP, Cin – PO	Orc – OD, Cin – PO	Orc – 12, Cin – 10	ICIPT

IN – Intra-nasal
IP – Intra-peritoneal
PO – *Per os*
OD – Once in a day
Orc – Orcinotriol
Cin – Cinnamaldehyde

Figure 2. Table for the description of the dosing regimen

A mouse model was developed for the sub-chronic toxicity assessment of Orc (Fig.1). Oral treatment with Cin was used for prophylaxis, started after 2 weeks of mycotoxin delivery, and carried out simultaneously up to 12 weeks (Fig.2). Mice were euthanized at the end of the regimen. Blood for DLC and eight organs for morphological, biochemical, and histological analyses were collected. A cell apoptotic marker (Caspase-3) was also measured spectrophotometrically. Further, plasma was collected and subjected to HRMS studies for the comparative determination of Orc in the control, MI (mycotoxicosis-induced), and prophylaxis groups.
PatchDock and FireDock web servers were used to determine *in-silico* interactions between Orc and tumour markers for brain and lung cancers.

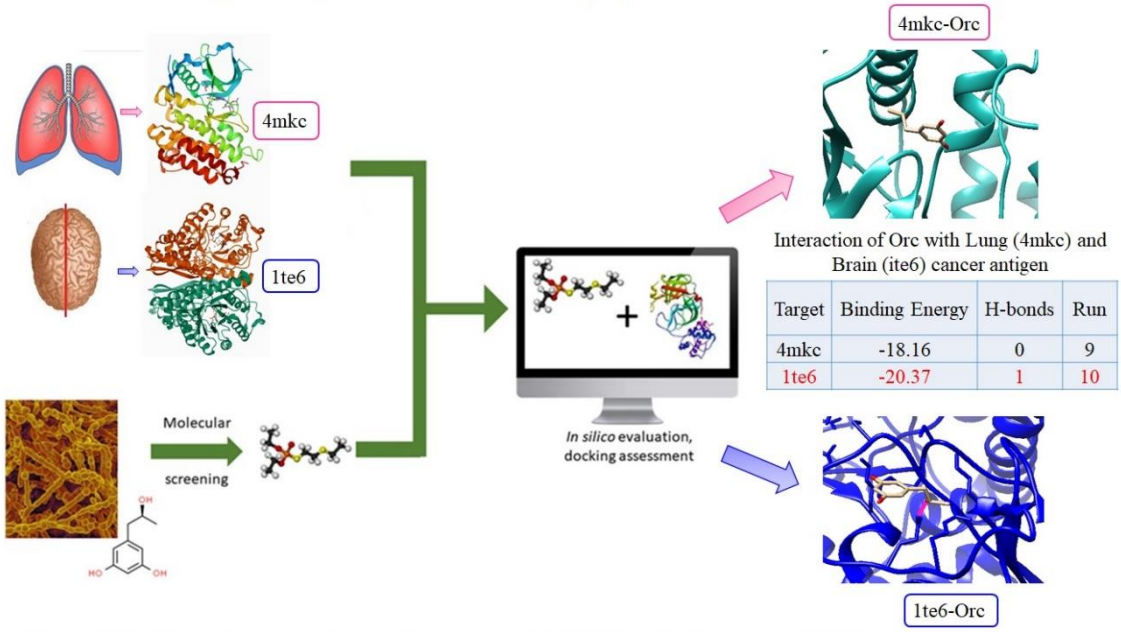
Results

Flow cytometric results of Orc with IC_{50}50 µg/ml represented the oxidative burst stimulation property.
Fig.3 shows the results of the toxicokinetic evaluation.
Lymphocytosis and neutropenia were observed in the MI groups. The prophylaxis groups, however, maintained the DLC to some extent. Morphologically, the size and weight of the spleen and liver in both MI groups increased, which corroborated with angiectasis (ICIN) and hyperplasia (ICIP) in the spleen and amyloid (ICIN) and inflammation (ICIN) in the liver

histopathologies. Increased lungs and brain weights suggested fluid build-ups, which can be correlated to oedema (MI) in lungs and axonopathy (ICIN) and necrosis (ICIP) in brain. The increased weight of the heart suggested dilated cardiomyopathy. Likewise, development of tumours in the stomach was further confirmed by hyperplasia in histological sections (MI). The increased weight of the kidneys was correlated with hypertrophy (ICIN) and nephroblastematosis (ICIP) in renal sections. No such pathologies were observed in the prophylaxis groups except for the testis. The MI groups showed reduced testis weight morphologically, and degeneration and atrophy histologically. Further, histopathology was concurrent with the reduced caspase 3 in MI groups. ROS studies marked the presence of oxidative stress by showing elevated levels of MDA, resulting in lipid peroxidation and lowered SOD and CAT activity. HRMS results showed a decrease in Orc concentration in the prophylaxis groups.

Physicochemical Properties		Toxicokinetics 1		Toxicokinetics 2	
Formula	$C_9H_{12}O_3$	Acute algae toxicity	0.0564288	GI absorption	High
Molecular weight	168.19 g/mol	Ames test (mutagenicity)	Mutagen	BBB permeant	Yes
Log $P_{o/w}$	1.16	Carcinogenicity(Rat)	Positive	Log K_p (skin permeation)	-6.46 cm/s
Log S (water)	-1.83	Carcinogenicity(Mice)	Negative	Bioavailability Score	0.55
Class	Soluble	Acute Daphnia toxicity	0.614744	Human intestinal absorption (%)	80.615925
Lipinski rule	0 violation	hERG inhibition (Human)	Low Risk		
Synthetic accessibility (very easy)	1.79	Acute fish toxicity (medaka)	0.437495		

Figure 3. Physiochemical and ADMET properties of Orcinotriol



Interaction of Orc with Lung (4mkc) and Brain (ite6) cancer antigen

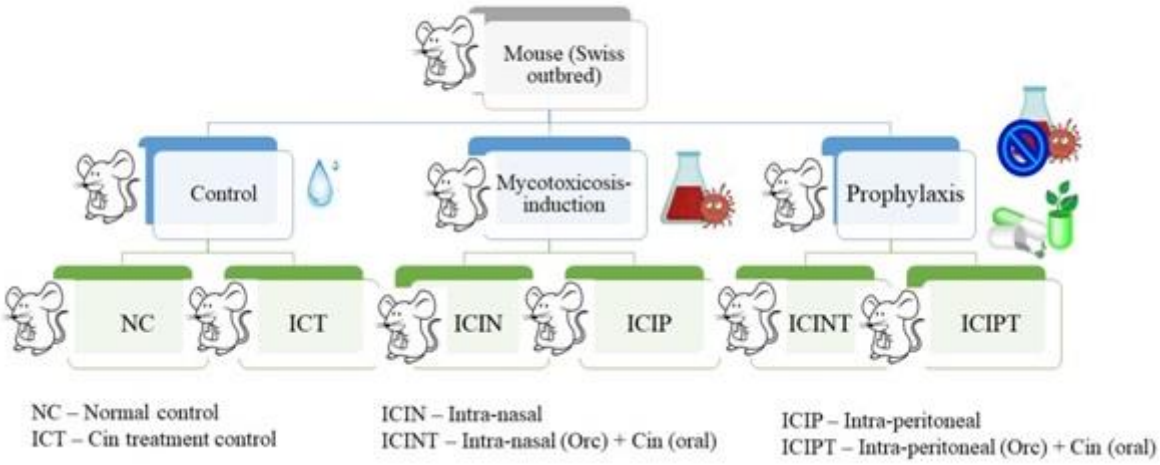
Target	Binding Energy	H-bonds	Run
4mkc	-18.16	0	9
1te6	-20.37	1	10

Figure 4. Docking of tumor markers of lung cancer (4MKC) and brain cancer (1TE6) and the Orc

Orc formed more stable docked structures with 1TE6 than 4MKC marker (Fig.4).

Conclusion

The toxicity of Orc was more pronounced when administered intranasally. However, Cin prophylaxis was found to be effective in both routes of administration.



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4	Orc	50 mg/kg BW	IN	OD	12	ICIN (5)
5	Orc	25 mg/kg BW	IP	OD	12	ICIP (5)
6	Orc + Cin	Orc – 50 mug/kg BW, Cin – 210 mg/kg BW	Orc – IN, Cin – PO	Orc – OD, Cin – PO	Orc – 12, Cin – 10	ICINT
7	Orc + Cin	Orc – 25 mug/kg BW, Cin – 210 mg/kg BW	Orc – IP, Cin – PO	Orc – OD, Cin – PO	Orc – 12, Cin – 10	ICIPT

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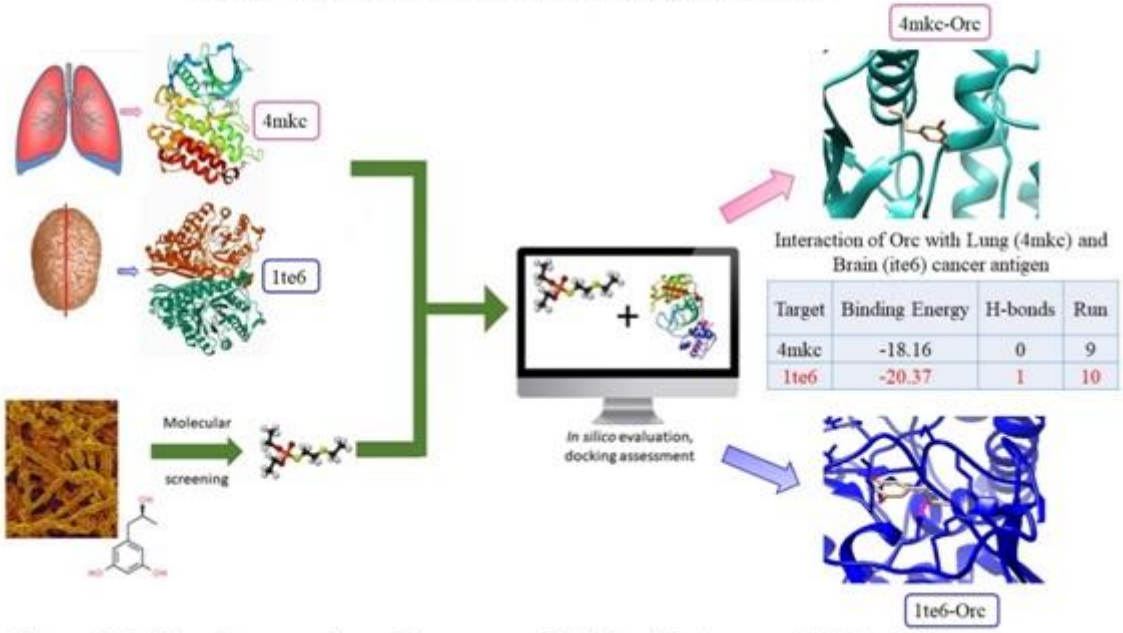

Interaction of Orc with Lung (4mkc) and Brain (ite6) cancer antigen

Target	Binding Energy	H-bonds	Run
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1te6	-20.37	1	10

Figure 4. Docking of tumor markers of lung cancer (4MKC) and brain cancer (1TE6) and the Orc

P006

Study of virulence factors in Trichophyton benhamiae using an optimized mouse skin infection model

Dr Wilfried Poirier[1], Emilie Faway[2], Françoise Maréchal[1], Célya Danzelle[1], Tsuyoshi Yamada[3], Yves Poumay[2], Bernard Mignon[1]
[1]Faculty of veterinary medicine, Liège, Belgium, [2]Faculty of medicine, Namur, Belgium, [3]Teikyo university - Institute of medical mycology, Tokyo, Japan

Introduction

Dermatophytosis are superficial cutaneous mycoses observed in both humans and animals caused by filamentous fungi called dermatophytes. Human dermatophytosis is widespread and most often caused by species belonging to the genus *Trichophyton*. The increasing emergence of strains resistant to currently available antifungals require the development of new treatments. In this context, a better knowledge of the pathogenesis of dermatophytosis, including the identification of fungal virulence factors such as subtilisins, is a key step for the identification of new therapeutic targets. A method for standardizing the production of dermatophyte spores to perform lab infections has been published by our research team (Faway *et al*., 2021). If this method based on the use of suspensions enriched in unicellular spores allows the development of standardized infections on human epidermis reconstructed *in vitro*, it is not optimal to produce relevant *in vivo* infections. The objective of this study is to develop a new relevant mouse model of infection with a reference strain of *Trichophyton benhamiae* and derived deleted strains, using a new standardized inoculum and to evaluate the expression of genes coding for subtilisins during the infection process.

Methods & Materials

Using tBLASTn and bioinformatics analysis, 12 genes coding for subtilisins were identified within the genome of *T. benhamiae*. To assess the involvement of these subtilisins in the infection, the *T. benhamiae* IHEM 20161 strain deleted at the Ku70 locus was used to produce six strains invalidated for one or more subtilisins (ΔSub6; ΔSub7; ΔSub8; ΔSub10; ΔSub6.ΔSub10 and ΔSub6.ΔSub8.ΔSub10). These strains were tested in a new epicutaneous mouse infection model, using a mixture of spores (1.10^8 CFU), germ tubes and mycelium (100 mg) as an inoculum. Kinetic monitoring of the infection was carried out by the establishment of a global clinical score based on the intensity (0 to 4) of three clinical signs, *i.e.* erythema, scales and crusts, while the expression of the 12 subtilisins was evaluated by real-time quantitative PCR at two and five post-infection day.

Results

The inoculum used in this new *in vivo* model generates clearly visible skin symptoms mimicking a self-resolving natural infection, lasting more than twice as long (16 days *vs* 7 days) as those obtained in the past with only spores as inoculum. Clinical scores did not differ significantly between WT and strains knocked out for one or more subtilisins. In comparison with the control condition (mycelium in liquid Sabouraud media), the Sub1, Sub3, Sub6 and Sub10 were overexpressed by the WT strain during the infection process while several compensation phenomena, notably the overexpression of Sub5 by strain ΔSub8, were observed.

Conclusion

Using a new optimized reproducible mouse model of *Trichophyton benhamiae* dermatophytosis allowing the study of fungal virulence factors, we show that the invalidation of subtilisin genes would induce compensatory mechanisms of overexpression of other subtilisins having a similar activity, testifying to the importance of these proteases during the

infection in mice. Other ongoing research will include histological analysis, quantification of fungal genomic DNA, and analysis of the expression of other virulence factors in skin lesions.

P010

Utilizing WHONET for Surveillance of Antifungal Resistance

Sidhya Choudhary[1], Smriti Parihar, Saurabh Sharma
[1]Government Medical College, Kota, India

Background:

Antifungal resistance has become a significant public health challenge, as it compromises the efficacy of antifungal agents and limits treatment options for fungal infections. Surveillance of antifungal resistance is essential to guide appropriate therapeutic decisions and implement effective infection control measures. WHONET, a software developed by the World Health Organization (WHO) for monitoring antimicrobial resistance, has shown promise in surveillance of bacterial resistance. However, its application in antifungal resistance surveillance remains understudied.

Objectives:

1. To assess the feasibility and adaptability of WHONET for monitoring antifungal resistance.
2. To evaluate the performance of WHONET in detecting trends and patterns of antifungal resistance.
3. To compare the results obtained from WHONET with other existing surveillance systems.

Materials and Methods:

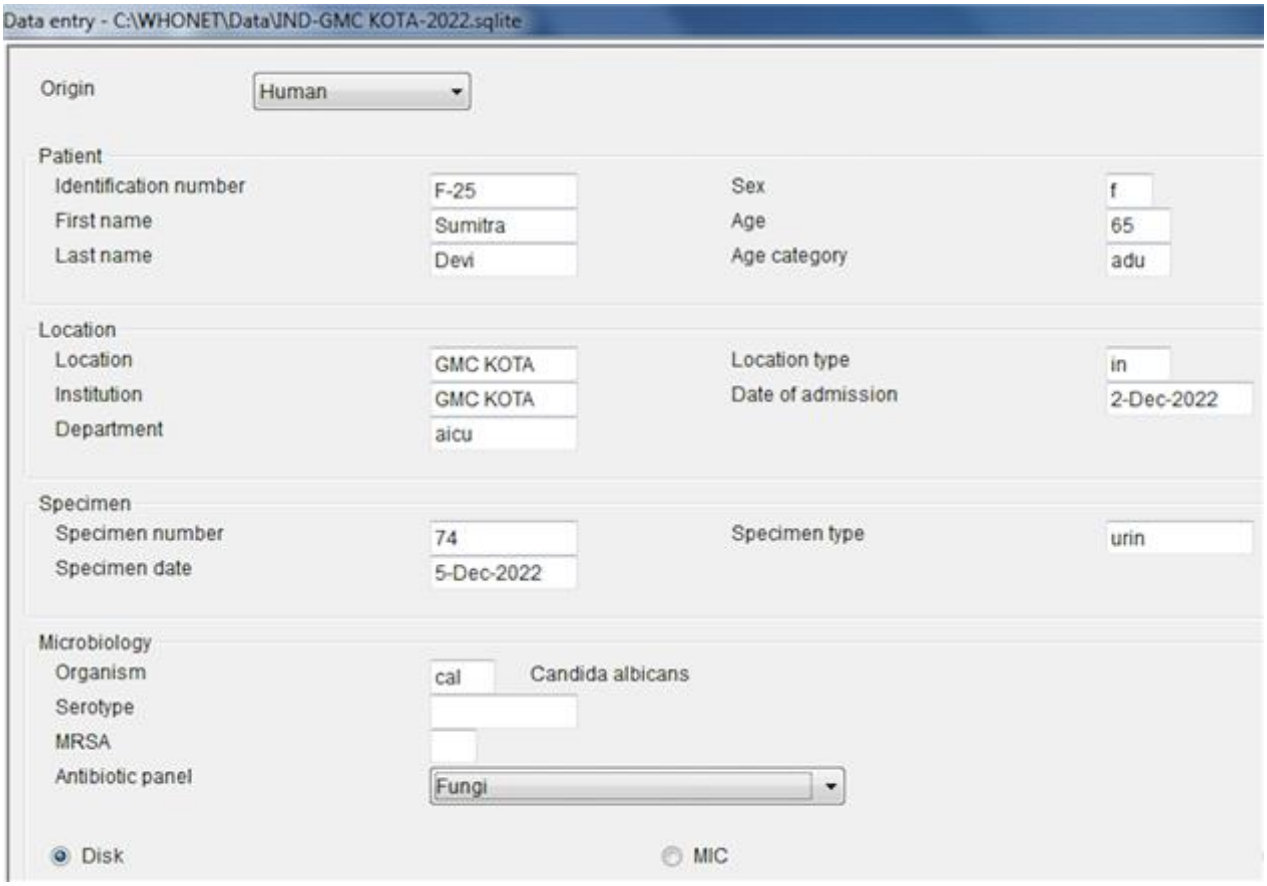
This study utilized retrospective data from clinical microbiology laboratories and hospital records. Since December 2022, our institution has implemented WHONET as our laboratory information system. The software is utilized to enter comprehensive patient details on the requisition form, including geographic location, specimen type, infection site, date and time of sample collection, provisional diagnosis, current drug therapy, and any existing immunocompromised conditions.

Results:

The results demonstrated the successful implementation of WHONET for antifungal resistance surveillance. The software proved adaptable for capturing and analyzing antifungal susceptibility data from multiple laboratory sources. The analysis revealed important trends, such as the emergence of resistance among specific fungal species and changes in susceptibility patterns over time. WHONET assists in the identification of both hospital and community outbreaks, while also facilitating the recognition of quality assurance issues in laboratory testing. One of the key advantages of WHONET is its cost-effectiveness, as it is a freely available software developed by the World Health Organization. This eliminates the need for purchasing and maintaining expensive data entry software.

Conclusion:

WHONET represents a valuable tool for surveillance of antifungal resistance. Its adaptability and ease of use make it suitable for monitoring and detecting trends in antifungal susceptibility across different healthcare settings. By incorporating WHONET into routine surveillance activities, healthcare professionals and public health authorities can improve the understanding of antifungal resistance patterns, facilitate early detection of emerging resistance, and guide appropriate therapeutic strategies. Future research should focus on expanding the scope of WHONET to encompass a broader range of antifungal agents and further validate its utility in different geographic regions.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Sumitra
Last name
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P012

Antifungal resistance of clinical Candida albicans isolates in Iran: A systematic review and meta-analysis

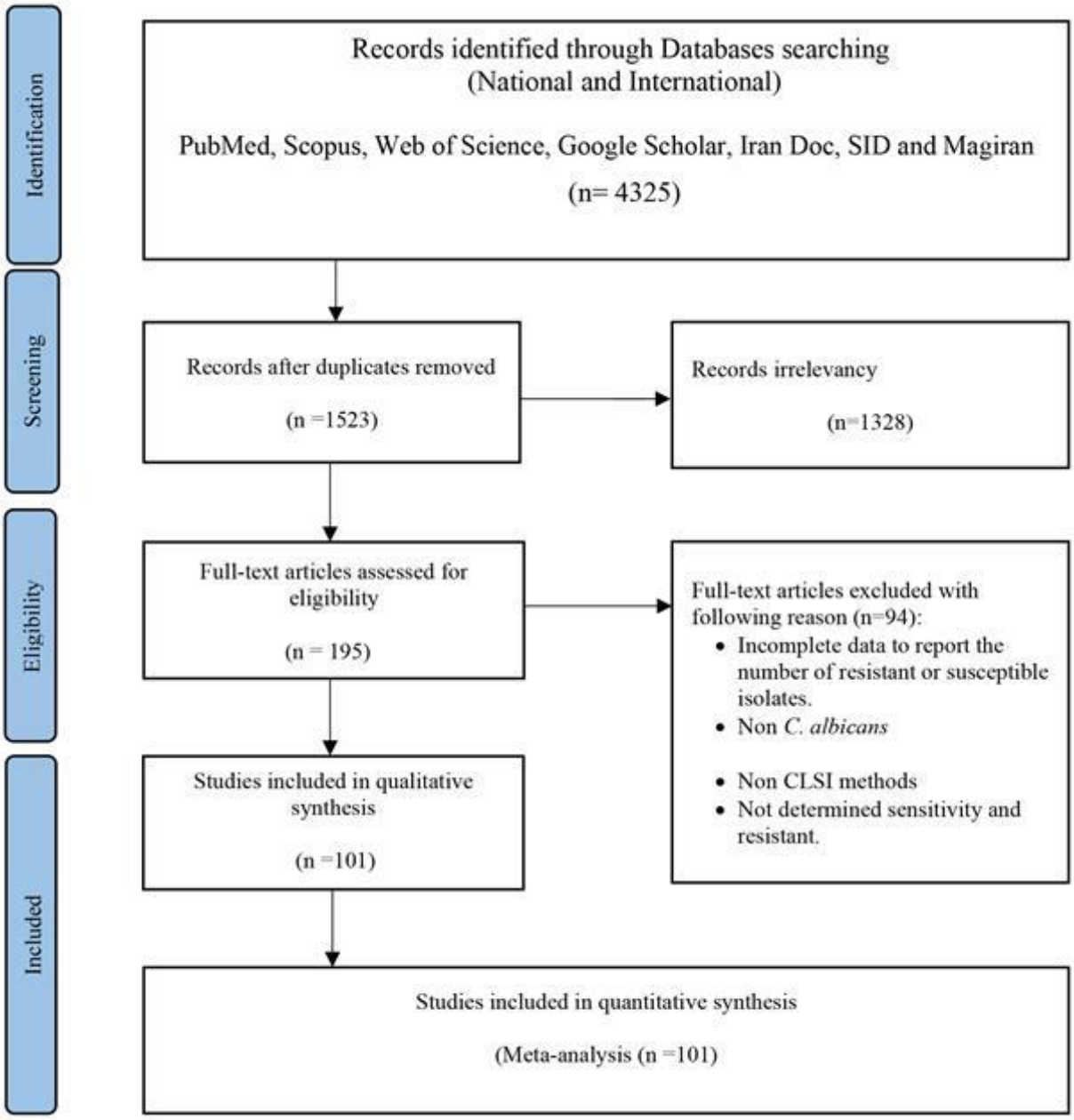
Firoozeh Kermani[1], Mojtaba Taghizadeh-Armaki[1], Seyed Abdollah Hosseini[2], Dr Nasrin Amirrajab[3], Javad Javidnia[4], Mahmoud Fami Zaghrami[5], Tahereh Shokohi[6]
[1]Department of Medical Myology /Parasitology, Babol University Of Medial Mycology, Sari, Iran, [2] Department of Medical Parasitology/Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran, [3] Department of Laboratory Sciences, School of Para-Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran, Ahvaz, Iran, [4]PhD, Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran, [5]Knowledge-based Co. Darui Daneshgar Zolang Respina Pharmaceutical Company, Sari, Iran, Sari, Iran, [6]Department of Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, Sari, Iran

Objectives: Antifungal susceptibility patterns of *Candida* infections can play an essential and decisive role in the treatment outcome. The present systematic review and meta-analysis aimed to investigate the drug susceptibility pattern of Iranian clinical *Candida albicans* isolates to antifungal drugs (azoles, polyenes, and echinocandins).

Materials & Methods: Six electronic databases including “PubMed,” “Scopus,” “Web of Science,” “IranDoc”, “SID”, “Magiran” were searched from May 2000 to June 2021.

Results: The susceptibility of 6322 *C. albicans* strains from 19967 patients against 14 antifungal drugs were evaluated according to Clinical and Laboratory Standards Institute (CLSI) methods. The pooled prevalence of antifungal resistance ranged from 0 to 26%. The lowest resistance levels among azoles were observed in luliconazole with a frequency of 0% and voriconazole of 3.94% (95% CI: 2.24 to 6.09%). An increase in trend of itraconazole (7-16.65%), voriconazole (1.4 to 6.52%), and amphotericin B resistance (7.5 to 9.11%) among *C. albicans* strains since 2006 in Iran, while a decreasing trend was observed in fluconazole resistance (23.42 to 14.42%).

Conclusions: Due to the resistance of *C. albicans* species to common antifungal drugs, antifungal stewardship strategy combining therapeutic drug monitoring to reduce the emergence of resistant multi-drug *Candida* species.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Screening
Eligibility
Included
Records identified through Databases searching
(National and International)
PubMed, Scopus, Web of Science, Google Scholar, Iran Doc, SID and Magiran
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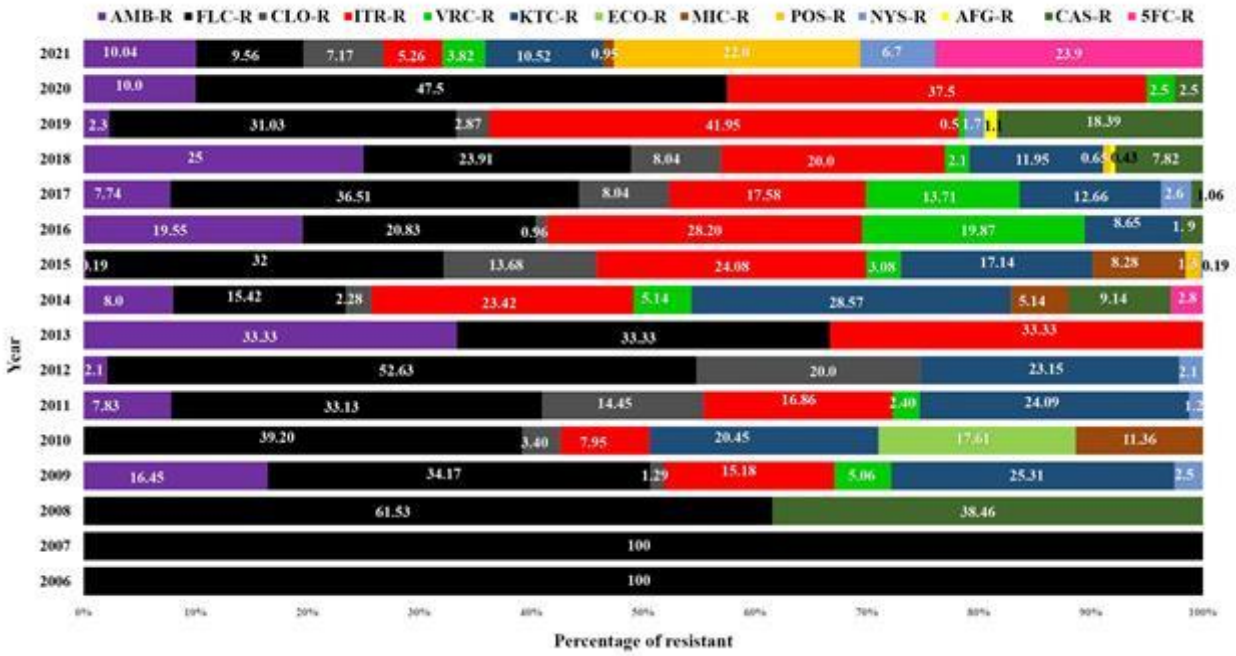
(n =1523)
Records irrelevancy
(n=1328)
Full-text articles assessed for eligibility
(n = 195)
Full-text articles excluded with following reason (n=94):
Incomplete data to report the number of resistant or susceptible isolates.
Non C. albicans
Non CLSI methods
Not determined sensitivity and resistant.
Studies included in qualitative synthesis
(n =101)
Studies included in quantitative synthesis
(Meta-analysis (n =101)


AMB-R FLC-R CLO-R ITR-R VRC-R KTC-R ECO-R MIC-R POS-R NYS-R AFG-R CAS-R 5FC-R
Year
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2020 10.0 47.5 37.5 2.5 2.5
2019 2.3 31.03 2.87 41.95 0.5 1.7 1.1 18.39
2018 25 23.91 8.04 20.0 2.1 11.95 0.6 0.43 7.82
2017 7.74 36.51 8.04 17.58 13.71 12.66 2.6 1.06
2016 19.55 20.83 0.96 28.20 19.87 8.65 1.9
2015 0.19 32 13.68 24.08 3.08 17.14 8.28 1.3 0.19
2014 8.0 15.42 2.28 23.42 5.14 28.57 5.14 9.14 2.8
2013 33.33 33.33 33.33
2012 2.1 52.63 20.0 23.15 2.1
2011 7.83 33.13 14.45 16.86 2.40 24.09 1.2
2010 39.20 3.40 7.95 20.45 17.61 11.36
2009 16.45 34.17 1.29 15.18 5.06 25.31 2.5
2008 61.53 38.46
2007 100
2006 100
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
Percentage of resistant

P013

Fruits Are Vehicles of Drug-Resistant Pathogenic Candida tropicalis

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Objectives:
Candida tropicalis is the leading cause of non-albicans candidemia in tropical/sub-tropical areas. We detected a predominant genotype of azole-resistant *C. tropicalis* clinical strains in Taiwan from 2014 to 2018. We would like to investigate the potential fomite transmission of drug-resistant *C. tropicalis*.

Materials & Methods:
We characterized yeasts recovered from 17 samples sourced from six different kinds of fruits from a supermarket in northern Taiwan in the present study. Fruits were gently washed with buffer, and the solution was then collected for centrifugation. Cell suspension was plated onto CHROMagar Candida medium. Representative yeast species was identified by rDNA sequencing. Drug susceptibilities of *C. tropicalis* were determined by the broth microdilution method.

Results:
We found that different types of fruit surface had different distributions of yeast species. Washing fruit can significantly eliminate number of yeasts from the surface. Of 123 identified isolates, *C. tropicalis* was the most frequently found species, followed by *Meyerozyma caribbica* and *Candida krusei*. Among 10 collected *C. tropicalis*, all 3 fluconazole-resistant ones were non-susceptible to voriconazole. Furthermore, the genotype of all azole-resistant *C. tropicalis* belonged to the same predominant genotype of azole-resistant *C. tropicalis* causing candidemia in patients in Taiwan.

Conclusions:
Hence, fruit can serve as a vehicle of azole-resistant *C. tropicalis* and other species, especially *C. krusei*, which is intrinsically resistant to fluconazole. Our findings provide an evidence that fruit should be washed before eaten , especially for immunocompromised individuals, not only to remove chemicals but also potential drug resistant pathogenic microbes.

P014

Phenotypic and genotypic characterization of azole-resistant Aspergillus fumigatus over 11 years

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Objectives
Invasive aspergillosis (IA) mostly occurs in immunocompromised patients, especially in patients with haematological malignancies or after allogeneic and solid organ transplantation. As first-line antifungal therapy, azole antifungal drugs are recommended which have been shown to be effective in the past. In recent years, the emergence of azole-resistant *Aspergillus fumigatus* strains (ARAF) has become a significant challenge in the treatment of IA. This study assessed the epidemiology of ARAF strains in the last eleven years within the University Hospital Essen, Germany.
Methods & Materials
The epidemiology of ARAF was investigated during 2012–2022. All respiratory samples were plated on malt extract agar and incubated for 7 days at 30°C. Identification of isolates was performed using classical macro- and micromorphological characteristics. During the years of collection, all isolates underwent susceptibility testing for at least itraconazole or for both, itraconazole and voriconazole by gradient test. ARAF was defined as non-wild-type minimal inhibitory concentration (MIC). Analysis of mutations mediating resistance was performed using PCR. Patient records were analysed retrospectively regarding sex, age, underlying disease and 30-day in-hospital outcome.
Results
Over the 11 years, 196 ARAFs (6.1%) and 3002 wild type (WT) isolates of *Aspergillus fumigatus* were found. The number of ARAF cases remained consistent in the years from 2015 to 2019 until an increase in the years 2020 and 2021. 2021 was the year with the highest ARAF rate of 10.8%. Regarding seasonal distribution, non-ARAFs occurred mostly in summer and fall whereas most of the ARAFs were isolated in spring and summer. In total, ARAFs were mostly detected in male patients (n=108, 55%) but the gender distribution was variable over time. Median age was 44 years in patients with ARAF and 50 years in patients with non-ARAF. L98H/TR34 was the most prevalent mutation (33%) followed by T289/Y121 (5%). Results on 30-day in-hospital outcome and underlying disease will follow.
Conclusion
The findings of this study provide valuable insights into the epidemiological development of infections with ARAF within the last decade. It highlights the emergence of azole-resistant IA in North Rhine-Westphalia and underlines the importance for systematic antifungal susceptibility testing of *A. fumigatus*.

P016

Update on the allele-specific TaqMan real-time PCR method of detecting triazole-resistant strains of Aspergillus fumigatus

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Objectives: The non-culture-based methods for molecular detection of triazole-resistant isolates of *A. fumigatus* directly from clinical samples can facilitate early diagnosis of resistance and the initiation of rational antifungal therapy by reducing turnaround times. In the previous work, we have described an in-house allele-specific TaqMan real-time PCR method consisting of 7 simultaneous assays that detect mutations (TR34/L98H, TR46/Y121F, G54R, G54W, and M220I) in *cyp51A* gene (DOI: 10.1128/JCM.00604-19). Recently, we obtained two triazole-resistant strains of *A. fumigatus*, one with novel *hmg1* (W272C) mutations, the other harboring combined *cyp51A* (M263I) and *hmg1* (E306K) mutations. Since triazole resistance caused by novel mutations in *hmg1* and *cyp51A* is increasingly reported worldwide, it is necessary to establish the corresponding real-time PCR assays targeting the above three mutations.

Materials & Methods: The specific primers, probes, and blockers were designed based on the sequences of the mutant alleles in *hmg1* and *cyp51A*. A 10-fold dilution range of genomic DNA (gDNA) spanning 300 fg to 30 ng (corresponding to *A. fumigatus* CFU of 1×10^2 to 1×10^8) was constructed for each real-time PCR assay. By plotting the logarithms of the serial dilution standard on the *x*-axis and the cycle threshold (Ct) values obtained as described previously (DOI: 10.1128/JCM.00604-19) on the *y*-axis, the standard curve and the equation for each assay were established.

Results: The slopes of the standard curves of these three assays were between - 3.690 and - 3.314 (87% to 100% efficiency), with a correlation coefficient of 0.99. The sensitivity of these three assays was less than 300 fg/well, corresponding to about 100 CFU per reaction mixture. There was no cross-reaction with human gDNA or gDNA from other pathogenic fungi tested, indicating good specificity.

Conclusions: These data demonstrate the good performance of the TaqMan real-time PCR method for detecting triazole-resistant isolates of *A. fumigatus* with the novel mutations in *cyp51A* (M263I) and *hmg1* (W272C and E306K), which is an important improvement to our previous work.

P017

Evaluation of Inoculum Preparation for Etest and EUCAST Broth Dilution to Detect Anidulafungin Polyresistance in Candida glabrata

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Objectives:

The aim was to evaluate the influence of inoculum preparation in EUCAST broth dilution and Etest on the detection of coexisting resistant and susceptible *Candida* subpopulations, which was defined as polyresistance.

Materials & Methods:

Two echinocandin-resistant and susceptible clinical *C. glabrata* strains were mixed together as a co-culture and were used to simulate the occurrence of mixed populations in clinical samples. Different approaches of inoculum preparation were performed for antifungal susceptibility testing of anidulafungin, including the suspension of 1 to 2 *C. glabrata* colonies, 5 colonies, and high turbidity suspensions prior to re-dilution for testing.

Results:

In Etests, polyresistant results manifested as microcolonies or double ellipses within the zone of inhibition, implying the representation of the resistant subpopulation in the test. In EUCAST broth dilution, polyresistant results manifested as single reduced optical density (OD) values, termed as a dip in OD. The inclusion of five distinct colonies, as recommended by procedural guidelines, led to higher rates of polyresistant and resistant results compared to including one to two colonies in inoculum preparation (30% and 26% for Etest and broth dilution, respectively). Increasing the inoculum turbidity (and by that the number of tested *C. glabrata* colonies) to a 2 to 4 McFarland standard before re-dilution to the required 0.5 McFarland standard reliably enabled the detection of resistance, with even better identification of polyresistance by Etest than by broth dilution (82% versus 32%, respectively). With these high turbidity suspensions, 18% of Etests and 67% of microdilutions found resistant minimum inhibitory concentration (MIC) values. As a main difference, polyresistant results were characterized by the apparent detection of resistant subpopulations, while in resistant results, no co-existence of resistant and susceptible strains was detectable. Overall, the highest identification of polyresistance succeeded with Etest and a modified 3 McFarland standard approach of inoculum preparation.

Conclusions:

Our results show that antifungal susceptibility testing as performed in clinical practice does not reliably identify resistant subpopulations in polyresistant *Candida* cultures, which is due to the use of only a few colonies in inoculum preparation. As a simple adaption, we propose to increase the number of suspended colonies in the inoculum to a 3 McFarland standard with subsequent re-dilution to overcome this issue.

Knoll MA, Samardzic E, Posch W, Lass-Flörl C. Evaluation of Inoculum Preparation for Etest and EUCAST Broth Dilution to Detect Anidulafungin Polyresistance in Candida glabrata. Antimicrob Agents Chemother. 2022 Aug 16;66(8):e0016822. doi: 10.1128/aac.00168-22

P018

The Aspergillus fumigatus DNA mismatch repair msh6 gene and its relation with antifungal resistance development

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Objectives: Fungi exhibit a diversity of mechanisms to create genetic variation that eventually can lead to the selection and spread of resistant fungal pathogens. Some studies have suggested the importance of genetic instability in *A. fumigatus* as a possible mechanism of evolving azole resistance. One of the systems responsible for the recognition and repair of the mistakes occurring during cell replication is the DNA mismatch repair (MMR) system. Two major protein complexes constitute the MMR pathway: MutS, which recognizes the mismatch, and MutL, which removes the strand with the mistake. In previous work, we examined the MMR gene variations in 300 *A. fumigatus* genomes including azole-susceptible and resistant strains. The *msh6* (Afu4g08300), *msh2* (Afu3g09850), *pms1* (Afu2g13410) and *mlh1* (Afu5g11700) genes were analyzed. Results showed that genes *msh2*, *pms1,* and *mlh1* had low genetic variability with only a few mutations detected in some strains, unrelated to their azole susceptibility phenotype. In contrast, the *msh6* gene had a nonsynonymous mutation (G240A) harbored by 42% of the strains, all of them closely related in the phylogenic tree and most of them also harboring the TR_{34}/L98H azole resistance mechanism in the *cyp*51A gene. Here, we investigate the *msh6* gene, and its possible relation with antifungal resistance development.

Materials & Methods: In this work, the gene *msh*6 was deleted in an *aku*B^{KU80} *A. fumigatus* strain and the Δ*msh6* mutant isolates were subjected to different fitness tests, azole susceptibility, and virulence assays using the *Galleria mellonella* alternative infection model. Mutagenesis experiments were carried out exposing wild-type (WT) *msh6* and Δ*msh6* strains to different concentrations of three azole drugs, posaconazole, voriconazole and prochloraz and other non azole antifungal drugs used in crop protection, benomyl, boscalid and azoxystrobin.

Results: There were no differences of the Δ*msh6*-deleted strains compared with the *aku*B^{KU80} parental strain in any of the conditions tested in terms of virulence or osmotic or cell wall stresses. In addition, there were no differences in susceptibility to different clinical or agricultural azole drugs nor in the development of resistance to these drugs. Azole resistant mutants were isolated only under posaconazole pressure, but not when voriconazole or prochloraz were used as selective agents. Interestingly, when non-azole antifungal selection was used, the Δ*msh*6 strains generated 10- and 2-fold more boscalid and benomyl-resistant mutants, respectively, showing a great variety of different mutations in each antifungal target genes.

Conclusions: The lack of *msh6* caused no apparent increase in the mutation rate to azole antifungals. In contrast, in the absence of *msh6* there was a higher mutation rate to other

agricultural fungicides, suggesting a role for this gene in the development of resistance in the fields

P019

ANTIFUNGAL SUSCEPTIBILITY OF FUNGI ISOLATED AT UNIVERSITY HOSPITAL OF POITIERS BETWEEN 2017 AND 2021

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Objectives:

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in vulnerable patients. The emergence of antifungal resistance threatens their treatment efficiency. Knowledge of the local epidemiology seems therefore essential to guide the therapeutic management. The aim of this study was to describe the susceptibility pattern of fungi isolated at the University Hospital of Poitiers.

Materials & Methods:

The minimum inhibitory concentration values, obtained by E-test, of isolates tested in Poitiers University Hospital laboratory between January 2017 and December 2021 were collected, interpreted with the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints and analyzed.

Results:

A total of 1193 strains (yeasts and filamentous fungi) were included in this study. Among the *Candida* species, *C. albicans* was the most represented (55.86%) followed by *C. glabrata* (15.35%) and *C. parapsilosis* complex (7.68%). The rates of acquired resistance in yeasts were low. However, few fluconazole-resistant and echinocandin-resistant *C. albicans* strains were isolated in pre-exposed patients. Several uncommon species of *Candida*, with decreased susceptibility to fluconazole were also isolated. For *Aspergillus* section *fumigati*, resistance to azoles was significant, with 4.8% of strains resistant to one or several azoles.

Conclusions:

Our study provides antifungal susceptibility patterns of fungi isolated in a French University Hospital and identified major risks of antifungal resistance. Pre-exposed patients, uncommon *Candida* species and azole susceptibility of *Aspergillus sp* must be closely monitored in our West European countries. Continuous monitoring of local epidemiology must be continued and appears essential to optimise the therapeutic management of patients and especially empirical treatments.

P020

Acquisition of echinocandin resistance in clinical settings

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Objectives: Echinocandins rank number one among systemic antifungal drugs used for the treatment of invasive fungal infections. In certain medical centres, this has been associated with a shift in the epidemiology of *Candida* species toward less-susceptible species and the emergence of echinocandin resistance mostly associated with point mutations in *fks* genes encoding the ß-glucan synthase.
Here, we retrospectively analysed the epidemiology of *Candida* species and the evolution of echinocandin MICs of *Candida* isolated since the introduction of those drugs in our centre. Resistant strains were genotyped for *fks* genes and microsatellite regions (i) to investigate the correlation between genotype and in vitro yeast fitness (ii) to distinguish between acquired resistance and superinfection with a previously susceptible strain.

Materials & Methods: The consumption of echinocandins and the epidemiology of *Candida* species in our centre during the study period were recorded. All *Candida* strains isolated from candidiasis between 01/2006 and 12/2018 for which echinocandins MIC have been determined were included. Antifungal susceptibility testing was performed by the mean of the Etest method. In the case of a non-susceptible phenotype, MIC was determined using the EUCAST method and direct sequencing of *fks1* and *fks2* genes was performed.
When strain series both susceptible and non-susceptible from a single patient were available, strains were analysed by means of microsatellite length polymorphism. The fitness of those strains was evaluated in presence or not of a stress agent (Calcofluor White or caffeine).
Finally, to investigate the structure-function relationship of described mutations linked to echinocandin resistance, a structure of Fks1:Fks2 heterodimer as well as FKS homodimer were modelled with AlphaFold and further inspected.

Results:
The consumption of echinocandins in our centre increased 3-fold between 2006 and 2015 and remained stable until 2018. The percentage of *C. parapsilosis* complex among the *Candida* species isolated increased from 3.6% in 2006 to 4.5% in 2018 (p=0.058). The percentage of patients infected with an echinocandin non-susceptible strain remained stable over time ranging from 0 to 3% each year.
Fifteen patients presented with a non-susceptible strain (*C. albicans* n=3, *C. glabrata* n=4, *C. guillermondii* n=2, *C. parapsilosis* n=1, *C. kefyr* n=3, *C. tropicalis* n=2).
All non-susceptible strains had non-synonymous point mutation in *fks* genes. Series of strains were available for microsatellite genotyping for 6 patients confirming the acquisition of mutation

in the previously susceptible strain. According to species, mutant strains had either better or worse growth rates than isogenic isolates in media with or without stress conditions.
Structural analysis showed a mapping of all mutated residues within a hydrophobic contact pocket positioned within the membrane domains of FKS proteins, suggesting an impact on the flow of the BDG released.

Conclusions: A huge increase in echinocandin consumption was observed between 2006 and 2018 with a concomitant modification of *Candida* epidemiology but without a significant emergence of echinocandin non-susceptible *Candida* strains. Fitness analysis supports the hypothesis that *fks* mutations may be associated with a modification in growth capacity accounting for this situation. Structure function of FKS proteins may explain in part those differences.

P021

Candida auris TAC1B regulon in high-level fluconazole resistance

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Objectives:
Candida auris has been named by the CDC and WHO as an urgent threat to public health in no small part due to its development of multidrug resistance. Previously we have shown that mutations in the *C. auris* zinc cluster transcription factor gene *TAC1B* are common among triazole resistant clinical isolates from a global collection, and that the A640V encoding mutation contributes directly to clinical triazole resistance. In the present study, we examined the differential gene expression conferred by the most prevalent *TAC1B* mutations associated with triazole resistance: A640V, A657V, and N773_L774del (referred to as "ADdel"), and the contribution of the *CDR1* overexpression to *TAC1B* mutation-mediated fluconazole resistance.

Materials & Methods:
An isogenic set of *C. auris* strains carrying each of the three *TAC1B* mutations of interest and a wildtype control allele (*TAC1B*-WT) were constructed using a Cas9-mediated gene editing system. RNA was isolated from three biological replicates of each strain grown to mid-log phase, and gene expression was analyzed by RNA-seq. Subsequent analysis of genes of interest was performed by qRT-PCR with cDNA synthesized from RNA from three biological replicates of each strain grown for 6 hrs in MOPS-buffered RPMI + 2% glucose supplemented with fluconazole 16mg/L or DMSO solvent control. Disruption of *C. auris CDR1* was performed in each of the *TAC1B* allele backgrounds using a Cas9-mediated gene editing system. FLC MICs were measured by both broth microdilution and Etest following CLSI methodology.

Results:
As compared to the *TAC1B*-WT strain, sixteen genes were observed to be differentially expressed in the A640V strain, 98 genes in the A657V strain, and 880 genes in the ADdel strain. Moreover, thirteen genes were commonly differentially expressed and included *CDR1*. Quantitative RT-PCR revealed *CDR1* gene expression was increased approximately 2-fold in *TAC1B*-A657V, 4-fold in *TAC1B*-A640V, and 7-fold in *TAC1B*-ADdel as compared to *TAC1B*-WT. *CDR1* expression could be induced further in all strains upon FLC treatment: 5-fold in FLC-treated *TAC1B*-WT, 6-fold in FLC-treated *TAC1B*-A657V, 7-fold in FLC-treated *TAC1B*-A640V, and 8-fold in FLC-treated *TAC1B*-ADdel as compared to untreated *TAC1B*-WT. FLC MICs were subsequently measured in all strains: *TAC1B*-WT= 2 mg/L, *TAC1B*-A640V= 32 mg/L, *TAC1B-A657V*= 64 mg/L, and *TAC1B* ADdel= 64 mg/L. Disruption of *CDR1* in these strains led to stark reduction in FLC MIC in each *TAC1B* allele strain: *TAC1B*-WT= 0.125 mg/L, *TAC1B*-A640V= 0.5 mg/L, *TAC1B*-A657V= 1 mg/L, and *TAC1B*-ADdel= 0.5 mg/L.

Conclusions:
These data demonstrate the most prevalent *TAC1B* mutations associated with triazole resistance significantly increase fluconazole MICs. And while vast differences in the number of differentially-expressed genes associated with each of the three *TAC1B* mutations were observed, all *TAC1B* strains up-regulated *CDR1* expression. Furthermore, disruption of *CDR1* in each of these mutant strains resulted in lower FLC MICs to levels comparable to those observed in the *TAC1B*-WT comparator strain. Therefore, these results indicate that *CDR1* has a direct, significant role in *TAC1B*-mediated fluconazole resistance in *C. auris*.

P022

Impact of COVID-19 pandemic on antifungal consumption in a tertiary referral hospital in Greece

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Objectives:
During the COVID-19 pandemic, an overall increase in antimicrobial consumption was noticed. However, little is known about the effect of the pandemic on antifungal consumption in hospitals. This study aimed to analyze the impact of the COVID-19 pandemic on antifungal consumption in a tertiary hospital of 750 /beds, which was characterized as the main referral hospital for Covid-19 in Athens, Greece.

Materials & Methods:
We compared antifungal consumption data for two periods: pre-pandemic, from January 2019 to December 2019, and during the COVID-19 pandemic (January to December 2021).
Inpatient antifungal consumption was determined and expressed as a defined daily dose (DDD) per 100 occupied bed days. Overall hospital and ICU consumption were evaluated and compared. Consumption by antifungal class was defined, and also for each antifungal drug seperately (in grams per 100 bed days).

Results:
The total antifungal consumption in 2021 increased by +145% compared to consumption in 2019. The median antifungal consumption in 2021 was 0.26 (IQR: 0.1-2.2) compared to median consumption of 0.04 in 2019 (IQR: 0.01 – 0.9) [p-value <0.05 for non-parametric comparison]. The increase in total antifungal consumption during COVID-19 pandemic was driven mainly by a 172% increase in consumption within ICUs , with a marginal degree of increase of 6,3% in non-ICU inpatients.
No statistically significant increase was noted among different classes of antifungals (comparing consumption of azoles and echinocandins pre- and during the pandemic). The use of Amphotericin B and isavuconazole was substantially increased for both during the pandemic, as none of these drugs was used in ICUs in 2019.

TABLE1. Antifungal consumption pre- and during COVID-19 pandemic

Antifungal consumption in DDD/100 beddays	Prepandemic (2019)	during COVID-19 pandemic (2021)	p value
Overall *	0,04 (0,01-0,9)	0,26 (0,1-2,2)	<0.05
In ICU**	8,73	23,75	N/A
In non-ICU**	1,72	1,83	N/A

*expressed as median(IQR) **expressed as total sum of consumption
N/A: non Applicable

Conclusions:
A substantial increase in antifungal consumption during the second year of COVID-19 pandemic was noticed in our hospital, especially in the ICUs. That can be explained partially by the increased incidence of invasive fungal diseases -especially pulmonary aspergillosis- in COVID-19 patients. Additionally, the rise of antifungal consumption can be attributed to the addition of 64 new ICU beds after the first wave of the pandemic. Moreover our hospital was a referral hospital for complicated COVID-19 cases.
That increase in antifungal consumption is of great concern and indicates the urgent need for updated antifungal stewardship programs in order to ensure appropriate antifungal use.

P023

High-level fluconazole resistance in Candida parapsilosis isolates driven by combination of Y132F substitution in Erg11 and G650E substitution in Tac1

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Objectives:
As *Candida parapsilosis* is the most common non-*albicans* agent causing candidemia in neonatal and pediatric populations, recent increases in fluconazole (FLC) resistance in this species are cause for concern. Mutations in FLC drug target gene *ERG11* along with increased expression of the ATP-binding cassette (ABC) transporter genes, *CDR1* and *CDR2*, via activating mutations in the gene encoding zinc-cluster transcription factor Tac1 represent two of the predominant adaptive mechanisms for azole resistance in *Candida albicans.* While the Y132F substitution in CpErg11 has been reported in clinical isolates with FLC minimum inhibitory concentrations (MIC) from 2 to ≥256 mg/L, we have previously observed that highly FLC-resistant clinical isolates Cp35 and Cp38 overexpressed *CpCDR1* and contained mutations leading to both CpTac1^{G650E} and CpErg11^{Y132F} substitutions. The objective of this study is to determine the contribution of CpErg11 and CpTac1 mutations to high-level FLC resistance in *C. parapsilosis*.

Materials & Methods:
Sequences for *CpTAC1*, *CpERG11* and *CpCDR1C* in clinical isolates were determined via PCR amplification and subsequent Sanger sequencing. Overexpression of ABC transporters *CpCDR1*, *CpCDR1B*, and *CpCDR1C* (CPAR2_300010) via *CpTEF1* promoter replacement was performed via Cas9-Ribonucleoprotein-facilitated transformation. Single nucleotide polymorphisms and short barcoded stop codon sequences were introduced into genes of interest using a plasmid-based CRISPR system. Positive transformants were confirmed by Sanger sequencing. MICs were determined by broth microdilution following CLSI methodology read visually at 24 hours for 50% growth reduction. For gene expression analysis, isolates and strains were grown in MOPS-buffered RPMI + 2% glucose to mid-log phase. Isolated RNA was sequenced using Illumina NovaSeq. Fold changes of |≥2| (FDR p-values ≤0.05) were considered differentially expressed. Increased gene expression was confirmed by qRT-PCR with cDNA generated from biological triplicate RNA not used for RNA-seq.

Results:
The mutation leading to the CpErg11^{Y132F} substitution was introduced into three susceptible clinical isolates, resulting in a 4- to 8-fold increase in FLC MICs. As these elevated MICs did not account for the high-level resistance observed in some clinical isolates, the mutation leading to the *CpTAC1*^{G650E} substitution was corrected to the wildtype sequence in FLC-resistant clinical isolates Cp35 and Cp38 which resulted in an 8-fold reduction in FLC MIC in each strain. RNA-seq of Cp35 vs. Cp35-CpTac1^{WT} and Cp38 vs. Cp38-CpTac1^{WT} revealed only 4 genes differentially expressed in both *CpTAC1*^{G650E} strains: *CpCDR1*, *CpCDR1B*, a predicted *CDR1*-like pseudogene, and a *CIP1* ortholog. Sequence analysis of the predicted *CDR1*-like pseudogene, referred to hereafter as *CpCDR1C*, revealed an intact open reading frame in multiple clinical isolates. Overexpression of each of the *CDR1* homolog genes in Cp13 led to a 4- to 16-fold increase in FLC MIC while disruption of all three *CDR1* genes in Cp35 led to a 4-fold decrease in FLC MIC.

Conclusions:
While CpErg11^{Y132F} results in decreased fluconazole susceptibility, it alone does not explain high-level fluconazole resistance in *C. parapsilosis.* Further, CpTac1^{G650E} contributes to fluconazole resistance and elevates *CpCDR1*, *CpCDR1B*, and *CpCDR1C* expression. We conclude that high-level fluconazole resistance in *C. parapsilosis* can be driven by CpErg11^{Y132F} working in concert with a *CpTAC1* activating mutation.

P024

Deletion of cdr1B gene attenuates triazole-resistant phenotypes in triazole-resistant clinical isolates of A. fumigatus harboring cyp51A or hmg1 gene mutations

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Objectives: Triazole resistance in clinical isolates of *Aspergillus fumigatus* were mainly attributed to the mutations in, or overexpression of, the *cyp51A* gene, followed by mutations in the *hmg1* gene encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. In addition, overexpression of efflux transporters has been observed in triazole-resistant clinical isolates of *A. fumigatus*. Among efflux transporters, the ATP-binding cassette (ABC) transporter Cdr1B was the most associated with triazole resistance. Loss of Cdr1B has been demonstrated to increase the susceptibility to triazoles in both triazole-susceptible *A. fumigatus* strains and triazole-resistant *A. fumigatus* strain with a TR_{34}/L98H mutation in *cyp51A* gene. Here, We investigate the contribution of Cdr1B to triazole resistance in triazole-resistant clinical isolates of *A. fumigatus* with mutations causing resistance in *cyp51A* or *hmg1*.

Materials & Methods: Deletion of *cdr1B* gene in triazole-resistant clinical isolates of *A. fumigatus* were performed by using the Cas9-RNP editing technique incorporating a split hygromycin B resistance marker cassettes. Antifungal susceptibility testing of *A. fumigatus* strains was performed using the broth microdilution method (CLSI M38-A3).

Results: (1) For triazole-resistant clinical isolates of *A. fumigatus* with a *cyp51A* point mutation: Deletion of *cdr1B* gene in the clinical isolate of *A. fumigatus* BMU02731 (with M220I mutation in *cyp51A*) remained resistant to itraconazole (ITC), but resulted in increased susceptibility to voriconazole (VRC), posaconazole (POS), and isavuconazole (ISZ); Deletion of *cdr1B* gene in the clinical isolate of *A. fumigatus* BMU09386 (with G54W mutation in *cyp51A*) remained resistant to ITC and POS, but enhanced the susceptibility to VRC and ISZ. (2) For triazole-resistant clinical isolates of *A. fumigatus* with both point mutation in the coding region and tandem repeat (TR) in the promoter region in *cyp51A* gene: Deletion of the *cdr1B* gene in BMU04835 (with TR_{34}/L98H mutation in *cyp51A*) lead to enhanced susceptibility to all of the triazoles tested; Deletion of the *cdr1B* gene in BMU07945 (with TR_{46}/Y121F/T289A mutation in *cyp51A*) resulted in increased susceptibility to ITC, VRC and ISZ. (3) For triazole-resistant clinical isolate of *A. fumigatus* with point mutations in both *cyp51A* (M263I) and *hmg1* (E306K) gene: Deletion of *cdr1B* gene in *A. fumigatus* BMU11335 remained resistant to ITC, but significantly increased the susceptibility to VRC, POS and ISZ. The results of minimum inhibitory concentrations (MICs) of triazoles against triazole-resistant clinical isolates of *A. fumigatus* and corresponding *cdr1B* gene deletion strains were shown in Table 1.

Conclusions: In summary, deletion of the *cdr1B* gene in triazole-resistant clinical isolates of *A. fumigatus* harboring *cyp51A* or *hmg1* gene mutations significantly increased susceptibility to triazoles, suggesting that the function of the efflux transporter Cdr1B plays an important role in the resistance to triazoles in *A. fumigatus*.

Table 1. Minimum inhibitory concentrations of *A. fumigatus* determined by the broth microdilution method (CLSI M38-A3)

Strains	*cyp51A*	*hmg1*	MIC/MEC (μg/ml)					
			ITC	VRC	POS	ISZ	AMB	CAS
BMU02731	M220I	-	>16	1	0.25	2	1	0.125
BMU02731-*Δcdr1B*	M220I	-	>16	0.25	0.125	1	1	0.125
BMU09386	G54W	-	>16	0.5	>16	0.5	1	0.125
BMU09386-*Δcdr1B*	G54W	-	>16	0.125	>16	0.125	1	0.125
BMU04835	TR_{34}/L98H	-	>16	4	1	>16	0.5	0.125
BMU04835-*Δcdr1B*	TR_{34}/L98H	-	1	1	0.25	1	0.5	0.125
BMU07945	TR_{46}/Y121F/T289A	-	1	>16	0.5	>16	0.25	0.125
BMU07945-*Δcdr1B*	TR_{46}/Y121F/T289A	-	0.5	16	0.5	16	0.25	0.125
BMU11335	M263I	E306K	>16	8	4	8	0.5	0.06
BMU11335-*Δcdr1B*	M263I	E306K	>16	2	2	2	0.5	0.06

P026

Genomic analysis of antifungal resistance in Candida parapsilosis in Canada

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Objectives: Azole resistance in *Candida parapsilosis* has recently emerged, and most reports have found that the mechanism of resistance arises from substitutions in the azole target, *ERG11*, such as Y132F, K143R, and G458S. Here, we used whole genome sequencing (WGS) analysis to elucidate the genomic epidemiology and to identify genetic determinants of resistance in fluconazole-resistant *C. parapsilosis* isolates.

Methods: Ninety-five *C. parapsilosis* isolates collected between 2016 and 2019 from patients across Canada (West, n = 19; Central West, n = 19; Central East, n = 45; East, n = 12) were provided by the ten provincial public health laboratories. Eighty-five isolates were collected from sterile body sites (blood, n = 78; sterile fluid, n = 4; undetermined, n = 2; and cornea, n = 1), while ten were from non-sterile sites. We conducted phylogenomic analysis based on single nucleotide variants (SNVs) in the core genome using the Single Nucleotide Variant Phylogenomic (SNVPhyl) pipeline in 21 fluconazole-resistant (MIC ≥ 8 ug/mL), 2 susceptible-dose dependent (MIC = 4 ug/mL), and 72 fluconazole-susceptible (MIC ≤ 2 ug/mL) *C. parapsilosis* isolates. We also examined variants of known potential resistance proteins, such as *CDR1*, *ERG11*, *MDR1*, *MRR1*, *TAC1*, and *UPC2*.

Results: Phylogenomic analysis revealed 12 clusters of genetically related isolates. Fluconazole-resistant *C. parapsilosis* isolates were associated with seven of the 12 clusters (cluster 1, n = 2; cluster 5, n = 7; cluster 7, n = 1; cluster 8, n = 1; cluster 9, n = 1; cluster 11, n = 2; and cluster 12, n = 6) and differed by five to 2577 SNVs. One fluconazole-resistant isolate did not cluster. Of the 21 fluconazole-resistant isolates, ten were found in clusters with at least one other fluconazole-resistant isolate from the same healthcare institution. For example, all seven fluconazole-resistant isolates in cluster 5 were from the same province in the Central East region of Canada, with five isolates from the same hospital (5–20 SNVs) collected between 2016 and 2018. *ERG11* variants, Y132F (n = 2) and G458S (n = 1) were identified in three fluconazole-resistant *C. parapsilosis* isolates.

Conclusions: These findings demonstrate the diversity of fluconazole-resistant *C. parapsilosis* isolates circulating in Canada. It appears that some fluconazole resistance in *C. parapsilosis* likely arises spontaneously due to selective pressure during treatment, while some fluconazole resistance results from the dissemination of resistant clones. Most

fluconazole-resistant isolates in this collection contained a wildtype *ERG11* gene suggesting that other mechanisms, such as transcription factors, efflux pumps or copy number variations, may mediate resistance.

P027

Azole resistance profiling of candidemia isolates of Candida krusei: ERG11 mutation and transcriptomics

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Objective:

Candida krusei accounts for 2.8% of invasive candidiasis worldwide. Recently, WHO fungal priority pathogens list categories C krusei among medium priority groups. In this study, we performed antifungal susceptibility profiling and investigated the underlying fluconazole (FLU) resistance mechanism. A total of 165 clinical isolates of *C.krusei* were collected from 10 hospitals in India over a period of eight years.

Material and method:

Isolates were identified by MALDI-TOF MS and their antifungal susceptibility test was done using CLSI-M27 broth microdilution. *ERG11* gene mutation and expression of *ERG11* gene and drug transporters *ABC1*, and *ABC2* were investigated in 35 *C. krusei* isolates [18 fluconazole-susceptible (FLU-S), & 17 fluconazole-susceptible dose dependant (FLU-SDD)]. Transcriptomics of one each FLU-SDD (MIC 32 mg/L) and FLU-S (MIC 4 mg/L) strain was performed. Whole genome sequencing of 17 isolates belonging to a single hospital outbreak was undertaken.

Results:

Over the eight years' study period, *C. krusei* was the 5th cause of candidemia in the northern region of India. Majority of the isolates were from blood (n=132; 80%) followed by urine and sputum and vaginal swab. Over the study span, two smouldering outbreaks of *C. krusei* in neonatal intensive care units (NICUs) of two different hospitals were observed. Antifungal susceptibility testing of 165 *C. krusei* isolates showed 86.6% isolates exhibit lower susceptibility to FLU (MIC ≥ 16 mg/L). In contrast to the intrinsic FLU resistance nature of *C. krusei*, 13.3% of tested isolates exhibited low FLU MIC values (≤8 mg/L). *ERG11* gene screening revealed absence of mutations contributing to the FLU resistance. Additionally, no difference in expression level of *ERG11* gene and transporter genes *ABC1* and *ABC2 w*ere observed. Transcriptomic data revealed, genes associated with transporter (10 genes), mitogen activated protein kinase (MAPK) signalling (8 genes), transcription factors (7 genes) and ergosterol biosynthesis (3 genes) were differentially expressed in FLU-SDD isolate compared to FLU-S. Interestingly, four genes involved in ergosterol synthesis and known to modulate FLU susceptibility in *C. albicans* namely; the transcription factor *UPC2*, and *ERG24*, *ERG 26*, and *ERG27* were significantly up-regulated in FLU-SDD *C. krusei* as compared to FLU-S. Furthermore, two-fold decreased expression of *PDR12*, plasma membrane ABC transporter was observed in FLU-SDD isolate. Genes involved in MAPK pathway i.e. *MSG5*, *PTP3*, *STE50*, *BNR1*, *OPY2*, *STE5*, *SKN7* & *RLM1* showed altered expression in FLU-SDD isolate compared to FLU-S. Another important genes that impart low azole susceptibility, *ICL1* (Isocitrate Lyase, a major glyoxylate-synthesizing enzyme) and *UME6* (filament-specific Zn (II) 2Cys6 TF) were found to be up-regulated in FLU SDD

isolate compared to FLU-S. WGS of 17 *C. krusei* strains recovered from single NICUs outbreak formed two clusters comprising of 12 highly related strains and the other comprising 5 strains

Conclusion:

C. krusei is an important species in the NICUs causing prolonged outbreaks. Unlike to other *Candida* species, the resistant mechanism in *C.krusei* seems to be more complex. Taken together, altered expression of *PDR12*, *UPC2* and the *MAPK* signaling network may partially account for the altered FLU susceptibility in *C. krusei*.

P028

Isavuconazole exposure and activity: Five year experience from a Reference Laboratory

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Introduction: Monitoring antifungal susceptibility patterns and clinical exposure for new antifungal agents is a very interesting analytical exercise to effectively draw the pattern of activity and efficacy of this recently approved antifungal.
Objective: The purpose of this study was to describe the real-word experience of exposure to isavuconazole (ISZ) in samples from treated patients, received in our reference laboratory over a period of five years. In addition, a descriptive summary of ISZ in vitro activity against most common yeast and filamentous fungi is presented to comparatively evaluate data of exposure.
Methods: A liquid chromatographic method coupled with ultra-violet detection (LC-UV) adapted from a multiplex validated method was complemented for ISZ characterization and exposure quantification. ISZ was detected using a PhotoDiodeArray (PDA) system that allows the characterization of its specific UV spectra. The method was validated according to international guidelines for efficient ISZ monitoring.
We also summarized the *in-vitro* susceptibility to ISZ against a large clinical collection of *Aspergillus* and *Candida* isolates received in our lab during the period of study (2017-2021). EUCAST susceptibility testing was performed according to E.Def7.3.2 for *Candida* spp and E.Def9.4 for *Aspergillus spp*. MICs were interpreted using the recently established EUCAST clinical breakpoints (CBP) and epidemiological cut-off values (ECOFF).
Results: The assay exhibited linearity between 0.25-16 mg/L for ISZ. Accuracy and intra- and inter-day precision were within acceptable ranges, and the method was successfully applied to quantify ISZ from clinical samples. The median ISZ concentration was 2.92 mg/L (IQR 5.33-1.82mg/L) with 88.9% of measurements classified as adequate exposure (>1 mg/L). Additionally, 71% of samples reached concentration values >2 mg/L. Great inter-patient variability (CV >50%) and different ISZ exposure between adults to children were found.
ISZ showed good in vitro activity (MIC_{90}=2 mg/L) against most common *Aspergillus* species (*A. fumigatus*, *A. flavus*, and *A. terreus*) with lower MIC_{mode} for *A. nidulans* and higher MIC_{mode} for *A. niger*. The percentage of ISZ resistant strains varied between species: 7% of *A.fumigatus* (MIC>2mg/L), 2% *of A.flavus* (MIC>2mg/L), 24% *A.terreus* (MIC>1mg/L), 23% of *A. nidulans* (MIC>0.25 mg/L). Although CBP or ECOFFs have not been formally established for ISZ and *Candida* spp, we found MIC_{90} values ranged from 0.06–2 mg/L. Of all *Candida* spp. studied, *C. glabrata* was the less susceptible strains, while ISZ demonstrated better activity against isolates of *C. albicans* and *C. parapsilosis*.
Conclusions: Our data, in line with previously described, reported that ISZ achieved excellent blood concentrations. These concentrations exceed the MICs of most common fungal pathogens. We also highlight the importance of having valid methods for efficient TDM monitoring to optimize therapy in specific scenarios and populations.

P029

Fluconazole-resistant Candida parapsilosis candidemia and analysis of mutations in the ERG11 gene from Pakistan

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Objectives:
The study aimed to report the emergence of fluconazole resistance in *C. parapsilosis* strains isolated from blood culture at a single centre in Pakistan.
Materials & Methods:
This was a retrospective observational single-centre study. Data related to fluconazole-resistant *C. parapsilosis* from July 2018- June 2020 were retrieved from the integrated laboratory management system of Clinical Laborotiries, Aga Khan University, Karachi, Pakistan. For admitted patients, the clinical details were retrieved from discharge summaries and for those admitted outside, histories were obtained over the telephone. Morphological identification of Candida species was done on BiGGY, ChromAgar Candida, and Cornmeal Tween80 Agar and biochemical reactions on API 20C AUX (BioMerieux, France). Antifungal sensitivity testing was performed by disc diffusion method according to CLSI M44-A. MICs were determined using Sensititre Yeast ONE YO10 according to the manufacturer's recommendations, while interpretation was done according to CLSI M60 ED1:2017. Serum Beta D-Glucan levels measured using Fungitell® assay at the time of active infection were also evaluated ERG11 gene region of seven *C.parapsilosis* strains was amplified and sequenced using sanger sequencing methodology.on 7 *C.parapsilosis* strains. ERG11 gene sequence was analyzed by MEGA 11 Software. Consensus sequences were translated into their corresponding amino acid sequence using the ExPASY Translate Tool and BioEdit Sequence Alignment Editor. nNon-synonymous and synonymous mutations were identified.
Results:
Out of 152 *C.parapsilosis* fungemia strains, 13 (8.55%) fluconazole-resistant isolates were identified in two years of study duration. Results of disc susceptibility were compatible with MICs in all isolates. Median MICs for fluconazole were 32 µg/ml, voriconazole 0.5 µg/ml. Out of 12 patients whose clinical data were available, 4 expired, 6 discharged in stable condition and 2 left against medical advice. Out of 8 patient whose Beta-D-glucan were performed, the result was positive in a single patient (12.5%). In *C. parapsilosis* non-synonymous mutation at position Y132F was observed in 6/7 (86%) isolates; other common mutations K143R, M178T and N283Y associated with resistance were not found in our isolates.
Conclusions:
C.parapsilosis resistance during years 2010-2014 evaluated by same group was 4.66% which has increased to 8.55% in blood stream infections. The ERG11 mutation Y132F, the most common one reported, was also found in our clinical isolates. To prevent the spread of fluconazole resistant *C.parapsilosis* strains, strategies with multidisciplinary approach integrating improved surveillance, diagnostics, antibiotic stewardship and adherence to infection prevention and control protocols are required.

P030

The role and regulatory mechanism of PDR1 mutation in triazole-resistant isolate of Candida glabrata

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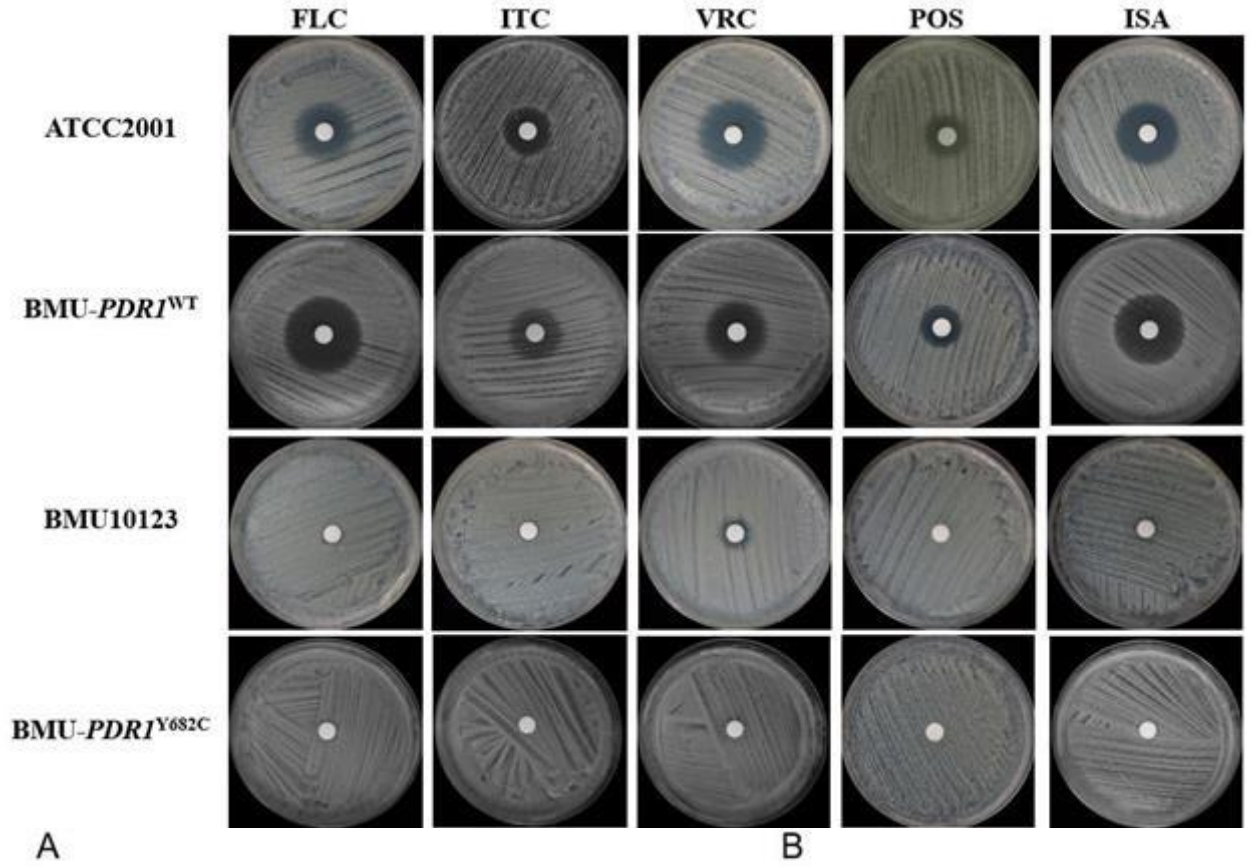
Objectives: *Candida glabrata* is one of the most prevalent causative pathogens of invasive candidiasis, and is of particular concern due to its prevalence of antifungal resistance. Triazole-resistance in *C. glabrata* is mainly mediated by functional mutations in the transcription regulator *PDR1* rather than drug target *ERG11*. In this study, the role and regulatory mechanisms of a novel mutation site of *PDR1* in triazole-resistance of *C. glabrata* was explored.

Materials & Methods: An isolate of *C. glabrata* was recovered from the ascites of a patient. Antifungal susceptibility test was determined by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M27-A4 document and by disk diffusion using disks prepared in-house. Transcription factor gene *PDR1* of BMU10123 was amplified and sequenced. The *PDR1* mutation allele and a *PDR1* wild-type control allele were directly introduced to the native *PDR1* locus of the triazole-susceptible *C. glabrata* reference strain ATCC2001 using the CRISPR-Cas9 gene editing system. To investigate the role of *PDR1* mutation in fluconazole response, global gene expression profiles in *PDR1* mutatnt stain were assessed through RNA-sequencing and compared with those of *PDR1* wild-type control strain. Differentially expressed genes (DEGs) were defined by a log2 fold change ≥ or ≤ 1, with an adjusted P value cutoff ≤ 0.05.

Results: The clinical isolate of *C. glabrata* BMU10123 was resistant to fluconazole, voriconazole, posaconazole (with MICs of 128, 4, and 2 mg/L), and exhibited high MICs of itraconazole and isavuconazole (4 and 2 mg/L). *PDR1* sequencing revealed an A2045G mutation resulting in a Y682C substitution. Antifungal susceptibility test showed that the constructed *PDR1* mutant strain BMU-*PDR1*^{Y682C} presented dramatically increased resistance to all clinically available triazole agents, with MICs of 128, 4, 4, 2, and 2 mg/L for fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole, respectively, same as those in BMU10123. While the strain harboring wild-type control allele, BMU-*PDR1*^{WT}, was triazole-susceptible with the same MICs as reference strain ATCC2001. Disk diffusion presented the similar results (figure 1). RNA-sequencing revealed that 98 DEGs were found between BMU-*PDR1*^{Y682C} and BMU-*PDR1*^{WT}. The Gene ontology (GO) enrichment analysis of DEGs revealed a significant enrichment of transporter activity, component of plasma membrane, ergosterol biosynthetic and metabolic process (figure 2A). In detail, significantly upregulated genes in BMU-*PDR1*^{Y682C} involved in transporter genes including *CDR1, SNQ2, QDR2, YOR1, YBT1, PXA1*, etc, and genes belonging to the ergosterol biosynthesis *ERG11, ERG3, ERG1, ERG2, ERG4, ERG5,* etc (figure 2B).

Conclusions: Mutation in transcription factor gene *PDR1* (*PDR1*^{Y682C}) confers triazole-resistance of *C. glabrata* by mainly upregulating transporter genes and ergosterol biosynthesis genes.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VRC
POS
ISA
ATCC2001
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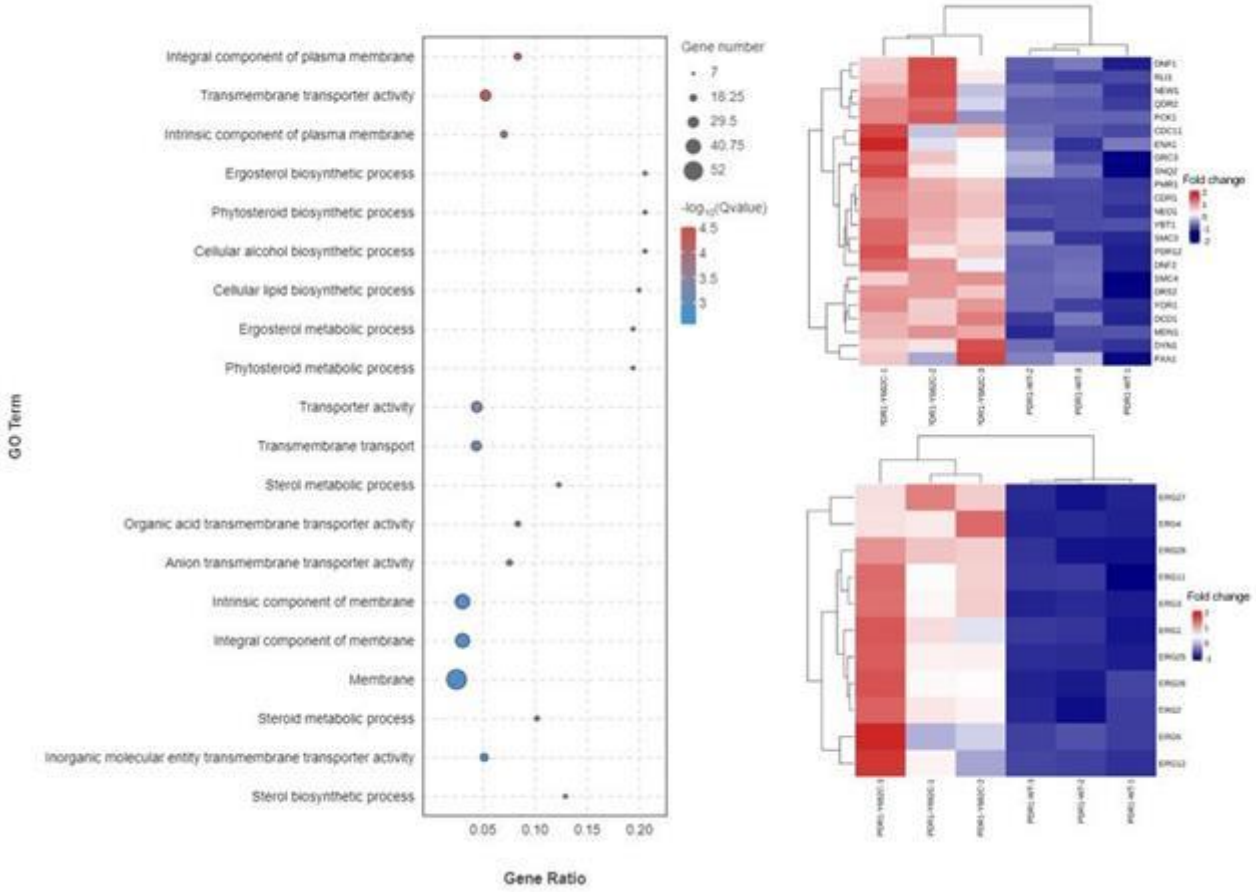
BMU10123
BMU-PDR1Y682C
A
B


Integral component of plasma membrane
Transmembrane transporter activity
Intrinsic component of plasma membrane
Ergosterol biosynthetic process
Phytosteroid biosynthetic process
Cellular alcohol biosynthetic process
Cellular lipid biosynthetic process
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Phytosteroid metabolic process
Transporter activity
Transmembrane transport
Sterol metabolic process
Organic acid transmembrane transporter activity
Anion transmembrane transporter activity
Intrinsic component of membrane
Integral component of membrane
Membrane
Steroid metabolic process
Inorganic molecular entity transmembrane transporter activity
Sterol biosynthetic process
GO Term
0.05
0.10
0.15
0.20
Gene Ratio
Gene number
7
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29.5
40.75
52
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4.5
4
3.5
3
Fold change
Fold change

P031

Does heteroresistance precede resistance emergence in serial Candida isolates from ICU patients?

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Objectives:

1. To assess the impact of ICU patient exposure to long courses of antifungal treatment with azoles or echinocandins (≥ 7 days) on the emergence of heteroresistant subpopulations in serial colonising and invasive *Candida* species isolated from these patients.
2. To assess the relationship between presence of heteroresistant subpopulations and the development of fully resistant *Candida* isolates in ICU patients.

Methods:

Candida isolates were cultured from twice weekly oral and perianal swabs from ICU patients at risk of invasive candidiasis enrolled in a multi-centre prospective observational cohort study (CandiRes, ISRCTN14165977). Isolate MICs were assessed using Sensititre Yeastone plates. Data on antifungal prescribing were collected. Serial *Candida* isolates showing a ≥4 fold increase in anidulafungin and/or fluconazole minimum inhibitory concentration (MIC), and/or cultured from patients receiving ≥7 days of antifungal treatment, were selected for population analysis profiling (PAP). PAP (an agar macrodilution method) was used to assess the presence of fluconazole or anidulafungin heteroresistant subpopulations showing ≥2 LOG decrease in population, beyond the isolate MIC. Heteroresistance was confirmed using broth microdilution (BMD) according to the EUCAST guidelines. Whole genome sequencing was used to look for known resistant mutations. The impact of antifungal exposure on the emergence of heteroresistant *Candida* subpopulations, and their impact on the emergence of isolates with heightened resistance, will be assessed.

Results:

34 serial isolates from 9 patients showing ≥4 fold increase in MIC from the baseline MIC, and 79 serial isolates from patients exposed to ≥7 days of fluconazole or anidulafungin were identified, which will all be assessed for heteroresistance using PAP.

Preliminary findings show 3 out of the 5 series of patient isolates tested so far demonstrate emergence of anidulafungin heteroresistant subpopulations, following exposure to this antifungal, such as that seen in figure 1, which shows the presence of a small subpopulation growing beyond the isolate MIC (0.03µg/ml). BMD testing can subsequently verify the heightened MIC of heteroresistant subpopulations identified by PAP, isolated by growth on drug-containing media, in comparison to the MIC of the isolate grown on drug-free media (fig. 2). *C. glabrata* isolated from the patient in fig. 1, 8 days after antifungal treatment, showed heteroresistance comparable to the baseline (pre-exposure) isolate levels, suggesting that reversion can occur upon removal of antifungal pressure.

Conclusions:
Presence of heteroresistance goes undetected by conventional susceptibility testing methods (e.g. BMD). Agar macrodilution methods like PAP can detect emergence of heteroresistant populations, which can then be confirmed by BMD. In the first ex vivo human serial isolate cohort study, we demonstrate that exposure to azoles or echinocandins in ICU patients selects for heteroresistant subpopulations, leading to an increase in the proportion of the population able to grow above the MIC even after relatively short treatment durations. In some cases, where antifungal prescription stops, this heteroresistant phenotype may dissappear due to reduced selection for it. However, continued pressure of antifungal monotherapy can drive these heteroresistant populations to become the dominant population, leading to colonisation by fully resistant isolates.

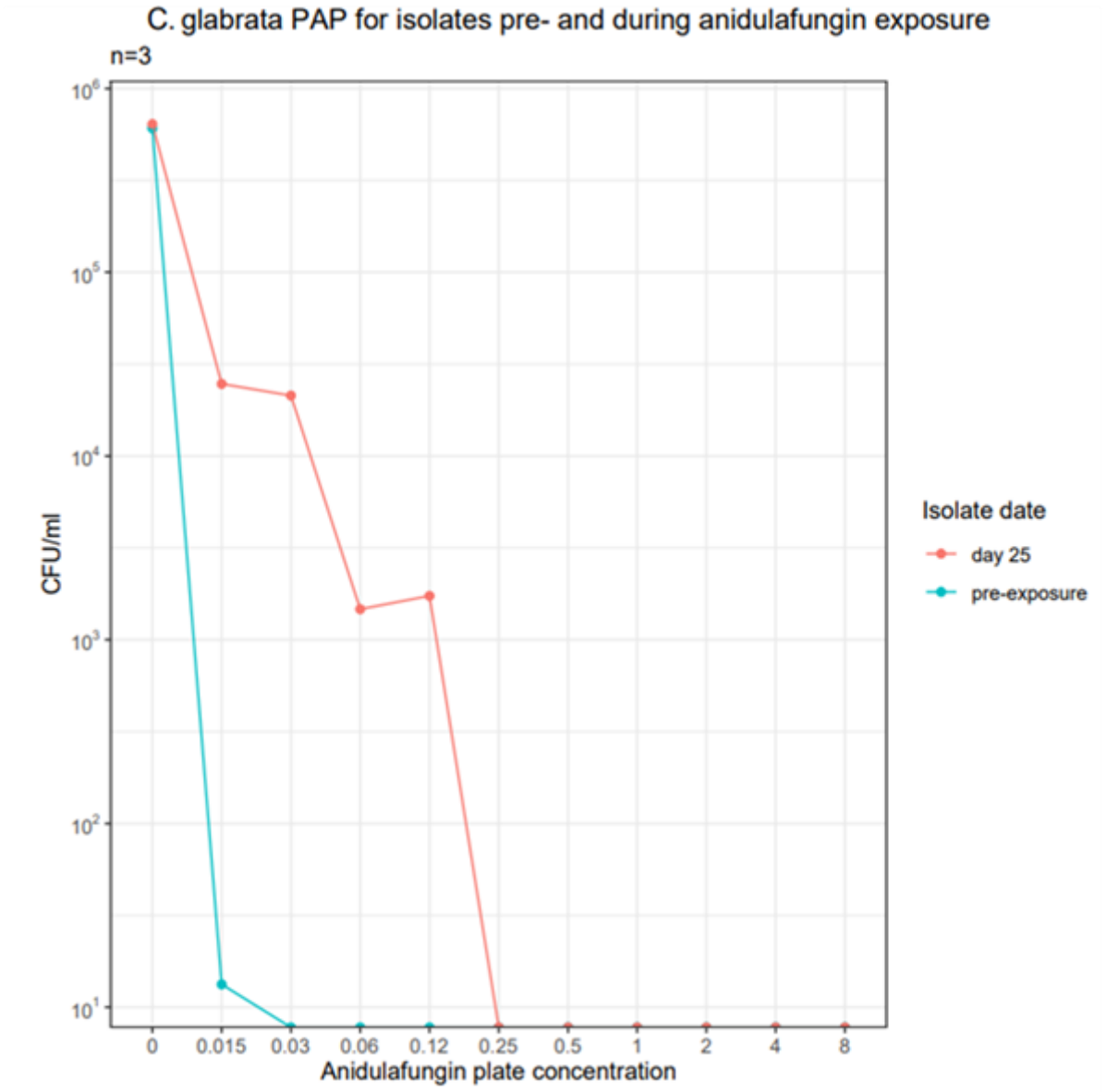

Figure 1 PAP of two serial C. glabrata isolates from patient, before the patient was exposed to anidulafungin treatment (blue line), and at day 25 of treatment (red line). Points represent mean CFU/ml from 3 technical replicates.

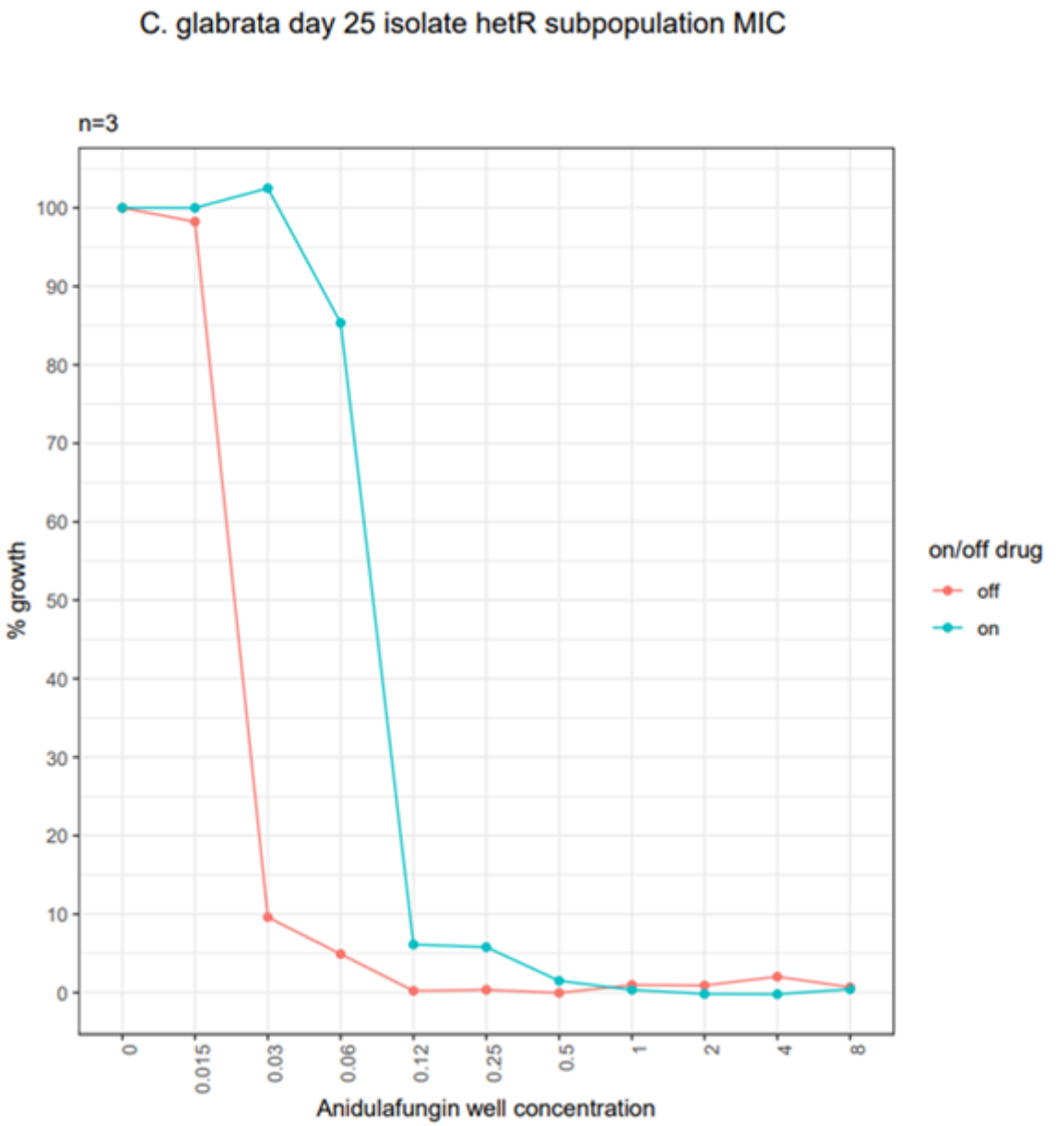

Figure 2 MIC of C. glabrata isolate from day 25 of anidulafungin exposure, when grown on SAB containing 0.25µg/ml anidulafungin (blue line) and on drug-free SAB (red line) prior to BMD testing. Points represent mean % growth relative to the untreated control from 3 technical replicates.

P032

2022-2023 Belgian national survey on terbinafine resistance among T. interdigitale/mentagrophytes/indotineae

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Objectives: The new emerging dermatophyte *Trichophyton indotineae* closely related to *T. interdigitale/mentagrophytes* has become a major concern in dermatology because of its high resistance to terbinafine. This species is at epidemic level in India giving rise to extended tinea corporis. Its spread in Europe has been already described. Mutations on the gene coding for squalene epoxidase (SQLE) have abundantly been observed among terbinafine resistant strains. Regarding this context, the National Reference Center for mycoses of the CHU of Liege (NRCML) conducted a study at the Belgian level to collect all *T. interdigitale/mentagrophytes/indotineae* isolated from skin and scalp. The aim of the study was to evaluate the situation in Belgium and characterize these strains in terms of identification and terbinafine susceptibility. A phylogenomic study based on whole genome sequencing (WGS) data was also performed with a particular focus on SQLE gene.
Methods: The study was conducted from 1 April 2022 and 1 April 2023. In total, 129 strains have been sent to the NRCML by 18 laboratories. The terbinafine susceptibility of these strains was evaluated by a four wells dilution on agar screening method containing 0, 0,05, 0,1 and 0,2 µg/ml of terbinafine. Minimal inhibitory concentration (MIC) of each strain was then determined by EUCAST E. Def.11.0 microdilution method. WGS was performed by Illumina sequencing (GIGA Genomics, Liège, NovaSeq S4 V1.5 300 cycles XP workflow). Assembly of the genome was done using SPAdes integrated in a custom made bioinformatic pipeline “WGS typer” (Hedera22, Liège). Similarity dendrogram was generated by WGS typer using the maximum likelihood method. Reference genomes of *T. indotineae/mentagrophytes/interdigitale* were included on the tree.
Results: Among the 129 strains, 4 (3,1%) showed a growth in well containing 0,2µg/ml of terbinafine with the dilution on agar method. MICs higher than 0.25µg/ml for terbinafine were confirmed by EUCAST microdilution method for these strains. The other 125 strains showed MICs <0,1µg/ml for terbinafine. At this day, only 85/129 strains have been characterized by WGS. Preliminary results show that among these strains 5 (5,8%) were *T. indotineae*, 20 (24,3%) were *T. mentagrophytes* and 60 (73,5%) were *T. interdigitale.* These three species were separated into three distinct clades on the dendrogram. The analysis of SQLE region permitted to identify the substitutions F397L (2), L393F (1) and L393S (1) among the five *T. indotineae,* one being a wild type. Our genomic study also highlighted that *T. interdigitale* even if mainly causing tinea pedis (47/60), was also isolated from tinea corporis (13/60). On the contrary, only 4/20 *T. mentagrophytes* were isolated from tinea pedis, the majority (16/20) being isolated from tinea corporis. *T. indotineae* was exclusively isolated from extended tinea corporis/cruris.
Conclusion: Our study demonstrates the presence of *T. indotineae* resistant to terbinafine on the Belgian territory. The phylogenomic analysis permitted to precisely identify *T. indotineae* among a distinct clade from *T. interdigitale/mentagrophytes* and to show that *T. interdigitale* is not only isolated from foot skin. The substitutions on SQLE gene F397L, L393F and L393S have been described in Belgian *T. indotineae* strains.

TIMM
Trends in Medical Mycology

P033

Emergence of Cyp51A variants in azole-resistant A. fumigatus

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Objectives:
Aspergillus fumigatus is the main cause of invasive aspergillosis and triazole antifungals represent primary treatment options. However, the effectiveness of triazole therapy is hampered by the emergence of resistance, mainly caused by TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in the *Cyp51A*-gene, which correspond with signature triazole resistance phenotypes. Our aims are to investigate the occurrence of variant genotypes and phenotype over a 29-year period in the Netherlands, the impact of individual SNPs and combinations thereof on the azole phenotype in TR_{34}-mediated resistance genotypes, and the characterizations of TR_{34} variants isolates against medical azoles by recombinant analysis and homology modeling.

Materials & Methods:

The Radboud University Medical Center Fungal Culture Collection contains 12,676 clinical *A. fumigatus* isolates from Dutch hospitals collected since 1994. Triazole agar-based screening was used to detect resistant isolates, which were characterized by sequencing of the *Cyp51A*-gene and in vitro susceptibility testing using the EUCAST microdilution reference method. Construction of the repair template and the dual Cas9-gRNAs system to acquire the interest of recombinants by protoplast transformation. The antifungal susceptibility test and growth measurement were performed.

Results:

In total, 1,981 (15.6%) *A. fumigatus* isolates harboured triazole-resistance mutations in the *cyp51A*-gene, predominately TR_{34}/L98H *sensu stricto* (*ss*) in 67.7% and TR_{46}/Y121F/T289A *ss* in 16.8% of resistant isolates. Overall, 34 (5%) variants were identified, including 7 TR_{34}-variants and 7 TR_{46}-variants. Four TR_{34}-variants showed a phenotype change compared to that of TR_{34}/L98H *ss*. The number of variants significantly correlated with year of isolation and annual number of isolates, with an annual variant frequency ≥10% since 2018.

We focus on the novel, recent mutations and explore the effects of these SNPs: L98H, T289A, I364V, G448S and the combination of these mutations TR34/L98H, TR_{34}/L98H/T289A/G448S, TR_{34}/L98H/T289A/I364V/G448S, on azole affinity and susceptibility. The mutation was introduced to the wildtype-cyp51A. *fumigatus* strain by the CRISPR-Cas9 gene editing technique and the same strategy was performed for early emerged variant TR_{34}/L98H/S297T/F495I. Finally, in vitro susceptibility testing of *A. fumigatus* strains carrying the aforementioned mutations was conducted to confirm the azole phenotypes observed in clinical isolates. The MICs of all four azoles against the mutated cyp51A strains harbored combination mutations were higher than that of the wild type, with highly elevated MICs of itraconazole, voriconazole, and isavuconazole. The TR34/L98H, and L98H/T289A strains were resistant to the highest tested itraconazole concentration (16 mg/l). TR_{34}/L98H/T289A/I364V/G448S showed consistent phenotype to the clinical strains, which are highly resistant to Voriconazole but susceptible to Itraconazole.

In CYP51A-azole complexes, the combination of T289A, I364V and G448S mutations are more likely to impact the VOR, TR_{34}/L98H/S297T/F495I might be more sensitive to longer ITR.

Conclusions:
Our study showed ≥10% *A. fumigatus* resistance variant frequency in recent years, some of which correspond with major shifts in phenotype. Emerging resistance variants represent a major challenge in triazole resistance management as our current molecular diagnostic tools will increasingly fail to predict the resistance phenotype. The individual SNPs have low impact on the azole phenotypes, while the combinations thereof contributed the Itraconazole and voriconazole phenotype significantly. SNPs accumulation and new resistant mechanisms conferring to resistance would be addressed by genomic surveillance.

P036

First report of azole-resistant Aspergillus species in poultry feed in Kabale, South West, UgandaP036

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Due to the widespread use of azoles in clinical and environmental settings favoring the selection of azole-resistant *Aspergillus* species, antifungal susceptibility studies have become increasingly important in understanding their prevalence and resistance profiles in the medical and environmental settings. Azole-resistant *Aspergillus* strains have been increasingly reported across the globe. However, there is still limited information available on these strains in Africa. This study was aimed at assessing the environmental prevalence of the Azole-resistance *Aspergillus* strains, particularly in Africa. This study aimed to assess the environmental prevalence of the azole-resistance in *Aspergillus* strains in the poultry feed in Kabale, South West Uganda. The study evaluated the susceptibility profile of *Aspergillus* and other fungi isolates from 10 poultry feed samples collected ten different commercial feed outlets. Isolates were first identified using ITS 4 and 5 and confirmed using Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). In total, we found twelve different isolates. *Aspergillus flavus* was the most prevalent fungi specie present in the feed followed by *A. niger*, *A. tamari*, *Penicillium corylophilum*, *A. terreus*, *A. oryzae*, *P. steckii*, *P. citrinum*, *A. chevalieri*, *Hypopichia burtonii*, *Meira nashicola*, and *Fusarium proliferatum*. From this all, *Aspergillus* and one *Penicillium* isolates were further screened for azole-resistance using azole-containing agar plates (itraconazole, voriconazole, posaconazole, isavoriconazole) and fluconazole antifungal susceptibility strip method, which was the first line of treatment). *Aspergillus chevalieri, A. tamari,* A. *flavus, P. citrinum* fungi isolates showed resistance to fluconazole. The EUCAST susceptibility testing method did not report isolates resistant to tested azoles (itraconazole, voriconazole, posaconazole, isavoriconazole) or amphotericin B. Overall, we found resistance to fluconazole. This study is the first investigative study of *Aspergillus* species in poultry feed in Kabale, South West, Uganda. There is, therefore, need for national surveillance programmes which are presently lacking in most African countries.

P037

Capability of commercial antifungal susceptibility testing methods to detect resistance in Aspergillus fumigatus

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Objectives: Susceptibility testing of filamentous fungi has become increasingly important, due to the emergence of strains with intrinsic or acquired resistance to available antifungal drugs. Although there are data for strengths and limitations of commercially available methods for antifungal susceptibility testing of yeasts as predictors of resistance, there is a gap in knowledge concerning filamentous fungi. The goal of the present study was to summarize commercial techniques for antifungal susceptibility testing concerning filamentous fungi and the potential ability to detect non-wild type (NWT) *Aspergillus fumigatus*, as this species are the most common and most explored.
Material/Methods: We review the literature in order to find studies where commercial methods were used for *in vitro* susceptibility testing of *A. fumigatus* isolates including cyp51a mutants. We focus on Etest and Sensititre Yeast One (SYO), as those methods are widely used to found triazoles or echinocandins NWT isolates based on available ECVs. We used method dependent ECVs derived from studies where Etest and SYO were used and calculated the % of NWT isolates with known cyp51a and fks mutations conferring resistance to azoles and echinocandins, respectively. Data were found for Etest and itraconazole, voriconazole, posaconazole, micafungin and caspofungin and for SYO and voriconazole and posaconazole.
Results: Applying Etest ECVs for *A. fumigatus* to itraconazole, we noticed that Etest was able to detect 91.4% (85/93) of NWT isolates with known cyp51a mutations. Concerning voriconazole, results obtained from different studies were somewhat less promising in detection of NWT isolates with Etest detecting 48% (52/109) of NWT isolates with cyp51a mutations. More promising were the results for posaconazole, with ability of the proposed ECV to detect the vast majority of NWT isolates (86%, 31/36) with different cyp51a mutations. Etest was able to detect 3/3 of fks mutants using caspofungin ECV, while for micafungin 2/3 NWT isolates were detected using the corresponding ECV. Applying the method specific SYO ECV for voriconazole, only 47% (31/66) of mutant isolates with known cyp51a substitutions, were able to be distinguished from WT isolates. The posaconazole SYO ECV was able to detect all NWT isolates (100%, 37/37).
Conclusions: Method-specific ECVs have been determined for many drugs and *A. fumigatus* isolates in order to define wild-type isolates and distinguish from known cyp51a mutants. Although, in some cases the number of mutant isolates used to evaluate proposed ECVs are low, some conclusions can be made: i) for Etest method, the proposed ECVs of posaconazole, itraconazole, micafungin and caspofungin were able to detect *A. fumigatus* mutants, while the ECV of voriconazole was unable to detect NWT with known cyp51a mutations (**Table 1**); ii) for the SYO method, the ECV of posaconazole was able to detect all mutants, whereas the ECV of voriconazole detected only 31/66 of *A. fumigatus* isolates with cyp51a mutations (**Table 2**).

Table 1. Relationship between Etest and triazoles/echinocandins gene mutations of non-WT *Aspergillus fumigatus* isolates

Species	No. non-WT isolates	Agents MICs (mg/L)	ECV (mg/L)	Mutations
		Itraconazole	2	
Aspergillus fumigatus	78/81	≥0.06		cyp51A mutants
	6/6	≥12		G54E, M220R, M220I, TR/L98H
	0/3	1.5		cyp51A mutants
	0/1	0.5		G448S
	1/1	2		M220K
	0/1	≤0.06		I301T
		Voriconazole	0.5	
	49/75	≥0.06		cyp51A mutants
	3/6	≥0.047		G54E, M220R, M220I, TR/L98H
	0/3	0.047		cyp51A mutants
	0/1	≤0.06		I301T
	0/3	≥0.125		TR_{34}
	0/12	≤0.5		G54E/R/W
	0/8	≤0.5		M220I/K//R/T/V
	0/1	≤0.25		G138C
		Posaconazole	0.25	
	6/6	≥1		G54E, M220R, M220I, TR/L98H
	13/13	≥0.5		TR_{34}/L98H
	5/5	≥2		G54E/R/V/W
	3/4	≥0.25		M220I/R/T/V/K
	4/5	≥0.25		G448S
	0/3	0.023		cyp51A mutants
		Micafungin	0.016	
	2/3 (MTS)	≥0.004		fks alterations S678P
		Caspofungin	0.25	
	3/3 (MTS)	≥2		fks alterations S678P

Table 2. Relationship between SYO and triazoles gene mutations of non-WT *Aspergillus fumigatus* isolates

Species	No. non-WT isolates	Agents MICs (mg/L)	ECV (mg/L)	Mutations
Aspergillus fumigatus		Voriconazole	1	
	21/39	≥0.125		cyp51A mutants
	0/1	0.125		I301T
	0/1	1		TR_{34}
	0/5	≤0.5		G54E/R/W
	0/5	≤0.5		M220I/K//R/T/V
	0/1	0.25		G138C
	4/5	≥1		TR_{34}/L98H
	5/5	≥8		TR_{46}/Y121F T289A
	0/2	0.125		G54R
	1/1	8		TR_{34}/L98H
		Posaconazole	0.06	
	5/5	≥0.5		TR_{34}/L98H
	5/5	≥1		TR_{46}/Y121F T289A
	24/24	≥0.25		TR_{34}
	2/2	1		G54R
	1/1	1		TR_{34}/L98H

P038

Prevalence of fluconazole-resistant Candida albicans: The option of local dietary spices

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Objectives:
Fluconazole remains one of the most widely used azole drugs in the clinical settings for the treatment of infections caused by *C. albicans*. However, fluconazole-resistant *C. albicans* strains have emerged to pose a threat in the treatment of opportunistic fungal infections.

Materials & Methods:
The prevalence of *C. albicans* was determined by culture of 103 samples (skin, n = 25; oral, n = 18; and high vaginal swabs, n = 60) on SDA plates. Identification of the isolates was carried out on chromagar (HardyCHROM Candida, Santa Maria CA, USA). Virulence of the isolates was evaluated based on their proteinase and hemolytic activities, and their resistance to fluconazole. The sensitivity of the fluconazole-resistant isolates to *Zingiber officinale* (Ginger) was determined using the CLSI M27 – A3 procedures.

Results:
C. albicans total prevalence was 54.4% (n = 56/103) from the three sample sources. The distribution of the positive samples showed that HVS had the highest prevalence (50%, n = 28/56), while 37.5% (n = 21) and 12.5% (n = 7) were recorded in oral and swab samples respectively. Fluconazole-resistance was observed in 62.5% of the isolates (n = 35/56). The Pz values of hemolytic and proteinase activities of the resistant isolates were in the ranges of 0.11 and 0.18; and 0.32 and 0.75 respectively. Also, *Zingiber officinale* (Ginger) aqueous crude extract showed good activity (MIC range = 12.5 – 25 mg/ml) against the fluconazole – resistant *C. albicans* isolates.

Conclusions:
This study shows that fluconazole-resistant *C. albicans* strains have continued to circulate as indicated by the increased prevalence. The susceptibility of the isolates to *Zingiber officinale* (ginger) extract suggest the potential of the local dietary spices as alternative medicinal remedy for infections due to pathogenic fungi such as *C. albicans*.

P039

Antifungal Drug Susceptibility and Molecular Epidemiology of invasive Candida glabrata strains in Pakistan

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Objective

Previously, invasive non-albicans *candida* infections have been a major cause of candidemia causing >80% of infections. There is a knowledge gap between its antifungal drug profiling and molecular epidemiology. Multilocus sequence typing (MLST) has proven to be a useful molecular method for sequence typing of *Candida glabrata* isolates. MLST system for *C. glabrata* utilizes six housekeeping genes.

Method

In this study, twenty *C. glabrata* isolates from patients with candidemia from the years; 2016-2020 were selected for MLST typing. They were analyzed for genetic diversity in six housekeeping genes i.e., *FKS, LEU2, NMT1, TRP1, UGP1* and *URA3.*

Result

Among the six studied loci, 65 (1.94%) polymorphic sites were identified, the most informative site is *NMT1* with 9 alleles, *UGP1* gave the highest discriminatory ratio (0.87) and*UGP3* has the highest d_N/d_S ratio (0.73). The data suggested 11 STs among the 20 studied isolates with ST 162 as the predominant ST.

Conclusion

It can be concluded that the population structure of *Candida glabrata* was highly diverse with the 9 ST. Collectively, this data will be a substantial addition in the fundamental database for better therapeutic approach.

P040

Etiological analysis and antifungal sensitivity profile of fungi causing keratitis in Sri Lanka

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Objectives:

Untreated fungal keratitis leads to catastrophic visual results. Early diagnosis and management are vital to prevent long-term complications. We evaluated the fungal species and the antifungal susceptibility patterns of fungal species isolated from corneal specimens received at the Department of Mycology, Medical Research Institute, Sri Lanka.

Materials & Methods:

Phenotypic identification (Microscopy, Colony morphology, slide culture, water agar, water plant leaf culture) of fungi isolated from corneal samples at the Department of Mycology, MRI from 2013 to 2016 was followed by the anti-fungal susceptibility testing (E strip and disk diffusion methods: voriconazole, itraconazole, and amphotericin b). The ethical clearance for this study was obtained from the Ethics Review Committee, MRI, Colombo, Sri Lanka.

Results:

The dominant pathogen was genus *Fusarium* (66%), with *F. solani* complex (96.5%), and *F. chlamydosporum* (3.5%) being common species. The genus *Aspergillus* (30%) was the next common pathogen, with the main species of *A. flavus* (85%) and *A. fumigatus* (8%). They were followed by less common species including *Pythium (2%), Acremonium* (1%), and *Coelomycetes* (1%). Ninety-one percent, 28%, and 16% of *Fusarium* isolates had minimum inhibitory concentration (MIC) above epidemiological cut-off values (ECVs) for itraconazole, voriconazole, and amphotericin B respectively. None of the *Aspergillus* isolates showed higher MIC values against itraconazole and voriconazole. The findings of MICs and inhibitory zone diameter were comparable only for itraconazole (*Aspergillus* sp. and *Fusarium* sp.) and voriconazole (*Aspergillus* sp.).

Conclusions:

Fusarium sp. followed by Aspergillus sp. are the common pathogen causing fungal keratitis in our study group. A significant number of *Fusarium* isolates should be considered non-wild type and may have an acquired mechanism of resistance against amphotericin B and itraconazole. The management of fungal keratitis is benefited by the guidance of antifungal sensitivity testing.

Keywords: Fungal keratitis, *Fusarium* sp., *Aspergillus* sp., Antifungal resistance

Source of funding: This study was funded by the Medical Research Institute, Colombo

Ethical clearance: The ethical clearance for this study was obtained from the Ethics Review Committee, MRI, Colombo.

P041

The Prevalence, Diagnosis, and Anti-fungal Susceptibility of Candida Species in Palestine

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Background: *Candida* species are the single most common cause of opportunistic fungal infection. Various healthcare implications are associated with *Candida* infections, including morbidity and mortality. The significantly increased occurrence of *Candida* species as human pathogens can be attributed to improved identification techniques. In Palestine, minimal data have been reported about *Candida* infection.

Methods: We performed a descriptive cross-sectional study at two hospitals in Palestine (Istishari Arab Hospital, and Najah National University Hospital). All patients diagnosed with candidiasis during the year 2022 have participated in the study. The prevalence of *Candida spp* and their distribution were statistically analyzed using Microsoft Excel.Also, the activity of selected antifungals against *Candida* pathogens was analyzed using Microsoft Excel. *Candida* isolates were subcultured on *Candida* deferential agar media. In combination with phenotypic properties, *Candida* isolates were identified and tested for antifungal susceptibility using the colorimetric VITEK-2 Compact system.

Results. Our preliminary results showed that the prevalence of *Candida spp* among infected samples was 11.37 % of 4216 participants. Eleven different *Candida species* were identified. Among these isolates, *C. albicans* (49.88%) was the most frequent, followed by *C. glabrata* (17.3%), *C. tropicalis* (14.83%), *C. parapsilosis* (5.16%), *C. krusei* (3.82%), *C. auris*(2.69%) , *C.dubliniensis* (2.24%), *C.ciferrii* (1.79%) *C. lusitaniae* (0.89%), *C. guilliermondii* (0.67%), , *C.Kefyer* (0.44%) and *Candida spherica* (0.22%) respectively. Among *C. albicans*, all isolates were 100% susceptible to fluconazole and voriconazole. The susceptibility rates to amphotericine B and caspofungin were 90.1 % and 98.03% respectively. As a comparison, the susceptibility rates of non-albicans *Candida* to fluconazole, voriconazole, amphotericine B, and caspofungin were 98.6 %, 72.9 %, 93.2 %, and 81.08 %, respectively. The intensive care unit patients are at a greater risk of infection with *Candida* than the rest of the patients since it makes up 31.5% of the total.

Conclusions. Palestine has a high prevalence of Candida infections. Four pathogens are responsible for the most invasive infections: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*. Those in an immunocompromised state are more likely to develop *Candida* infections. A notable characteristic of this study was the high frequency of non-albicans *Candida* species, which were often more resistant to antifungal agents. A quick and accurate technique like Vitek 2 compact was suggested for careful species identification of clinical isolates of *Candida*. We suggest that continued surveillance of species distribution and susceptibility to antifungals will enhance future burden estimates and assist in evaluating preventative measures' effectiveness.

Key words: *Candida*, Prevalence, Anti-fungal and Palestine.

P042

An Emerging Concern: Reduced Susceptibility and Aflatoxigenic Fungi Causing Fungal Rhinosinusitis in Sudan

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Objective: This study aimed to analyze strains collected over a period of 5 years to determine the prevalence, molecular characterization, antifungal susceptibility, and aflatoxin production of FRS-causing opportunists in Sudan.

Materials and Methods: The isolates were identified through the sequencing of β-tubulin and calmodulin. Antifungal susceptibility profiles were evaluated. The presence of aflatoxins was detected through thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Results: Over five-year period, *Aspergillus flavus* complex (n=244) was the most prevalent, followed by *A. terreus* complex (n=16), *A. fumigatus* complex (n=7), and other fungi (n=17). Among a total of 88 *A. flavus* isolates, 2.3-4.5% (n=88) exhibited reduced susceptibility to azoles. Despite several mutations revealed in *cyp51A* of these isolates, none could be directly linked to azole resistance. Aflatoxin production was detected in 42% (37/88) and 49% (43/88) of the clinical *A. flavus* isolates, as assessed by TLC and HPLC analyses, respectively. The total amount of aflatoxin produced varied significantly, ranging from 34.39 ± 0.01 to 0.02 ± 0 mg/kg. It is noteworthy that aflatoxigenic clinical isolates exclusively produced aflatoxin B, comprising aflatoxin B1 and aflatoxin B2.

Conclusion: This study highlights the public health concern of emerging azole resistance in *A. flavus* isolates causing FRS in Sudan. Monitoring antifungal susceptibility is crucial to guide treatment decisions, especially in regions with limited treatment options. The presence of aflatoxin-producing A. flavus isolates emphasizes the health risks associated with A. flavus infections. These findings emphasize the need for comprehensive monitoring and management strategies.

P044

Surveillance of Aspergillus section Nigri over a 4-year Period in a General Hospital

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Objectives: *Aspergillus* section *Nigri* is less likely to cause invasive aspergillosis (IA) than some other *Aspergillus* species. Data on the antifungal susceptibility patterns of most of the species in section *Nigri* is scarce. Breakthrough IA have been observed under voriconazole therapy and data on antifungal susceptibility testing seem to be contradictory. Then, their identification and susceptibility profiles have clinical interest since we observed several patients had IA due to *Aspergillus* section *Nigri* at our institution.
The present study aims to evaluate the epidemiology and antifungal susceptibility of *Aspergillus* section *Nigri* isolated in our institution over a 4-year period.

Materials & Methods: A total 194 *Aspergillus* section *Nigri* strains from 137 patients were analysed. Charts were found from January 2019 to February 2023 by reviewing clinical data. The antifungal susceptibility of all isolates was determined by E-test (Biomerieux) according to the manufacturer and taking into account current available CLSI ECVs (CLSI M59-ED3, 2020) with amphotericin B (AMB), itraconazole (IZ), voriconazole (VZ), posaconazole (POS), and isavuconazole (ISV).

Results: Of the total patients, 26 (19%) had invasive aspergillosis caused by *Aspergillus* section *Nigri*. The majority of IA caused by *Aspergillus* section *Nigri* were monofungal (80.8%), although 19.2% were polyfungal with other *Aspergillus* spp, mainly *Aspergillus* section *Fumigati*. The distribution by year of monofungal and polyfungal IA caused by *Aspergillus* section *Nigri* was as follows respectively: 2019 (1/0), 2020 (10/2), 2021 (0/2), 2022 (9/0) and 2023 (1/1).
The MICs range (µg/ml) of the available clinical isolates were as follows: AMB (0.125-2), IZ (0.03-64), VZ (0.016-4), POS (0.03-2), and ISV (0.03-8). The overall geometric mean (GM) and MIC90 (µg/ml) for the antifungals tested were as follows: AMB (1.090/1), IZ (4.335/8), VZ (1.155/1), POS (1.259/0.5), and ISV (1.728/2).

Conclusions: We observed a significant proportion in the number of patients with invasive aspergillosis due to *Aspergillus* section *Nigri* in our institution. *Aspergillus* section *Nigri* shows particularly high MICs to azoles, especially to itraconazole and isavuconazole, whether this translates into a poorer clinical response need to be evaluated. However amphotericin B showed good activity in vitro.

P045

In vitro activities of antifungal drugs against a large collection of Trichophyton tonsurans isolated from wrestlers

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Objectives:
Trichophyton tonsurans is the most common agent causing tinea gladiatorum in wrestlers, and limited data on susceptibility profiles of Trichophyton tonsurans are available.We aimed to assess the *in vitro* activity of the common antifungal drug against a large collection of *T tonsurans.*

Materials & Methods:
The *in vitro* activities to eight common antifungal drugs (sertaconazole, itraconazole, clotrimazole, fluconazole, butenafine, tolnaftate, terbinafine and griseofulvin) against 128 clinical isolates of *T tonsurans* strains, obtained from wrestlers with dermatophytosis, were performed according to CLSI M38-A2 broth microdilution document.

Results:
The geometric mean minimum inhibitory concentration was the lowest for tolnaftate (0.022 µg/mL), followed by itraconazole (0.026 µg/mL), terbinafine (0.033 µg/mL), butenafine (0.088 µg/mL), griseofulvin (0.566 µg/mL), sertaconazole (2.875 µg/mL), clotrimazole (3.419 µg/mL) and fluconazole (12.540 µg/mL).

Conclusions:
Evaluation of antifungal susceptibility of dermatophytes showed that tolnaftate and itraconazole were the most effective drugs against *T tonsurans* and fluconazole had the least effect.

P046

Aspergillus flavus and azole resistance in clinical and environmental isolates from Pakistan

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Objectives:
Aspergillus flavus is the most common *Aspergillus* spp. from both humans and soil samples in Pakistan. A study from Asia reported over 85% resistance to at least one azole in *Aspergillus flavus*. Considering this high azole resistance in the region, it is important to determine the susceptibility of *A. flavus* against azoles in Pakistan. In this study, we determined minimum inhibitory concentrations (MICs) of itraconazole, voriconazole, and posaconazole in *A. flavus* isolated from clinical and environmental specimens using broth microdilution (BMD).

Materials & Methods:
MICs of 203 (144 clinical and 59 environmental) *A. flavus* strains from different regions of Pakistan were determined by BMD using the Clinical and Laboratory Standards Institute (CLSI) M38 guidelines. The results were interpreted according to the epidemiological cut-off values (ECVs) established in 2022 by CLSI.

Results:
None of the isolates had azole MICs above ECVs. The clinical isolates had MIC_{50} and MIC_{90} of 0.25/0.5 ug/ml for itraconazole, 0.5/1 ug/ml for voriconazole, and 0.25/0.5 ug/ml for posaconazole. The environmental isolates had MIC_{50} and MIC_{90} of 0.125/0.25 ug/ml for itraconazole, 0.5/0.5 ug/ml for voriconazole, and 0.125/0.25 ug/ml for posaconazole.

Conclusions:
In our study, no evidence of resistance to itraconazole, voriconazole, and posaconazole in *Aspergillus flavus* was observed. Determination of *CYP51*gene mutation in these strains is ongoing and results are awaited. Future antifungal resistance surveillance studies are needed to monitor the emergence of azole resistance in *A. flavus*.

P048

Correlation Between Antifungal Zone Diameters and MICs, and Categorical Agreement for C. auris Isolates in Pakistan

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Objectives:
This study aimed to assess the correlation between zone diameter (ZD) measurements and minimum inhibitory concentrations (MICs) of fluconazole (FLU), caspofungin (CAS), and voriconazole (VOR) against *C. auris* isolates. The study also aimed to determine the categorical agreement between ZD and MIC results for these antifungal agents. The findings of the study were intended to contribute to existing knowledge on *C. auris* susceptibility and provide evidence to support development of clinical breakpoints.
Methods:
Data on *C. auris* isolates were collected from the Aga Khan University Laboratory database for the period from Jan-2020 to Nov-2022. Identification of *C. auris* was based on colony morphology and API 20C AUX (2020-Jun2021) and Vitek2 system (Jul2021 onwards). Antifungal susceptibility testing was performed using broth microdilution on Sensititre® YeastOne, and results interpreted according to CDC guidelines. Disc diffusion testing was by Kirby–Bauer method, and zone diameter interpretation of < 12 mm was considered resistant based on the study by Nunnally et al. (JCM 2021).
Result:
Between 2018 and 2023, 217 patient specimens were obtained; 106 had both MICs and zone diameters recorded. The ages varied from 2 days to 91 years, 64.2% were adults >18. The male population made up 67.9% of the cases. Most common source of isolate was blood (59.4%), followed by urine (11.3%) and ear samples (9.4%). Fifty six percent of the isolates (~~82, or 77.4%~~) came from our own center, 15.8% from other hospitals in Karachi, 18.6% from other locations in Karachi and 9.3% other cities of Pakistan. Fluconazole resistance was in 87.7% of strains by disc diffusion, and 88.6% by broth microdilution; Voriconazole resistance was detected in 2.8% by disc diffusion and 4% by broth microdilution. All strains were susceptible to Caspofungin by disc diffusion and to Anidulafungin and micafungin by broth microdilution. FLU ZD-MIC by Spearman correlation was weak (-0.291, p=0.006). There was no significant relationship between VOR ZD and MIC (p=0.804). CAS ZD was significant with MICs of neither ANI (p=-0.173) nor MFG (p=-0.202). Categorical agreement for FLU disc diffusion two major and 6 very major errors, whereas VOR had 3 major errors and 1 very major error.
Conclusions:
The findings of Nunally et al. differ from the isolates collected in our study. These disparities could be attributed to certain limitations, considering the data on ZD and MIC was retrospective. Additionally, it is noteworthy that the isolates in our collection all belong to the possibly Clade I and most to the same clonality and heavily skewed towards a single center, which may contribute to difference in the results from Nunally et al.'s findings. Further studies are needed to explore corelation between disk diffusion and MICs using a more diverse set of isolates.

P049

An insight into the antifungal susceptibility of Candida species from animals to four antifungal agents

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Objectives:

Candida species are known opportunistic infectious agents in humans but are not as common in veterinary medicine. They are part of the resident microflora of domestic and wild animals, and sporadic cases of infection in animals have been reported. Their azole resistance is well known in human medicine, but reports of azole-resistant *Candida albicans*, and the emergence of resistance in non-*Candida albicans* isolates (NCA) not only in infected but also in healthy animals are increasing. The One Health concept calls for the need for susceptibility testing of animal isolates to provide information on susceptibility patterns and possible resistance in veterinary medicine, which is still lacking. The aim of this study was to investigate the susceptibility of *Candida* species isolated from different animal samples to four antifungal agents: miconazole (MCZ) and clotrimazole (CTZ), commonly used in veterinary medicine for topical treatment, and two oral formulations, itraconazole (ITZ) and fluconazole (FCZ).

Materials & Methods

Candida species were identified by classical mycological methods, followed by conventional polymerase chain reaction (PCR) using the fungal universal primers ITS-1 and ITS-4 for amplification and sequencing of ITS1, ITS2, and the 5.8S ribosomal region. Antifungal susceptibility testing for MCZ, CTZ, ITZ, and FCZ was assessed using the broth microdilution method according to EUCAST document E. DEF.7.3.2. with recommended test ranges for ITZ and FCZ, while concentrations of 0.004-2 mg/L were used for MCZ and CTZ (Mesquida et al., 2021). Results were interpreted as minimum inhibitory concentration (MIC) values, which represented the lowest drug concentration that inhibits ≥50% of fungal growth compared to the drug-free control well. All samples were tested in triplicate.

Results:

Twenty-five *Candida* strains were isolated from: external ear canal (9), bronchoalveolar lavage (4), skin (2), feces (2), urine (1), anus (1), udder (1), nasal plaque (1), pharynx (1), punctate (1), wound (1), unknown origin (1) of different animal species. The most frequently isolated species was *Candida albicans* (10) followed by NCA species: *C. parapsilosis* (4), *C. tropicalis* (4), *C. palmioleophila* (2), and one isolate each of *C. krusei*, *C. lusitaniae*, *C. zeylanoides*, *C. fermentati* and *C. guilliermondii*. Antifungal susceptibility testing of all isolates revealed MIC ranges of 0.008-1 mg/L for MCZ, 0.004-1 mg/L for CTZ, <0.008->4 mg/L for ITZ, and 0.125-≥64 mg/L for FCZ. *C. albicans* species were more susceptible compared to NCA species with the highest MIC values found in one strain for FCZ (2 mg/L), one for ITZ (0.125 mg/L), one for MCZ and two for CTZ (0.06 mg/L). In addition, the highest MIC values for FCZ (2 to ≥64 mg/L) were observed in eight NCA species tested, followed by one for ITZ (>4 mg/L), one for CTZ (1 mg/L), and six for MCZ (1 mg/L). The lowest susceptibility to FCZ was observed in three *C. tropicalis* (8 and ≥64 mg/L).

Conclusions

The reduced susceptibility of *Candida* isolates observed in this study requires further studies to provide more information on the susceptibility pattern and molecular mechanisms that may indicate the presence of potential resistance in these animal strains.

Mesquida, A., T. Vicente, E. Reigadas, M. Palomo, C. Sánchez-Carrillo, P. Muñoz, J. Guinea, P. Escribano (2021): *In vitro* activity of ibrexafungerp and comparators against *Candida albicans* genotypes from vaginal samples and blood cultures. Clin. Microbiol. Infect. 27(6):915.e5-915.e8.

P050

Evaluation of Impact of the COVID-19 Pandemic in the Incidence and Characteristics of Candidemia: A Single Center Study

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Objectives:

The purpose of this study was to assess the impact of the COVID-19 pandemic on the incidence of Candidemia and to evaluate the clinical and microbiological characteristics and outcomes of patients with *Candida* bloodstream infection admitted to the University Hospital of Udine, Italy.

Materials & Methods:

A cohort of 221 patients with at least one positive blood culture positive for *Candida* spp. from January 2020 to October 2022 was compared with a population of 151 patients with *Candida* bloodstream infections hospitalized between January 2018 and December 2019. We analyzed medical charts to extract information about demographics, underlying conditions, microbiological data, patient management and outcome. A statistical analysis was conducted in order to evaluate significant between-group differences and correlations.

Results:

Demographics, clinical and microbiological characteristics are summarized in Table 1. Considering main risk factors, the pre-pandemic group (PRE) present less abdominal problems (6.0% vs 15.7%, p=0.005) and any intravascular device (central venous catheters (CVC) 13.9%, midline 29.0%, p<0.001) compared to post-pandemic group (POST)). On the contrary, antibiotic exposure, parenteral nutrition and *Candida* colonization were significantly more frequent in the PRE group. The most frequent species of *Candida* isolated was *albicans* (PRE: 53.6%, POST 57.5%), followed by *glabrata* (PRE: 18.5%, POST: 14.5%), *parapsilosis* (PRE: 14.6%, POST: 16.7%), *tropicalis* (PRE: 12.6%, POST 6.3%), *lusitaniae* (PRE: 0.0%, POST:1.8%), *krusei* (PRE: 0.0%, POST:1.4%), *guillermondii* (PRE: 0.0%, POST: 0.9%), *dubliniensis* (PRE: 0.7%, POST: 0.0%), *lambica* (PRE:0.0%, POST:0.5%), and *kefir* (PRE:0.0%, POST: 0.5%). CVC/midline removal was performed more frequently in the POST group (75.3 %, 86.5%), but positive tip cultures were found more in the PRE one. Moreover, echocardiography was performed less frequently in the PRE group (25.3% vs 35.7%, p=0.040) but the evidence of infective endocarditis was higher in the PRE group than in the POST group (10.5% vs 1.2%, p=0.035). As shown in Table 2, *C. albicans* rarely presented resistance to echinocandin in both groups; *C. parapsilosis* and *C. glabrata* exhibited a low resistance rate to fluconazole. In addition, 78.9% of *C. parapsilosis* isolates were susceptible to echinocandins. Fifty three percent of patients in the PRE group and 49.8% of patients in the POST group died during hospitalization (p=0.598). Among patients who died, attributable mortality was more common in the POST group than in the PRE group (34.3% vs 19.0%, OR=2.214, p=0.031). Overall 30-day survival was 89.3% (PRE: 88.6%, POST: 89.8%, p=0.808), 90-day survival was 79.5% (82.9%, 76.7%, p=0.427).

Conclusions:

Tthis study has several limitations, mainly related to its retrospective design, but the analysis evidenced that in our center, The COVID-19 pandemic did not have any impact on the prevalence and susceptibility profiles of *Candida* species, nor did it affect the patient outcome.

Table 1. Some demographics, clinical and microbiological characteristics.

	COVID-19 Pandemic		
	PRE	**POST**	**Comparison**
Patients	151 (40.6%)	221 (59.4%)	
2018	8.3%	-	NC
2019	51.7%	-	NC
2020	-	22.6%	NC
2021	-	41.2%	NC
2022	-	36.2%	NC
Sex (females)	38.4%	43.4%	p=0.391
Age at Candidemia diagnosis	72.0 ±16.53 [6, 96]	73.3 ±14.17 [19, 98]	p=0.429
Charlson comorbidity index	5.8 ±3.09 [0, 14]	5.9 ±2.92 [0, 13]	p=0.785
Admission ward			
Medical	72.8%	65.2%	p=0.140
Surgical	14.6%	20.8%	p=0.135
Anaesthesia and Resuscitation	12.6%	14.0%	p=0.758
Intensive Care admission	23.2%	29.9%	p=0.191
Abdominal Surgery	43.6%	36.7%	p=0.194
Antibiotic therapy	96.6%	75.1%	p<0.001*
Parenteral nutrition	46.3%	33.8%	p=0.021*
Candida colonization	20.7%	8.7%	p=0.002*
Device			
CVC/midline	61.8%	56.5%	p=0.328
Port-a-cath	9.9%	3.6%	p=0.016*
High dose corticosteroid therapy	8.7%	9.7%	p=0.852
SARS.COV2 infection/	-	10.9%	NC
Fever at diagnosis	67.4%	88.1%	p<0.001*
Haemodynamic stability	93.9%	93.7%	p>0.999
Candida species			
albicans	53.6%	57.5%	p=0.524
glabrata	18.5%	14.5%	p=0.317
parapsilosis	14.6%	16.7%	p=0.665
tropicalis	12.6%	6.3%	p=0.042*
lusitaniae	-	1.8%	p=0.150
krusei	-	1.4%	p=0.275
Follow-up blood cultures			
Not performed	14.1%	9.0%	p=0.133
Negative	58.4%	66.1%	p=0.154
Positive	27.5%	24.9%	p=0.629
CVC/Midline			
Removal	75.3%	86.5%	p=0.103
Positive CVC tip cultures	69.4%	43.5%	p=0.026*

Table 2. Main antifungal drug susceptibility of most isolated *Candida* species

				COVID-19 Pandemic		
			All	PRE	POST	Comparison
C. albicans	*Fluconazole*	S	206 (100.0%)	81 (100.0%)	125 (100.0%)	-
		I	-	-	-	-
		R	-	-	-	-
	Anidulafungin	S	195 (98.0%)	72 (94.7%)	123 (100.0%)	p=0.020*
		I	-	-	-	-
		R	4 (2.0%)	4 (5.3%)	-	p=0.020*
	Micafungin	S	157 (85.8%)	48 (81.4%)	109 (87.9%)	p=0.261
		I	-	-	-	-
		R	26 (14.2%)	11 (18.6%)	15 (12.1%)	p=0.261
	Amphotericin-B	S	206 (100.0%)	81 (100.0%)	125 (100.0%)	-
		I	-	-	-	-
		R	-	-	-	-
C. glabrata	*Fluconazole*	S	1 (1.7%)	-	1 (3.1%)	p>0.999
		I	56 (93.3%)	26 (92.9%)	30 (93.8%)	p>0.999
		R	3 (5.0%)	2 (7.1%)	1 (3.1%)	p=0.594
	Anidulafungin	S	58 (96.7%)	27 (96.4%)	31 (96.9%)	p>0.999
		I	-	-	-	-
		R	2 (3.3%)	1 (3.6%)	1 (3.1%)	p>0.999
	Micafungin	S	58 (100.0%)	26 (100.0%)	32 (100.0%)	-
		I	-	-	-	-
		R	-	-	-	-
	Amphotericin-B	S	57 (96.6%)	26 (92.9%)	31 (100.0%)	p=0.221
		I	-	-	-	-
		R	2 (3.4%)	2 (7.1%)	-	p=0.221
C.parapsilosis	*Fluconazole*	S	56 (94.9%)	21 (95.5%)	35 (94.6%)	p>0.999
		I	-	-	-	-
		R	3 (5.1%)	1 (4.5%)	2 (5.4%)	p>0.999
	Anidulafungin	S	45 (78.9%)	9 (45.0%)	36 (97.3%)	p<0.001***
		I	12 (21.1%)	11 (55.0%)	1 (2.7%)	p<0.001***
		R	-	-	-	-
	Micafungin	S	45 (78.9%)	9 (45.0%)	36 (97.3%)	p<0.001***
		I	12 (21.1%)	11 (55.0%)	1 (2.7%)	p<0.001***
		R	-	-	-	-
	Amphotericin-B	S	57 (98.3%)	21 (95.5%)	36 (100.0%)	p=0.379
		I	1 (1.7%)	1 (4.5%)	-	p=0.379
		R	-	-	-	-

P051

An experimental combination therapy to combat azole resistance in Candida auris

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Objectives: The emergent fungal pathogen *Candida auris* can cause severe invasive infections. Such outbreaks can be difficult to eliminate and the Centers for Disease Control and Prevention (USA) has expressed concern about this pathogen because: (1) it is difficult to identify with standard microbiological methods, (2) it is frequently highly resistant to commonly used antifungal drugs, and (3) it causes outbreaks in health care settings. The World Health Organization identified *C. auris* as a global priority in its first list of fungal health threats, highlighting the need for new treatment options.The monoamine oxidase A inhibitor Clorgyline inhibits ABC and MFS efflux pumps in *Candida albicans* and increases the efficacy of antifungal drugs in a *Saccharomyces cerevisiae* model. The aim of this study was to investigate the effect of Clorgyline and structurally related analogs on (I) recombinant *S. cerevisiae* strains overexpressing CauCdr1 and CauMdr1, and (II) azole resistant and susceptible clinical isolates of *C. auris* from clade I and II.

Materials & Methods: Disk diffusion and 2-dimensional liquid microdilution assays were used to determine the efficacy of Clorgyline and six structural analogs in combination with azole drugs. Azole resistant *C. auris* isolates from clade I, susceptible isolates from clade II, and recombinant *S. cerevisiae* strains functionally overexpressing the CauMdr1 and CauCdr1 efflux pumps were used in screens. Nile Red efflux was used to assess the inhibitory effect of Clorgyline and its analogs on efflux pumps in recombinant strains. The uncoupling effects of Clorgyline and its analogs were evaluated by determining their inhibition of the Oligomycin-sensitive CauCdr1 ATPase activity in membrane preparations from recombinant strains.

Results: Two of the six Clorgyline analogs (M19 and M25) synergized with the azoles Posaconazole and Voriconazole against azole resistant clade I *C.auris*. M19 and M25 inhibited Nile red effflux by CauCdr1 and CauMdr1 efflux pumps in recombinant strains. M19 showed synergy with Posaconzole or Voriconazole against recombinant CauCdr1 while M25 gave synergy with Posaconazole against both CauCdr1 and CauMdr1. Clorgyline, M19, and M25 uncoupled the Oligomycin-sensitive ATPase activity of CauCdr1.

Conclusions: The combination of M19 or M25 with azole drugs, particularly Posaconazole which is also unaffected by the CauErg11 Y132F or K143R mutations, synergistically increased the susceptibility of azole resistant *C. auris* and circumvented azole resistance in clade I *C. auris*. The uncoupling effects of Clorgyline and its analogs are not understood, but covalent modification of CauCdr1 or changed membrane fluidity due to these compounds affecting the membrane close to CauCdr1 are possibilities. Further research is needed to determine the clade specificity, mode of action and toxicity of M19 and M25.

P052

The impact of pH on susceptibility testing of azoles against Candida vaginal isolates using the EUCAST reference methodology

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Objectives: Vulvovaginal candidiasis (VVC) is commonly treated with azoles, which are fungistatic against *Candida* spp. and less effective against non-*albicans Candida* spp. Previous studies have shown that the medium pH can alter the azoles' CLSI MIC of *Candida* spp. (Sobel *AAC* 2022). However, the impact of pH on the EUCAST antifungal susceptibility testing (AFST) of *Candida* vaginal isolates has not been investigated. We therefore evaluated the effect of low-pH environment on the *in vitro* activity of fluconazole and itraconazole recommended as systemic therapeutic regimens for VVC using the EUCAST broth microdilution reference methodology.

Materials/methods: A total of 226 isolates (126 *C. albicans*, 69 *C. parapsilosis*, 24 *C. glabrata*, 7 *C. krusei*) were tested. Species were identified by MALDI-ToF MS. AFST of fluconazole and itraconazole was performed following the EUCAST-E.DEF7.3.2 but including both pH 7.0 and 4.5 in order to mimic the pH in laboratory conditions and in the vagina, respectively. The microtiter plates were incubated at 35±2°C for 24h and the MICs were defined as the lowest drug concentrations at which ≥50% growth inhibition, compared to the growth control well, was recorded spectrophotometrically. MIC data generated at different pH levels were compared with paired t-test, while differences in categorical interpretation were assessed by Fisher's exact test.

Results: The *in vitro* activity of antifungals at acidic and neutral pH values are shown in **Figure**. At pH 4.5, a species-specific impact on the fluconazole MICs was observed. Namely, the MIC values of *C. parapsilosis* and *C. glabrata* were significantly increased (p<0.0001), as well as those of *C. albicans* (p=0.07), as opposed to the MICs of *C. krusei* (p=0.11) that were reduced. The ±1 twofold dilution agreement between the MIC data obtained at different pH levels was 93% for *C. albicans*, 70% for *C. parapsilosis*, 38% for *C. glabrata* and 71% for *C. krusei*, with a median difference of 0, 1, 2 and -1 twofold dilutions, respectively. While 6/126 (5%) *C. albicans* and 11/24 (46%) *C. glabrata* isolates were fluconazole-resistant at pH 7.0, 8/126 (6%) *C. albicans* and 24/24 (100%) *C. glabrata* isolates exhibited fluconazole resistance at pH 4.5 (93% overall categorical agreement,p<0.0001). Itraconazole MIC values of all *Candida* spp. were significantly increased at pH 4.5 (p≤0.02). The ±1 twofold dilution agreement between the MICs generated at different pH levels was 4% for *C. albicans*, 6% for *C. parapsilosis*, 22% for *C. glabrata* and 14% for *C. krusei*, corresponding to a median difference of 3, 3, 2 and 3 twofold dilutions, respectively. Only 4/24 (17%) *C. glabrata* isolates demonstrated a non-wild type phenotype at pH 7.0, contrary to pH 4.5 were 21/126 (17%) *C. albicans*, 2/69 (3%) *C. parapsilosis* and 10/24 (42%) *C. glabrata* isolates were itraconazole-resistant/non-wild type (87% overall categorical agreement,p=0.0004).

Conclusions: The ordinary EUCAST AFST pH value did not reflect the susceptibility rates of *Candida* vaginal isolates, particularly *C. glabrata*, to fluconazole and itraconazole at normal vaginal pH levels. The clinical relevance of the increased MICs obtained to the isolates' real growth environment needs to be prospectively correlated with treatment outcome.

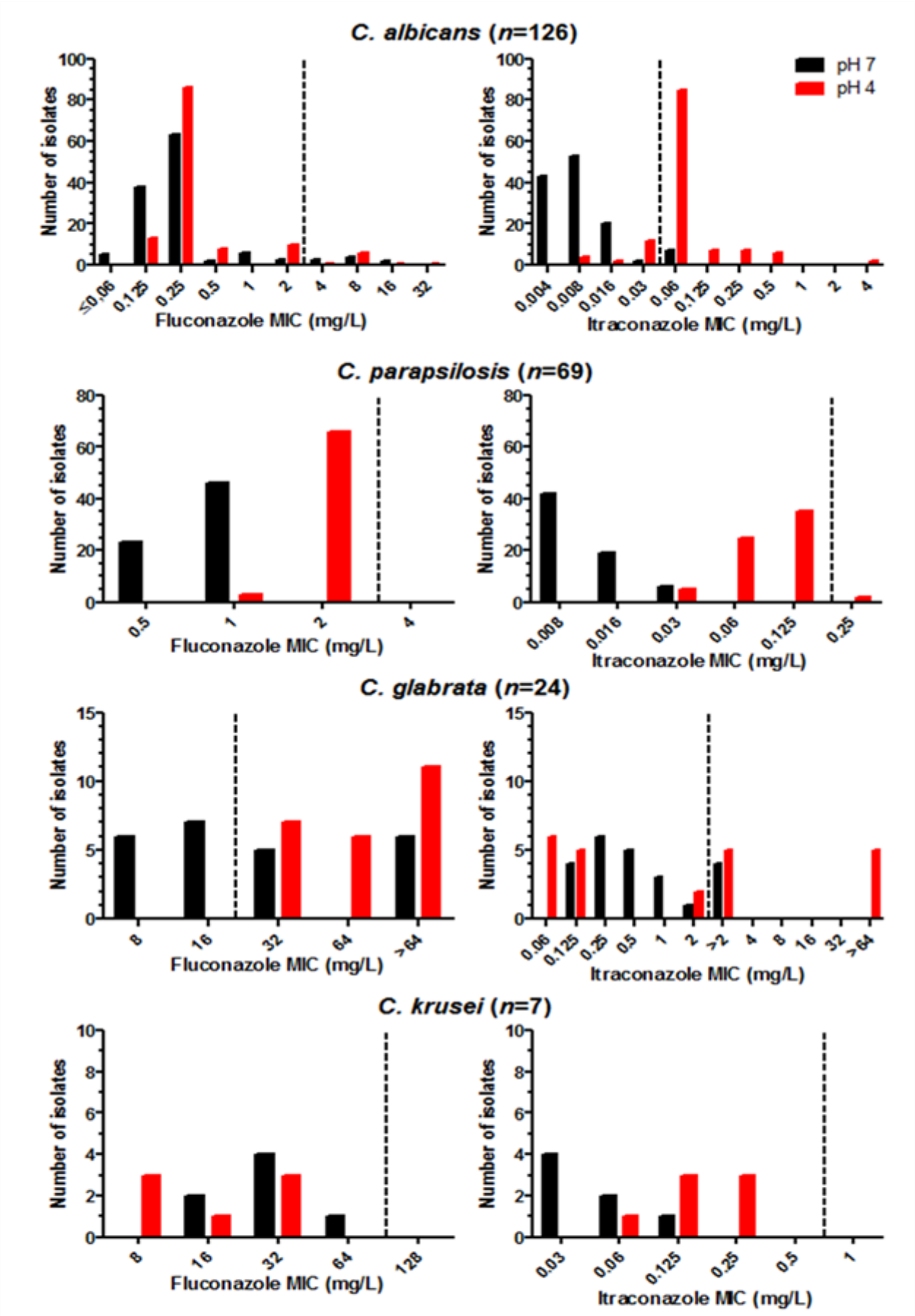

Figure. EUCAST fluconazole (left) and itraconazole (right) MIC distributions of *Candida* vaginal isolates generated at pH level 7.0 and 4.5. The broken lines indicate the clinical breakpoints or epidemiological cut-off values (if clinical breakpoints are not available) for each *Candida* spp.

P053

Characterization of Triacsin C - Susceptibility to Malassezia Acyl-CoA Synthetase

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Objectives:

Malassezia species are opportunistic pathogens associated with cutaneous diseases and systemic infections. Since this fungus lacks in fatty acid synthesizing enzyme (FAS), it needs to assimilate exogenous long-chain fatty acids (LCFA) and then activate LCFA to LCFA-CoA by long-chain acyl-CoA synthetase (ACS) for the downstream metabolic processing. The previous reports showed that Triacsin C (TriC) inhibits ACS in Raji cells and *Malassezia* fungus. On the other hand, TriC does not suppress *Saccharomyces cerevisiae* proliferation even when their endogenous fatty acid synthesis is blocked by cerulenin (Cer). The study aims to clarify the amino acid sequence region in fungal ACSs that are essential to be inhibited by TriC and annotate *Malassezia* ACS genes *(FAA*) for future studies to develop drugs attacking ACS.

Materials & Methods:

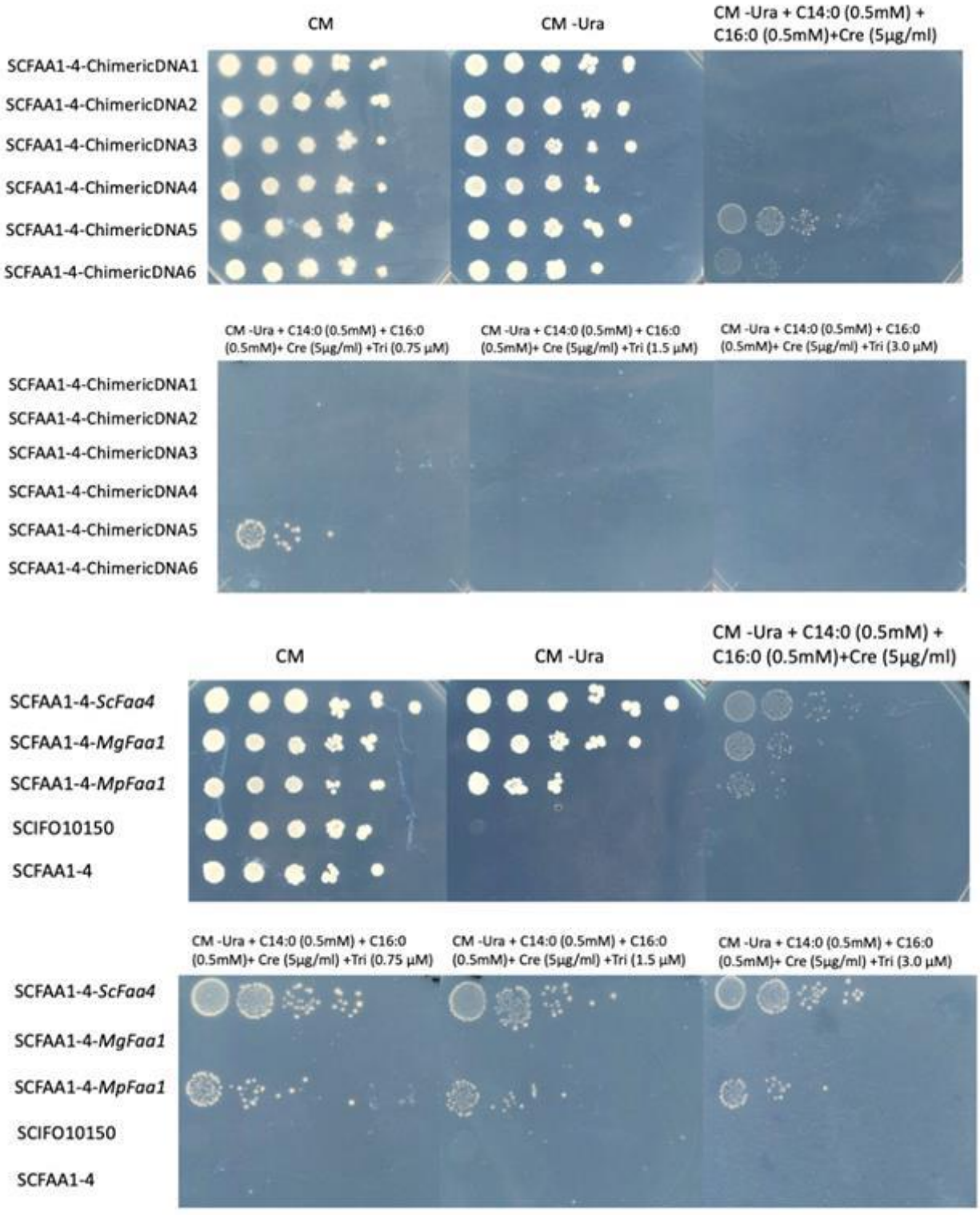
S. cerevisiae Δfaa1 Δfaa4 (SCFAA1-4) was used as the recipient cell. *M. globosa FAA1* (*MgFAA1*) and *M.pachydermatis FAA1* (MpFAA1) were expressed in SCFAA1-4. To construct chimeric DNAs of the *FAA* gene, the overlap extension PCR method was performed. These chimeric genes were also introduced into SCFAA1-4. The growth of these recombinant yeast cells was observed.

Results:

The observation of growth of SCFAA1-4 expressing *MgFAA1*, *MpFAA1,* and *ScFAA4* in the medium with TriC and Cer showed that only the strain harboring *MgFAA1 could not* grow, and this result differed from previous information. Next, two *Malassezia* chimeric FAA genes were designed (f*FAA5*: the N-terminal region of *MgFAA1* and the C-terminal region of *MpFAA1*, f*FAA6*: the N-terminal region of *MpFAA1* and the C-terminal region of *MgFAA1*) and were introduced into SCFAA1-4. These transformants could grow in the medium with Cer, and it was confirmed that these chimeric ACSs could function in the cells (Figure 1). Besides, the strain harboring f*FAA6* could not grow in the medium with TriC and Cer (Figure 1). From these results, it was suggested that the C-terminal region of *Malassezia FAA1* is closely related to the function of TriC.

Conclusions:

M. pachydermatis ACS was not inhibited by TriC, which differed from the previous information. *Malassezia* Chimeric ACSs were able to function in *S. cerevisiae*. Furthermore, the C-terminus (1783-2028nt) of *MpFAA1* contributes to TriC resistance in *Malassezia* fungus, indicating a lower affinity for Triacsin C to bind. We are processing further experiments to narrow down the C-terminus region of *Mp*ACS to determine the amino acid sequence related to the TriC resistance.


CM
CM -Ura
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+Cre (5µg/ml)
SCFAA1-4-ChimericDNA1
SCFAA1-4-ChimericDNA2
SCFAA1-4-ChimericDNA3
SCFAA1-4-ChimericDNA4
SCFAA1-4-ChimericDNA5
SCFAA1-4-ChimericDNA6
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (0.75 µM)
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (1.5 µM)
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (3.0 µM)
SCFAA1-4-ChimericDNA1
SCFAA1-4-ChimericDNA2
SCFAA1-4-ChimericDNA3
SCFAA1-4-ChimericDNA4
SCFAA1-4-ChimericDNA5
SCFAA1-4-ChimericDNA6
CM
CM -Ura
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+Cre (5µg/ml)
SCFAA1-4-ScFaa4
SCFAA1-4-MgFaa1
SCFAA1-4-MpFaa1
SCIFO10150
SCFAA1-4
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (0.75 µM)
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (1.5 µM)
CM -Ura + C14:0 (0.5mM) + C16:0 (0.5mM)+ Cre (5µg/ml) +Tri (3.0 µM)
SCFAA1-4-ScFaa4
SCFAA1-4-MgFaa1
SCFAA1-4-MpFaa1
SCIFO10150
SCFAA1-4

P054

Comparative Assessment of Sensititre YeastOne and Micronaut-AM for Antifungal Susceptibility Testing in Candidaemia Isolates

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Objectives:

Antifungal susceptibility testing (AFST) is essential to ensure appropriate antifungal therapy in candidaemia. This study compared two commercial colorimetric broth microdilution tests: Sensititre YeastOne (YO10) (Thermo Scientific) against Micronaut-AM EUCAST AFST (M-AM) (Bruker) for AFST of *Candida* spp. isolates causing bloodstream infections in our hospital.

Methods & Materials:

All strains were clinical isolates obtained from blood cultures of adult patients admitted to CHU UCL Namur site Godinne, a tertiary care hospital in Belgium, from 2017 to 2023. A total of 74 yeast strains were tested, including *C. albicans* (n=40), *C. glabrata* (n=6), *C. guilliermondii* (n=2), *C. kefyr* (n=1), *C. krusei* (n=8), *C. lusitaniae* (n=1), *C. parapsilosis* (n=11) and *C. tropicalis* (n=5). Reference strains *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 24433, were also included. The isolates were identified by using MALDI-TOF (Bruker). AFST by YO10 and by M-AM were performed according to the manufacturers' protocols and interpreted using CLSI and EUCAST guidelines respectively. Two operators visually determined MICs values. Essential agreement (EA), categorical agreement (CA), very major error, major error and minor error were calculated considering YO10 as the reference method.

Results:

In total, 441 and 392 isolate-antifungal results were evaluable for EA and CA, respectively. EA and CA for amphotericin B were 100% for all *Candida* species. EA and CA of echinocandins and azoles for *C. albicans* and *C.* non-*albicans* are shown in Figures 1 and 2, respectively. All *C. albicans* isolates demonstrated 100% CA between YO10 and M-AM. For *C.* non-*albicans* isolates, results showed no resistance to anidulafungin with YO10 *vs*. 13,3% with M-AM. We found no resistance to micafungin using either YO10 or M-AM. Concerning

azoles, 4% of isolates were reported as resistant (R) to fluconazole with YO10 *vs.* 8% with M-AM. For posaconazole, 18,7% of isolates were tested R with YO10 *vs.* 0% with M-AM. YO10 and M-AM did not show resistance to voriconazole. While YO10 allows for nearly fully automated testing of nine antifungals with one isolate per plate, M-AM is manual, requires additional preparation steps and allows testing of six antifungals for two isolates per plate. M-AM required longer incubation (36 hours) of 13,5% of plates due to a negative growth control after 24 hours, not observed with YO10.

Conclusion:

Our study showed perfect agreement between YO10 and M-AM for AFST of *C. albicans*, while the concordance was lower for *C.* non-*albicans* species. These discrepancies were likely due to the different interpretation criteria used by CLSI and EUCAST, and differences in the raw MICs values obtained by the two methods. Therefore, the AFST method should be carefully selected, considering the results might impact the choice of antifungals for non-*albicans* candidaemia treatment.

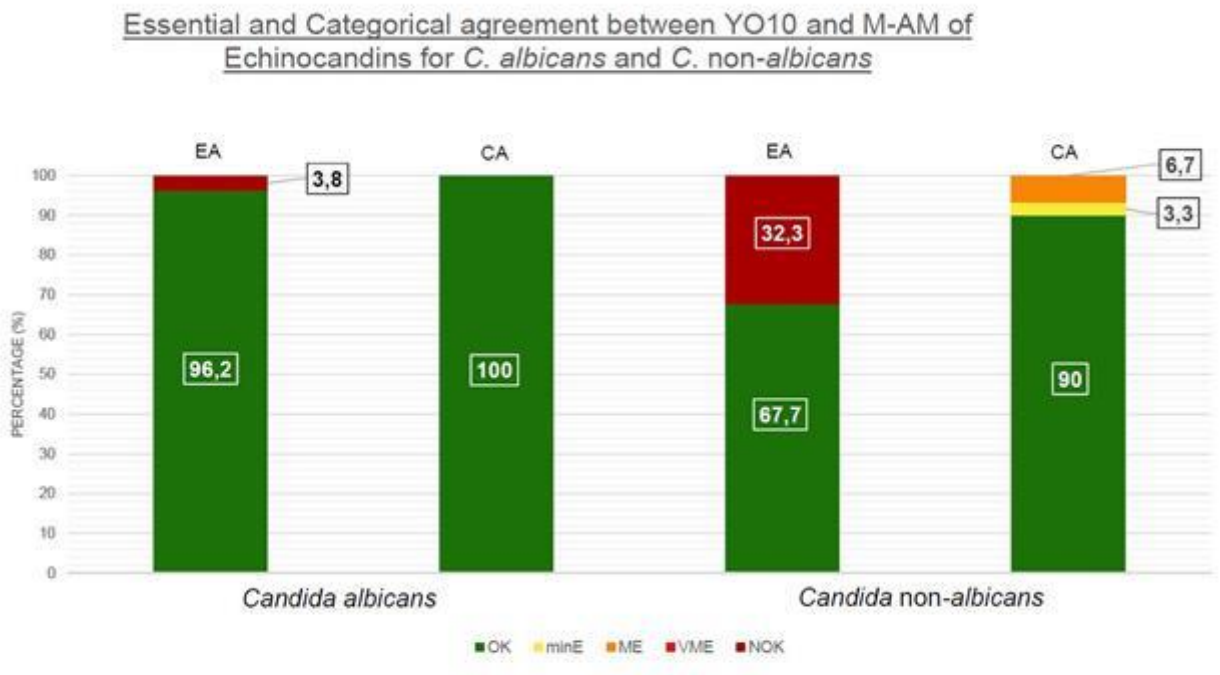

Figure 1 : EA and CA between YO10 and M-AM of echinocandins (anidulafungin and micafungin) for *C. albicans* and *C. non-albicans*
Very major error (VME) was defined as situation where an isolate was classified resistant (R) with YO10 and susceptible (S) with M-AM. Major error (ME): isolate classified S with YO10 and R with M-AM. Minor error (minE): isolate classified intermediate (I) with one method and S or R with the other.

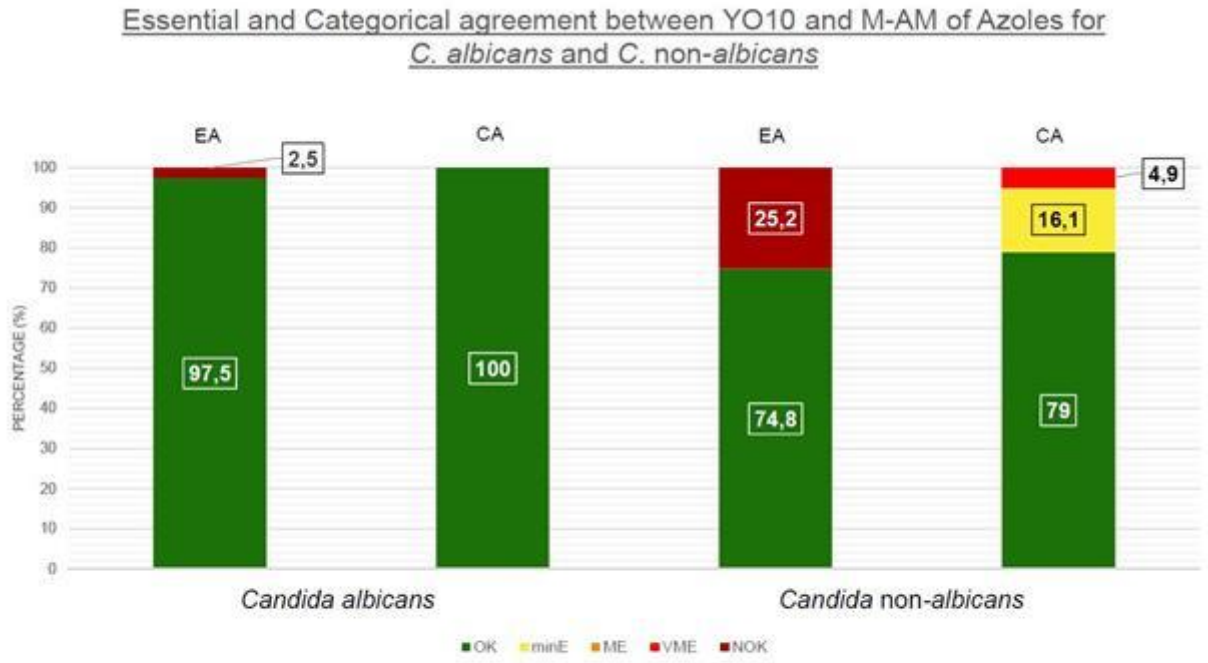

Figure 2: EA and CA between YO10 and M-AM of azoles (fluconazole, posaconazole and voriconazole) for *C. albicans* and *C. non-albicans*
Very major error (VME) was defined as situation where an isolate was classified resistant (R) with YO10 and susceptible (S) with M-AM. Major error (ME): isolate classified S with YO10 and R with M-AM. Minor error (minE): isolate classified intermediate (I) with one method and S or R with the other.

P055

Antifungal susceptibility testing using an agar-based screening method for Trichophyton spp.

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Objectives:
Resistance in *Trichophyton* spp. has emerged as a global public health issue. The current high recovery rate of terbinafine (TRB) non-wild type (WT) *T. indotineae* strains (formerly identified as *T. mentagrophytes* ITS genotype VIII) from Greek residents raises concerns (Siopi et al. *JoF* 2021). While a broth microdilution method for susceptibility testing of microconidia-forming dermatophytes has been standarized by the EUCAST (E.Def 11.0), its implementation in laboratory routine is hampered by its labor-intensive nature. Siopi et al. (TIMM 2021) have developed and validated an agar-based screening method for susceptibility testing of *Trichophyton* spp. We therefore used the aforementioned screening assay and investigated the *in vitro* susceptibility patterns of *Trichophyton* spp. recovered from patients attending the largest Dermatology Greek hospital (“Andreas Sygros”) over the past two years.

Materials & Methods:
A total of 136 clinical isolates of *Trichophyton* spp., whereof 93 *T. rubrum*, 20 *T. tonsurans*, 9 *T. mentagrophytes*, 12 *T. interdigitale* and 2 *T. indotineae*,, which were recovered from skin scrapings (*n*=31; 23%) and nail clippings (*n*=105; 77%) from individual patients attending our hospital during the period 2021-2022, were tested. All isolates were identified to the genus and species level by standard phenotypic methods based on their colonial and microscopic morphology as well as using two different MALDI-TOF MS platforms (Microflex LT platform, Bruker Daltonics, Bremen, Germany and Autof ms1000, Autobio, Zhengzhou, China). A 0.5 McF conidial suspension prepared in sterile water with 0.1% Tween 20 was inoculated onto RPMI (+2% glucose+50 mg/L chloramphenicol+300 mg/L cycloheximide) agar plates containing either no antifungal or one of the antifungals at a previously found selective concentration, i.e. 0.125 mg/L TRB and 1 mg/L itraconazole (ITZ). Plates were incubated for 5 days at 30°C and fungal susceptibility was evaluated using the absence of visual growth on the surface of the drug-containing agar as an endpoint.

Results:
Overall, no cross-resistance to TRB and ITZ was observed. 6/93 (6%) and 2/93 (2%) *T. rubrum* isolates recovered from nail clippings grew to agar plates containing TRB and ITZ, respectively. Regarding *T. tonsurans*, 4/20 (20%) and 2/20 (10%) strains isolated from skin scrapings appeared to have non WT phenotypes to TRB and ITZ, respectively. All *T. interdigitale* strains appeared to be TRB-WT, whereas 1/12 (8%) isolated from skin scrapings seemed to be ITZ-non WT. Concerning *T. mentagrophytes*, 2/9 (22%) and 1/9 (11%) isolates recovered from skin scrapings seemed to be TRB- and ITZ-non WT, respectively. Lastly, 2/2 *T. indotineae* strains, both recovered from skin scrapings appeared to exhibit

reduced susceptibility only to TRB. Regardless the species, 14/136 (10%) and 6/136 (4%) isolates tested seemed to have non WT phenotypes to TRB and ITZ, respectively.

Conclusions:

High incidence (10%) of Greek *Trichophyton* spp. clinical isolates with TRB non WT appearance was recorded.This finding underscores the consideration for performance of antifungal susceptibility testing at a local scale, particularly in non-responding to empirical treatment *Trichophyton*-induced infections.

P056

Azole resistance and cyp51A gene mutations in clinical and environmental isolates of Aspergillus fumigatus from Pakistan

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Objectives:
Azole resistance in clinical and environmental *Aspergillus fumigatus* isolates has been reported globally. Azole resistance in *A. fumigatus* has not been reported so far from Pakistan. We determined voriconazole, posaconazole and itraconazole minimum inhibitory concentrations (MICs) in clinical and environmental *A. fumigatus* strains and sequenced *cyp51A* gene to determine the presence of mutations conferring resistance to azoles.

Materials & Methods:
This study was conducted at the Aga Khan University Laboratory, Karachi, Pakistan. Clinical isolates were identified from both hospitalized and non-hospitalized patients from November 2021-June 2022. Environmental isolates were recovered from 135 soil samples collected from across Pakistan during the study period. MICs were performed and interpreted for posaconazole, voriconazole and itraconazole by broth microdilution (BMD) using CLSI standards. *cyp51A* gene was amplified and sequenced to observe presence of mutations known to confer resistance to azoles. Sequences were compared to the reference sequences available in GenBank (accession number AF338659) using sequence analysis software BioEdit.

Results:
A total of 147 clinical and 28 environmental *A. fumigatus* strains were obtained during the study period. Among the clinical strains, 1 isolate was resistant (MIC= 2 µg/mL) and 17 (11.5%) were intermediate (MIC= 1 µg/mL) to voriconazole. 14 (9.5%) isolates had non-wild-type MICs for posaconazole, and all isolates had wild-type MICs for itraconazole. Of the environmental isolates, all were sensitive to voriconazole, 3 (12%) had non-wild-type MICs to posaconazole and all had wild-type MICs for itraconazole. All isolates with higher MICs to azoles were retested. Patient with the voriconazole-resistant isolate had received voriconazole prophylaxis and succumbed despite receiving antifungal treatment. The *cyp51A* gene was amplified and sequenced for 18 clinical and 9 environmental isolates including those resistant or intermediate to voriconazole. No mutations known to confer resistance to azoles (TR/L98H, G54W, M220I and F219C) were detected in these strains.

Conclusions:
We report here, for the first time, *A. fumigatus* strains with voriconazole MIC of 2 µg/mL from Pakistan. This finding is of concern, considering the limited capacity of antifungal susceptibility testing and limited antifungal options in Pakistan. Continued genetic surveillance of mutations known to confer resistance to azoles is needed to monitor emergence of resistant strains of *A. fumigatus*.

P058

Functional Genomics Reveal Determinants of Amphotericin B Resistance in Candida auris

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Objectives

Candida auris is an emerging multidrug-resistant human fungal pathogen, often refractory to treatment by all classes of antifungal drugs. Amphotericin B (AmB) is a fungicidal drug that, despite its toxic side effects, remains a drug of choice for the treatment of drug-resistant fungal infections, including those caused by *C. auris*. However, the molecular mechanisms underlying AmB resistance are poorly understood. Therefore, the objective of this study is to understand the mechanism(s) of AmB resistance in *C. auris*.

Methods and Materials

To determine the plausible cause(s) of increased AmB resistance, we performed RNA-seq analysis of logarithmically growing AmB resistant isolates in comparison to AmB susceptible isolates in the Yeast Extract Peptone Dextrose broth medium (YPD; 1% yeast extract, 2% peptone, and 2% dextrose) at 37°C. Four independent AmB-resistant (AmB^R) and a two AmB susceptible (AmB^S) isolate belonging to the South Asian clade were used in this study.

Results

For RNA-seq experiments we used 4 independent AmB^R isolates in comparison to 2 susceptible *C. auris* isolates. Prior to RNA isolation, all strains were grown to logarithmic growth phase in YPD broth at 37^0C. From the RNA-seq analysis, we found 751 DEGs common to all AmB^R isolates vs two distinct sensitive isolates. Importantly, gene expression pattern varies very little between the two unrelated AmB^S isolates of distinct patient origin. These data strongly suggest that AmB resistance may be regulated by a set of “core” genes across distinct clinical *C. auris* strains in all clades. We therefore hypothesize that the conserved set of core genes can help in prediction of Multi Drug Resistance (MDR) signatures in clinical drug resistant isolates. The RNA-seq data identified several genes, including those involved in lipid and ergosterol biosynthesis, adhesion, drug transport, chromatin remodeling, and MAP kinase signaling, that were differentially expressed in AmB^R *C. auris* isolates. The transcriptomics data correlates well with increased adhesion and reduced lipid membrane permeability of AmB-resistant strains compared to the sensitive isolates. From the RNA-seq experiments, we have identified *SSK1,* a two-component response regulator and *HOG1* MAP kinase, to be highly expressed in AmB^R *C. auris* strains. The deletion of *SSK1* and *HOG1* restores susceptibility to AmB in AmB^R strains. Our data also show that genetic removal of *SSK1* and *HOG1* affects fungal fitness *in vitro*. This may at least in part arise from increased susceptibility to killing by macrophages. Additionally, we noticed an increased phosphorylation of Mkc1 cell integrity MAP kinase upon AmB

treatment. Thus, based on these data, we hypothesize that altered membrane lipids or differential expression of MAPK signaling pathway genes may contribute to AmB resistance in *C. auris*.

Conclusions

Collectively, these data identify differences in the transcriptional landscapes of AmB-resistant vs AmB-senstive isolates and provide a framework for the mechanistic understanding of AmB resistance in *C. auris*. Our study also reveals that changes in membrane lipid permeability is one of the major mechanisms for AmB^R in *C. auris*.

P059

Comparative evaluation of Sensititre YeastOne and CLSI reference method for antifungal susceptibility testing of Candida auris

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Objectives: *Candida auris* is an emerging and frequently multidrug-resistant fungal pathogen rendering antifungal susceptibility testing (AFST) crucial for the choice of the optimal treatment. Sensititre YeastOne (SYO) is a widely used commercial colorimetric assay for the AFST of yeasts due to its ready-to-use nature and its high concordance with the reference CLSI broth microdilution (BMD) methodology. Nevertheless, it has not yet been validated for *C. auris* AFST. We therefore evaluated the SYO performance for *C. auris* AFST compared to the CLSI BMD method using an international panel of isolates.

Materials/methods: A total of 100 genetically distinct clinical strains belonging to all 5 *C. auris* clades and being isolated from various geographical regions, namely 47 strains from clade I (Brazil, Greece, India, Kuwait, Oman, Pakistan), 3 strains from clade II (Japan, South Korea), 23 strains from clade III (South Africa, Spain), 22 strains from clade IV (Venezuela, Colombia) and 5 strains from clade V (Iran), were tested (de Groot *mBio* 2020). The CLSI and SYO AFST was performed according to the M27A4 protocol guidelines and the manufacturer's recommendations using the YO10 panel, respetively. The CLSI and SYO MICs were evaluated by two blinded observers (discordances were arbitrated by a third reader), while the SYO interday reproducibility was assessed by retesting 20 isolates on different days. The generated MIC data sets were compared quantitatively (paired t-test) for calculating the CLSI-SYO essential agreement (EA) within ±1/±2 twofold dilutions and qualitatively for determining the methods' categorical agreement (CA) following the CDC's fluconazole (≥32 mg/L), amphotericin B (≥2 mg/L) and echinocandins (≥4 mg/L for anidulafungin, ≥2 mg/L for caspofungin and ≥4 mg/L for micafungin) tentative MIC breakpoints for *C. auris*. Major errors (MaEs) and very major errors (VmEs) were considered in case of false resistance (CLSI susceptibility-SYO resistance) and false susceptibility (CLSI resistance-SYO susceptibility), respectively.

Results: The CLSI and SYO MIC distributions of all 9 antifungals are shown in **Figure**. The ±1 twofold dilution interobserver agreement was excellent (100%) for SYO, whereas the absolute/±1 twofold dilution interexperimental agreement between replicates of the SYO color endpoint was 63%/94%. Overall, the CLSI-SYO EA within ±1/±2 twofold dilutions was poor for posaconazole (13%/24%), fluconazole (27%/85%) and amphotericin B (36%/94%), moderate for voriconazole (48%/74%), itraconazole (63%/77%) as well as echinocandins (43-55%/70-86%) and good for flucytosine (80%/91%). All isolates were interpreted as CLSI amphotericin B-susceptible, whereas 70/100 strains were SYO AMB-resistant (30% CA, 70% MaE). On the contrary, significant interpretation discrepancies were not recorded for fluconazole (96% CA,

1% VmE, 4% MaE) and echinocandins (99% CA, 1% MaE for both anidulafungin and micafungin), except caspofungin (87% CA, 1% VmE, 12% MaE).

Conclusions: SYO could accurately exclude or predict fluconazole, anidulafungin and micafungin resistance among *C. auris* isolates. Nevertheless, it overestimated amphotericin B and caspofungin resistance underscoring that the respective SYO MICs need to be interpreted with caution.

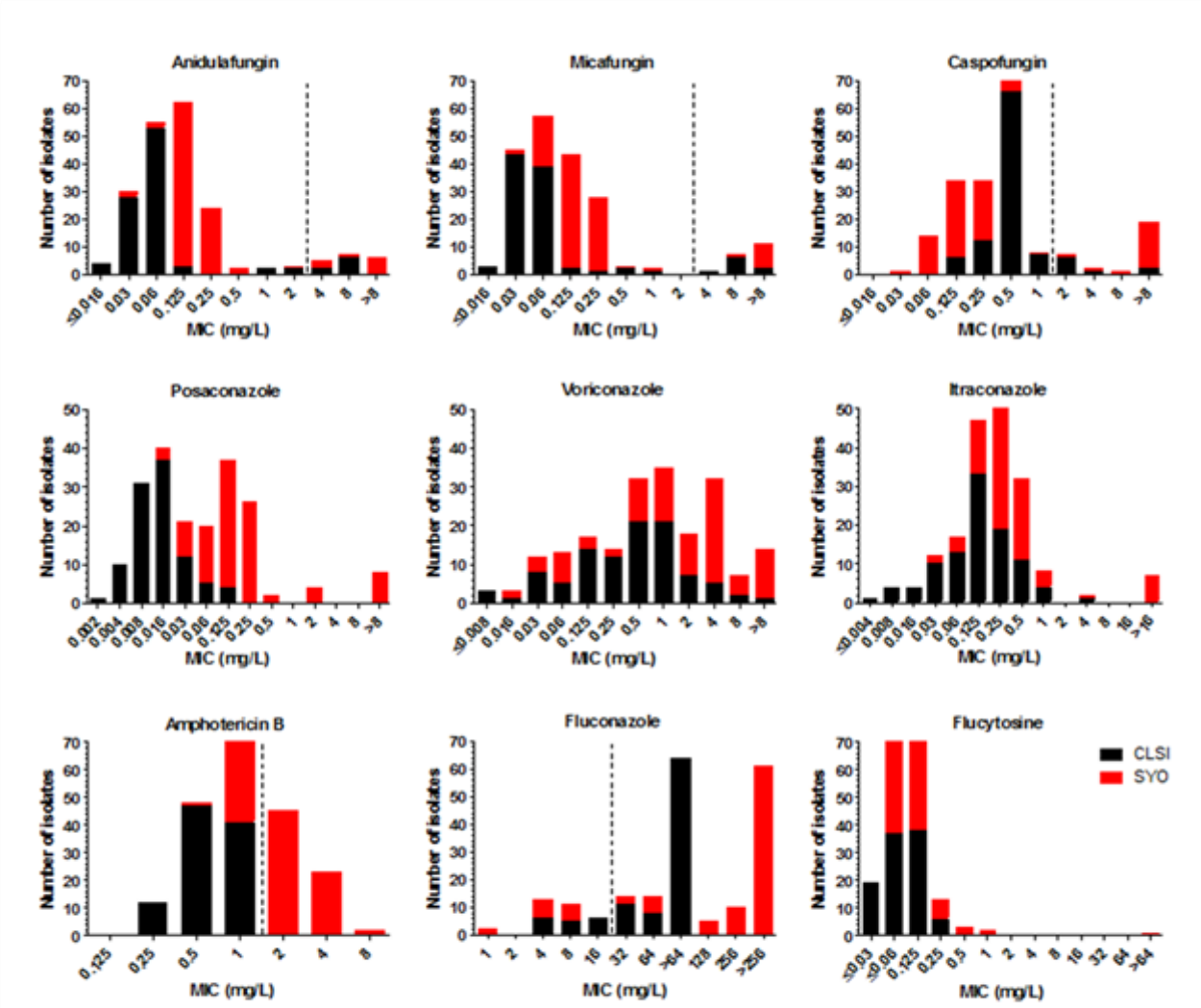

Figure. CLSI and Sensititre YeastOne (SYO) MIC distributions of *C. auris* isolates. The broken lines indicate the CDC's tentative breakpoints for *C. auris* (where available).

P060

CRISPR-Cas9 mutagenesis for deciphering the role in azole-resistance of new amino-acid substitutions in Candida albicans Erg11, Tac1 and Mrr2 .

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Objectives:

Candida albicans is the predominant yeast species isolated in invasive infections of patients with risk factors such as immunodeficiencies, recipients of solid organ transplantation or major abdominal surgery. Invasive candidiasis is mainly treated with azoles, and the emergence of azole-resistant strains poses a threat to successful treatment and recovery of patients. The development of resistance to certain antifungals has been linked to point mutations in the sequence of some enzymes, efflux pumps and transcription factors. However, there are still clinical isolates whose resistance is not due to any of the mutations known so far. In this work, we searched for previously undescribed amino acid changes in Erg11, Tac1, Upc2, Mrr1 and Mrr2 proteins of azole-resistant *C. albicans* clinical isolates, and evaluated the possible implication of the newly found mutations in azole resistance by CRISPR-Cas9 gene-editing.

Materials & Methods:

We determined the antifungal susceptibility of 43 *C. albicans* clinical isolates by CLSI M27-A3 and Sensititre YeastOne methods, and CRISPR-Cas9 derived strains were assayed by dot-spot . *ERG11*, *TAC1*, *UPC2*, *MRR1* and *MRR2* genes were sequenced and the expression of the *CDR1*, *CDR2* and *MDR1* genes was measured by RT-qPCR. CRISPR-Cas9 gene-editing was used to introduce new amino acid substitutions into the azole-susceptible *C. albicans* SC5314 genetic background.

Results:

Nineteen out of 43 *C. albicans* isolates showed reduced susceptibility to one or more azolic compounds; eleven isolates overexpressed either *CDR1*, *CDR2*, and/or *MDR1*, but not all of them harboured GOF mutations in their respective Tac1, Mrr1, and Mrr2 transcription factors. Conversely, 7 resistant isolates carried amino acid substitutions in Erg11 related to azole resistance. We identified three new mutations, in homozygosis, that were only present in azole-resistant isolates, and that could be associated with their reduced susceptibility (Erg11 Y477C, Tac1 S758F, and Mrr2 A311V). In order to unveil their role empirically, we introduced separately these 3 new mutations into the *C. albicans* SC5314 genome using the CRISPR-Cas9 gene-editing tool. Afterwards, we measured *CDR1* and *CDR2* gene expression levels for

the Tac1 and Mrr2 mutants, but we did not observe changes in expression. On the other hand, antifungal susceptibility tests confirmed that none of the newly identified mutations were associated with azole resistance in the SC5314 genetic background.

Conclusions:

In summary, we identified three mutations not previously reported; however, none of them was found to be associated with azole resistance on a stand-alone basis in a reference strain. Nevertheless, since resistance can be a cumulative effect of several processes in the same organism, an alternative approach would be testing whether the reversion of the mutated amino acid in the respective resistant isolate is accompanied by an increase in azole sensitivity. For this purpose, the CRISPR-Cas9 editing tool would also be useful.

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P062

National survey on azole-resistant clinical Aspergillus fumigatus collected in 2022: TR34-L98H substitutions are dominating an expanding resistance across Spain

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Objectives: We recently showed a 7% rate of azole resistance in *Aspergillus fumigatus* complex isolates collected in 2019 in 29 hospitals located in Spanish cities. We here report the burden of azole resistance in *A. fumigatus* complex isolates collected in 2022 and also expanded the number of participating hospitals to 84 centres.

Methods and Materials: A total of 84 participating hospitals, covering all Spanish regions, stored and identified *Aspergillus* spp clinical isolates – regardless their clinical significance – collected from the 1st of February to the 31st of March 2022. Antifungal susceptibility to amphotericin B and azoles was performed according to EUCAST 9.4 methodology. Here we report data from the *A. fumigatus* complex isolates collected (n=1,249) collected from 81 hospitals. Resistant isolates were molecularly identified and the c*yp51A* gene sequenced in azole-resistant *A. fumigatus sensu stricto*.

Results: Isolates distributed as fully antifungal susceptible (n=1,165, 93.3%) or resistant to one or more antifungal agent (n=84, 6.7%) that distributed among *A. fumigatus sensu stricto*, n=51, *A. lentulus*, n=20, *A. fumigatiaffinis*, n=4, *A. novofumigatus*, n=3, *Neosartorya udagawae*, n=2, and *A. clavatus*, *A. felis*, *N. udagawae* + *A. felis*, and *N. thermomutatus* + *A. felis*, one isolate each. Overall, the amphotericin B resistance rate was 2.4%, only found in cryptic species, whereas the azole resistance rate was 6.4%. Resistance to one or more azole was found in 4.2% and 87.8% of *A. fumigatus sensu stricto* and cryptic species isolates, respectively. Individual rates of resistance to itraconazole, posaconazole, isavuconazole, or voriconazole against *A. fumigatus* complex, *A. fumigatus sensu stricto*, and cryptic species are shown in Figure 1. Azole-resistant *A. fumigatus sensu stricto* isolates harboured the TR_{34}-L98H substitutions (n=40/51), other *cyp51A* gene substitutions (F46Y/M172V/N248T/D255E/E427K, n=4; G54R+G448S, n= 3; N248K, n=2; G54E, n=1), or were wild type *cyp51A* gene sequence (n=1). Isolates harbouring the TR_{34}-L98H substitutions resulted in a phenotype of pan-azole resistance except two isolates that were voriconazole-susceptible and harboured two additional *cyp51A* substitutions (S297T and F491I). Isolates harbouring the TR_{34}-L98H

substitutions were detected in patients admitted to 17 hospitals located in six Spanish regions. The presence of such isolates was already reported in five regions (Madrid, Catalonia, Castilla León, Valencia and Andalusia) in the previous survey whereas a new region (Aragón) is here added to the list of Spanish regions where isolates harbouring the TR_{34}-L98H substitutions can be detected (Figure 2).

Conclusion: Our study showed that 6.4% of *A. fumigatus* complex Spanish isolates collected in 2022 were azole resistant, proving that azole resistance has not decreased since the last survey conducted in 2019. Resistance was dominated by the presence of the TR_{34}-L98H *cyp51A* gene substitutions; the detection of such isolates was confirmed in some regions (especially in Barcelona and Madrid) and a new region is now affected.

	MIC distributions (number of isolates at each MIC, in mg/L)											Resistance	
	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥ 16	No. of isolates	%
***A. fumigatus sensu lato* (n=1,249)**													
Amphotericin B	0	1	6	19	433	668	92	**11**	**10**	6	3	30	2.4
Itraconazole	1	4	4	137	719	308	12	5	5	**1**	**53**	64	5.1
Voriconazole	0	0	0	22	172	769	210	30	39	6	**1**	76	6.1
Posaconazole	7	190	741	238	23	**42**	5	0	0	0	3	58	4.6
Isavuconazole	0	0	0	1	20	705	437	38	**11**	32	5	68	5.4
***A. fumigatus sensu stricto* (n=1,216)**													
Amphotericin B	0	1	5	19	432	667	92	0	0	0	0	0	0
Itraconazole	1	4	4	136	714	299	9	0	1	0	**48**	49	4
Voriconazole	0	0	0	22	172	768	207	**11**	29	6	**1**	47	3.9
Posaconazole	7	190	739	219	15	39	4	0	0	0	3	49	4
Isavuconazole	0	0	0	1	20	703	426	21	8	32	5	48	4
Cryptic species (n=33)													
Amphotericin B	0	0	1	0	1	1	0	**11**	**10**	6	3	30	90.9
Itraconazole	0	0	0	1	5	9	3	5	4	**1**	5	15	45.5
Voriconazole	0	0	0	0	0	1	3	**19**	**10**	0	0	29	87.8
Posaconazole	0	0	2	19	8	3	**1**	0	0	0	0	9	27.3
Isavuconazole	0	0	0	0	0	2	11	17	3	0	0	20	60.1

Values shaded in grey indicate MICs within the area of technical uncertainty (ATU) translated as resistant isolates as follows: posaconazole (*A. fumigatus sensu lato* [n=8/23]; *A. fumigatus sensu stricto* [n=3/15]; cryptic species [n=5/8]) and isavuconazole (*A. fumigatus sensu lato* [n=20/38]; *A. fumigatus sensu stricto* [n=3/21]; cryptic species [n=17/17]). Values in bold indicate resistant isolates according to EUCAST clinical breakpoints (table v 10.0)


Hospitals in which isolates harbouring the TR34 -98H substitutions were detected

P063

Discrepancies in susceptibility testing of Candida auris with the Vitek2 system using a representative international panel of clinical isolates

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Objectives: *Candida auris* is an emerging and frequently multidrug-resistant fungal pathogen emphasizing the need for accurate and widely accessible antifungal susceptibility testing (AFST) to optimize treatment decisions. Vitek2 is a broadly used commercial system for the AFST of *Candida* spp. However, data on its performance for *C. auris* AFST are currently scarce, show contrary results and are based on evaluations testing a limited number of isolates recovered from restricted geographic areas (Ceballos-Garzon *Int J Antimicrob Agents* 2022, Known *J Clin Microbiol.* 2019). We therefore assessed the Vitek2 performance for *C. auris* AFST compared to the reference CLSI broth microdilution method using an international collection of isolates.

Materials/methods: A total of 100 genetically distinct clinical strains belonging to all 5 *C. auris* clades were included (de Groot *mBio* 2020). The CLSI and Vitek2 AFST was performed according to the M27A4 protocol guidelines and the manufacturer's recommendations using the AST-YS08 card (bioMérieux, Athens, Greece), respetively. As the Vitek2 system does not provide MICs for *C. auris*, the species was changed to *C. glabrata* in order to retrieve the MIC for each *C. auris* isolate. The CLSI MICs were evaluated by two blinded observers (discordances were arbitrated by a third reader), while the Vitek2 interday reproducibility was assessed by retesting 20 isolates on different days. The generated MIC data sets were compared quantitatively (paired t-test) for calculating the CLSI-Vitek2 essential agreement (EA) within ±1/±2 twofold dilutions and qualitatively for determining the methods' categorical agreement (CA) following the CDC's fluconazole (≥32 mg/L), amphotericin B (≥2 mg/L) and echinocandins (≥4 mg/L for anidulafungin, ≥2 mg/L for caspofungin and ≥4 mg/L for micafungin) tentative breakpoints for *C. auris*. Major errors (MaEs) and very major errors (VmEs) were considered in case of false resistance (CLSI susceptibility-Vitek2 resistance) and false susceptibility (CLSI resistance-Vitek2 susceptibility), respectively.

Results: The CLSI and Vitek2 MIC distributions of the 6 used antifungals are shown in **Figure**. The absolute/±1 twofold dilution interexperimental agreement of the Vitek2 MICs was 77%/92%. Overall, the CLSI-Vitek2 EA within ±1/±2 twofold dilutions was poor for flucytosine (3%/40%), fluconazole (16%/78%) and amphotericin B (29%/40%), with a median difference of 3, -2 and 3 twofold dilutions, respectively, and the MICs obtained by the two methods being significantly different (p<0.0001). On the other hand, the EA between the methods was moderate for voriconazole (63%/78%), micafungin (65%/97%) and caspofungin (79%/93%), with a median difference of 0, 1 and 1 twofold dilutions, respectively. Significant interpretation discrepancies were recorded for amphotericin B (31% CA, 69% MaE) and fluconazole (69%

CA, 31% VmE), but not for echinocandins (99% CA, 1% MaE for both micafungin and caspofungin).

Conclusions: The Vitek2 MICs correlated with CLSI MICs for echinocandins allowing correct categorization of all echinocandin-resistant *C. auris* isolates. MIC correlation was poor for flucytosine, fluconazole and amphotericin B with 31% VmE and 69% MaE for the last two drugs, respectively, indicating that the Vitek2 needs further optimization before being used to guide therapeutic decisions.

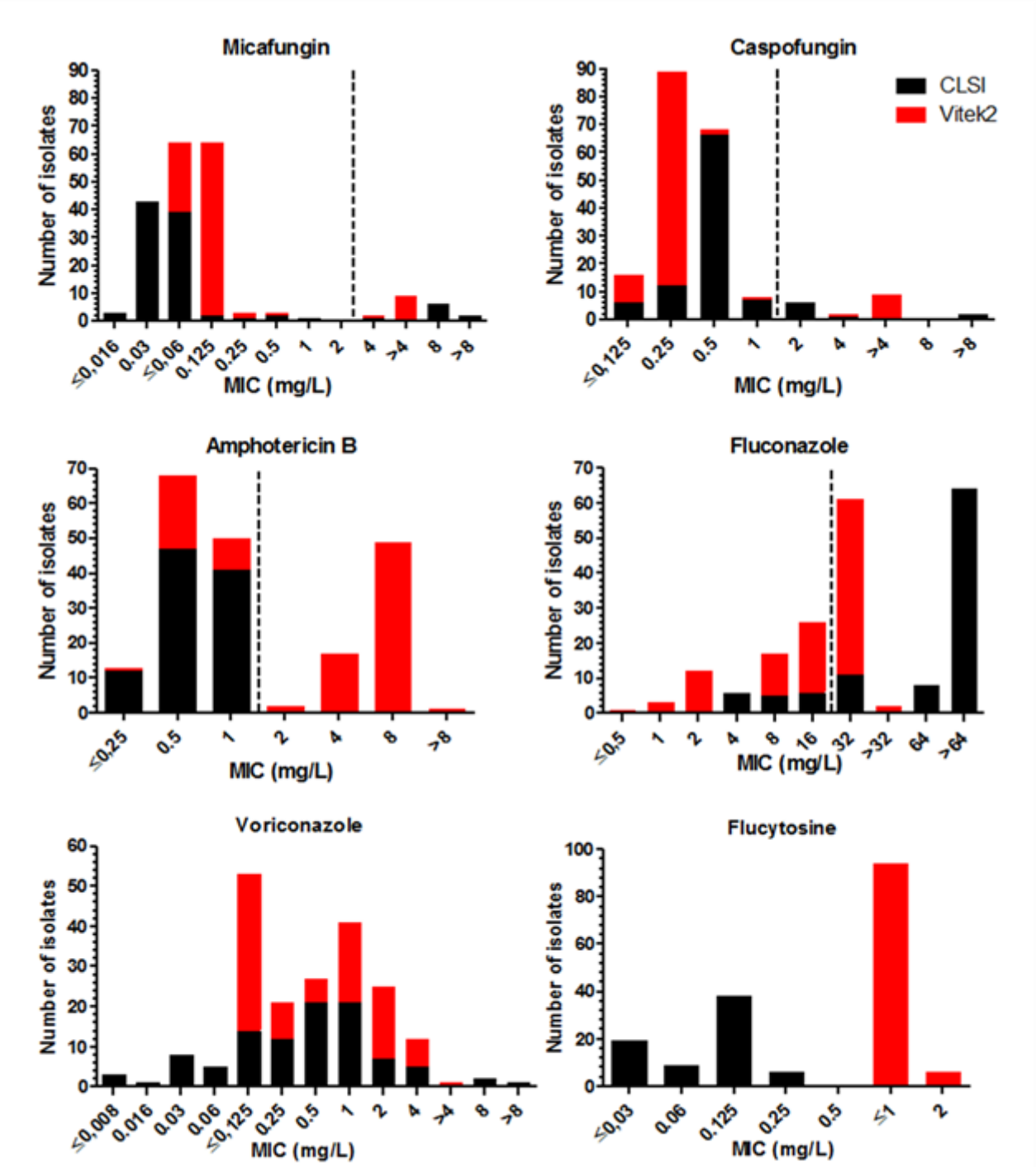

Figure. CLSI and Vitek2 MIC distributions of *C. auris* isolates. The broken lines indicate the CDC's tentative breakpoints for *C. auris* (where available).

P065

Terbinafine resistance in Tricophyton species from patients with recalcitrant infections detected by the EUCAST method and DermaGenius® Resistance PCR

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Objectives:
The first-line treatment for *Trichophyton* infections of the skin and nails is terbinafine. In the last few years terbinafine resistance in *Trichophyton* spp. is increasingly reported in several parts of the world. The aim of this study was to describe the prevalence of terbinafine resistance in *Trichophyton* isolates from patients with terbinafine treatment failure, by the EUCAST reference method and DermaGenius® Resistance PCR.

Materials & Methods:
Prospective study of the prevalence of resistant *Trichophyton* isolates in clinical skin- and nail samples submitted with the indication terbinafine treatment failure. Samples were submitted from general practitioners, dermatology specialists and hospitals in the Central Denmark Region in the period 01-12-2020 until 31-12-2021. The isolates were initially cultured on Sabouraud glucose agar (Oxoid) and Sabouraud dextrose agar with actidione and chloramphenicol (Oxoid). For *T. indotineae* final identification was obtained by sequencing. Antifungal susceptibility testing for terbinafine and itraconazole was performed according to the EUCAST E.Def 11.0 guideline. Interpretation was done according to the tentative ECOFFs set by EUCAST. DermaGenius® Resistance PCR was performed according to the manufacturer's instructions. The PCR detects the 5 most frequent mutations in the squalene epoxidase (SQLE) target gene.

Results:
33 isolates from 32 patients (10 females and 22 males) were included in the study. One patient had two isolates from different locations. Of the 33 received isolates, 15(45.5%) were from toenails, 7(21.2%) from foot skin samples and 11(33.3%) were skin samples from the body. The samples were submitted from general practitioners (n=16, 48.5%), dermatology specialist practice (n=13, 39.4%), a dermatology department (n=3, 9.1%) and a private hospital (n=1, 3.0%).

The species distribution of the isolates was as follows: *T. rubrum*: 27(81.8%), *T. interdigitale*: 2(6.1%), *T. mentagrophytes*: 1(3.0%), and *T. indotineae*: 3(9.1%).

Susceptibility testing by the EUCAST method was possible for 31/33 isolates. Resistance PCR was performed for 32/33 isolates. Of the 31 isolates tested by the EUCAST method, terbinafine resistance was detected in 16(51.6%) isolates. Resistance mutations were detected by PCR in 11(34.4%) of 32 isolates. All of the 11 isolates with detected mutations by PCR, also displayed terbinafine resistance in the EUCAST susceptibility testing. For the 2 samples, that did not grow in the EUCAST medium, no mutations were detected in the resistance PCR. Four isolates had resistance detected by the EUCAST method, but not by PCR. Apart from the 3 *T. indotineae* isolates, terbinafine resistance was detected in 13(48.1%) of the 27 *T. rubrum* isolates. All isolates were susceptible for itraconazole.

Conclusions:

In our diagnostic mycology laboratory, we found terbinafine resistance in >50 % of *Trichophyton* isolates from skin- and nail samples from patients with recalcitrant infections. *T. rubrum* was the most common species among the resistant isolates (81.25%).

DermaGenius® Resistance PCR was found to be reliable for the initial screening of terbinafine resistance and makes it possible for the clinician to initiate appropriate therapy in an early point, awaiting the EUCAST susceptibility testing.

Our results strongly support, that antifungal susceptibility testing is indicated in case of terbinafine treatment failure.

Table 1.xlsx (could not be inserted)

P066

Fungicidal activities of amphotericin B and AmBisome against Candida auris

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Objectives: *Candida auris* is rapidly emerging as a global public health challenge, and a multi-drug (MDR) resistant pathogen. Amphotericin B is one of the options for treating *C. auris* infections. However, isolates with high amphotericin B MIC values have been reported whereas there are no data on fungicidal activity. Therefore, we assessed the *in vitro* fungicidal activity of amphotericin B deoxycholate (d-AMB) and its liposomal formulation (L-AMB) against *C. auris* isolates from different clades.

Materials & Methods: A total of 69 clinical strains representing the four major *C. auris* clades (Clade I, n=29; Clade II, n=3; Clade III, n=19; Clade IV, n=18) isolated from various geographical locations, were tested. Susceptibility testing was performed according to CLSI M27A4 guidelines for both d-AMB (Sigma-Aldrich, Greece) and L-AMB (AmBisome, Gilead Sciences, Greece). Minimal fungicidal concentrations (MFCs) were obtained following MIC determination (inoculum size, 10^4 CFU/mL), by subculturing the entire volume of all clear wells, onto drug free Sabouraud dextrose agar (SDA) plates. All plates were incubated at 37^0C for 48h. The MFC was the lowest concentration producing ≥ 99.9% killing of the initial inoculum. Strong fungicidal activity was considered when MFC/MIC ratio was ≤4.

Results: Table 1 summarizes the *in vitro* susceptibilities and fungicidal activities of the two different formulations of amphotericin B against the 69 *C. auris* isolates. L-AmB was highly active against *C. auris* and comparable to d-AMB. All *C. auris* isolates were interpreted as amphotericin B - susceptible according to CDC's tentative susceptibility breakpoint (≥ 2 mg/L), irrespective of their origin or clade type. D-AMB MFC ranges were 1- >4 mg/L for Clade I and IV, 0.5-1 mg/L for Clade II and 1-2 mg/L for Clade III, while for AmBisome, MFC ranges were 0.5-8 mg/L for Clade I, 0.25-0.5 mg/L for Clade II and 0.5-1 mg/L for Clade III and IV. Strong fungicidal activity was found against all strains of Clade II and III and only 79% and 78% against

Clade I and IV *C. auris* isolates for d-AMB. In comparison, strong fungicidal activity was found against >94% for all Clades for AmBisome.

Conclusions: All *C. auris* isolates showed a rather uniform susceptibility pattern to d-AMB and AmBisome, within CDC's tentative breakpoint range. Strong fungicidal activity was found for both compounds against most strains except ~20% of isolates from Clade I and IV with d-AMB. Whether this *in vitro* differential activity is correlated with *in vivo* activity, remains to be further investigated.

Clade	no of isolates	amphotericin B-deoxycholate (d-AMB) [mg/L]			AmBisome (L-AMB) [mg/L]		
		MIC (mg/L)	MFC (mg/L)	MFC/MIC ratio[b]	MIC (mg/L)	MFC (mg/L)	MFC/MIC ratio[b]
Clade I	29	1(0.5-1)	4(1- > 4)	4(1-8) (79)	1(0.125-2)	2(0.5-8)	4(1-8) (100)
Clade II	3	0.25(0.25-0.5)	1(0.5-1)	4(1-4) (100)	0.125(0.125)	0.5(0.25-0.5)	4(2-4) (100)
Clade III	19	0.5(0.25-0.5)	1(1-2)	4(1-4) (100)	0.25(0.125-0.25)	0.5(0.5-1)	4(4) (100)
Clade IV	18	0.5(0.5-1)	4(1- > 4)	4(2-16) (78)	0.25(0.125-0.25)	1(0.5-1)	4(2-8) (94)

[b] numbers in brackets represent percentage of isolates with MFC/MIC ratio ≤4 indicating fungicidal activity

P067

Thorough Characterization of the Emerging Pathogen Trichophyton indotineae: Resistance Profile and MALDI-TOF MS Identification

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Objectives: The emerging pathogen, *Trichophyton indotineae* is known to cause severe dermatophytoses such as *tinea corporis* and *tinea cruris*. Often resistant to terbinafine (TBF), it is plaguing India and its neighboring countries and is swiftly spreading to other parts of the world. In order to prevent further increase of this species and to achieve successful treatment, correct identification and rapid antifungal susceptibility testing for TBF and/or other antifungal agents is necessary. In this research, MALDI-TOF MS was tested as a quick and accurate tool for identification of *T. indotineae*, as it can be challenging to distinguish it from closely related species such as *T. mentagrophytes* and *T. interdigitale* using only morphological characteristics. A thorough resistance profile of *T. indotineae* was set up to facilitate treatment of mycoses caused by this pathogen.

Materials & Methods: MALDI-TOF MS was performed on 20 *T. indotineae* strains to determine its strength as an identification tool for this species and its capacity to spot TBF resistance. Once correctly identified, a specific screening medium, consisting of Sabouraud agar and TBF, was tested as an indication tool forTBF resistance. The obtained results were confirmed by antifungal susceptibility testing (EUCAST) on all *T. indotineae* strains for TBF and eight other antifungal drugs: fluconazole, itraconazole, voriconazole, ketoconazole, griseofulvin, ciclopirox olamine, naftifine and amorolfine. DNA sequencing was performed on the squalene epoxidase-ecoding gene to examine which mutations were present.

Results: Hundred percent of *T. indotineae* strains (n = 20) was correctly and reliably identified on species level via MALDI-TOF MS using the BCCM/IHEM in-house database as reference, while this percentage was 95% for the online available MSI V2.0 database. Nevertheless, MALDI-TOF MS could not be used successfully to detect if the strains were resistant or susceptible to TBF. Using the screening medium, 45% of the strains was determined resistant to TBF after four days of incubation, which was confirmed by antifungal susceptibility testing following the EUCAST protocol. The TBF resistant strains showed elevated minimal inhibitory concentration (MIC) values for naftifine and amorolfine as well. DNA sequencing of the squalene epoxidase-encoding gene showed that the TBF resistance was a consequence of missense point mutations, which led to amino acid substitutions F397L and L393F.

Conclusions: In conclusion, this research demonstrated the strength of MALDI-TOF MS as a reliable tool for the identification of *T. indotineae strains*, as it is able to distinguish them from their closely related species when conventional methods sometimes fall short. Nevertheless, a distinction between TBF resistant and susceptible strains could not be made by this spectrometric technology. Additionally, the screening medium was able to determine TBF resistance in *T. indotineae* strains within four days, which can be considered as a rapid and easy alternative for broth microdilution antifungal susceptibility testing. Significantly elevated MIC-values were observed in terbinafine resistant *T. indotineae isolates* for naftifine and amorolfine, which could be an indication of (future) cross-resistance.

P068

Can fluconazole susceptibility be used as a surrogate marker for second generation triazole susceptibility for Candida auris?

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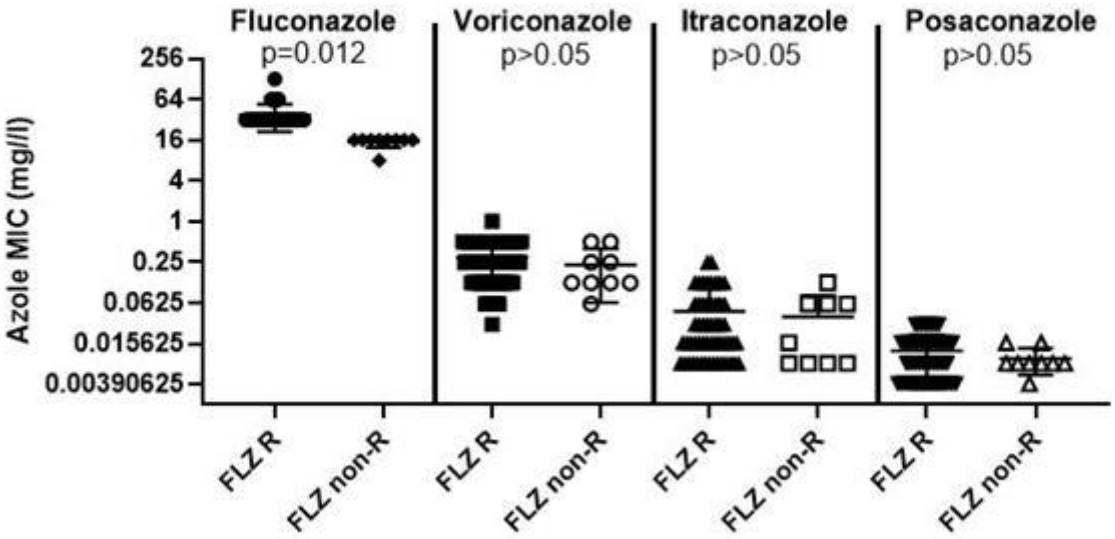
Objectives: *Candida auris* is an emerging and frequently multidrug-resistant fungal pathogen, which is responsible for life-threatening invasive infections and nosocomial outbreaks worldwide. Given its high prevalence and regional pattern of resistance, antifungal susceptibility testing (AFST) is crucial in the guidance of therapeutic decisions. Currently the only available breakpoints are those proposed by CDC based on CLSI reference broth microdilution method for fluconazole (FLZ), amphotericin B and echinocandins whereas for other azoles FLZ is suggested a surrogate marker of susceptibility. However, MIC correlation and cross resistance studies between FLZ and other azoles are lacking. We therefore, correlated FLZ MICs with the MICs of other azoles and compare the azoles MIC between FLZ susceptible and resistant isolates.
Material/Methods: A total of sixty-four *C. auris* mainly bloodstream clinical isolates, were collected from individual patients who were hospitalized in a Greek tertiary care academic reference hospital from December of 2021 till January 2023. CLSI AFST was performed, according to M27A4 protocol, using grade pure drug powders at concentrations of FLZ (64-0.06 mg/l), voriconazole (VRZ) (8-0.008 mg/l), posaconazole (PCZ) (16-0.016 mg/l) and itraconazole (ITZ) (8-0.008 mg/l). The microtiter plates were incubated at 35±2 ^{o}C, and the MICs were defined as the 50% of growth inhibition compared to the growth control after 24h. The recommended *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 strains were used as quality control. As tentative BP of resistance, MIC of ≥32 mg/l was used according to CDC guidelines. Fluconazole MICs were correlated with other azoles MICs with Pearson correlation after log2 transformation. Furthermore, VRZ, ITZ and PSZ MICs between FLZ non-resistant (<32 mg/l) and resistant (≥32 mg/l) isolates were compared with ANOVA.
Results: The median (range) MIC of FLZ were 32 (8-≥64) mg/l, of VRZ 0.25 (0.03-1) mg/l, ITZ 0.016 (≤0.008-0.25) mg/l and PCZ 0.008 (≤0.004-0.3) mg/l. Based on FLZ susceptibility, 14% (9/64) were susceptible with FLZ MICs 8-16 mg/l. Although FLZ MICs were statistically significantly correlated with VRZ, ITZ and PSZ MICs, the correlation was weak (r=0.338 and p=0.006 for VRZ, r=0.336 and p=0.007 for ITZ, r=0.280 and p=0.025 for PSZ). No statistically significant differences (p>0.05) were found between FLZ resistant with FLZ non-resistant isolates for VRZ [median (range) MIC 0.25(0.03-1) vs 0.125(0.06-0.50) mg/l], ITZ (0.016(≤0.008-0.25) vs 0.016(≤0.008-0.125) mg/l] and PSZ (0.008(≤0.004-0.03) vs 0.008(≤0.004-0.016) mg/l] (Figure).
Conclusions: Although, CDC suggest the consideration of using fluconazole susceptibility as a surrogate marker for second-generation triazole susceptibility assessment, we observed that FLZ MICs were poorly correlated with MICs of other azoles and no significant differences were found of other azoles MICs between FLZ resistant and FLZ non-resistant isolates. Thus, FLZ resistance may not predict resistance to other azoles and isolates that are resistant to fluconazole may respond to other triazoles. Further pharmacodynamic and clinical studies are needed to explore the clinical significance of this finding.

Keywords: *Candida auris*, CLSI, Susceptibility testing

Words: 464/500

Figure. Comparison of VRZ, ITZ and PCZ MICs between FLZ resistant and FLZ non-resistant *C. auris* isolates.



P070

Comparison of synergistic interactions between posaconazole and caspofungin against azole-resistant isolates of Aspergillus fumigatus using CLSI and EUCAST methodologies

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Objectives: The opportunistic fungal pathogen *Aspergillus fumigatus* has been linked to multiple life-threatening infections in immunocompromised patients. Despite the fact that azoles are the cornerstone of antifungal therapy, treating aspergillosis is a challenging endeavor that is made even more challenging by the lack of therapeutic efficacy caused by multi-azole resistance in *A. fumigatus.* Combination therapy of an azole with an echinocandins can be used to combat those infections. *In vitro* combinations are usually assessed with broth microdilution checkerboard methods. There are two reference methodologies for antifungal susceptibility testing of *Aspergillus* spp., the CLSI and the EUCAST reference methods. We therefore tested the *in vitro* combination of posaconazole (POS) and caspofungin (CAS) against twenty wild-type and resistant *A. fumigatus* isolates with different resistance mechanisms, using both CLSI and EUCAST methodologies, in order to find any method depended differences.

Material/Methods: Eighteen clinical isolates of *A. fumigatus* were chosen based on their azole-resistance mechanisms carrying TR34/L98H (n=3), M220 (n=5) and G54 (n=3) known cyp51a substitutions, as well as wild type (WT) (n=3) and isogenic isolates (n=4). Antifungal susceptibility testing was carried out using both the CLSI M38-A2 method and EUCAST E. DEF 9.4. The drug combination experiment was carried out using an 8x12 checkerboard design. The concentrations of POS and CAS ranging between 8-0.002 mg/l and 4-0.06 mg/l, respectively. Following a 48-hour incubation period, fungal growth was measured by spectrophotometry using the modified XTT method. The FICi was calculated for 10% (FICi-0), 25% (FICi-1) or 50% (FICi-2) growth endpoints. Synergy and antagonism were concluded when the $\log_2$FICi of the three independent replicates were lower than 0.5 and higher than 4, respectively. FICis were compared between EUCAST and CLSI datasets for all different growth endpoints, as well as for all different group of isolates.

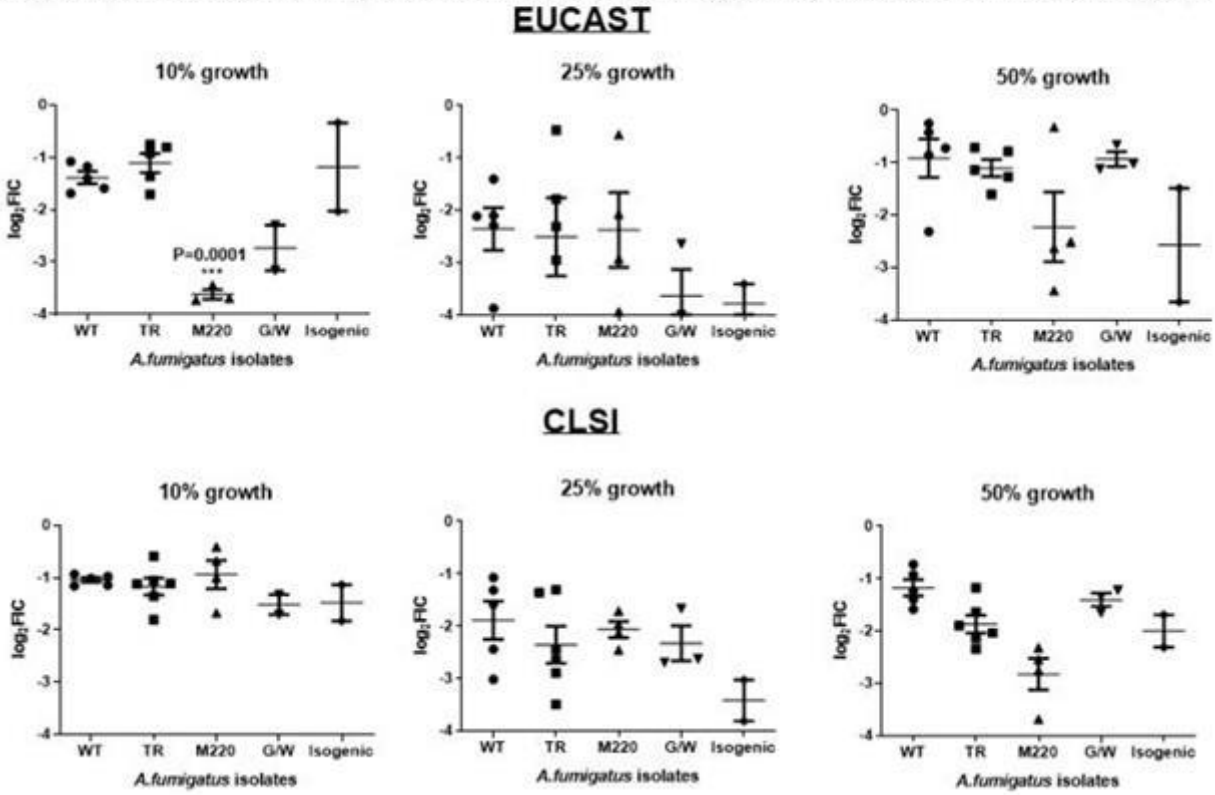
Results: FICi were significantly lower than 0.5 for 78%, 89% and 56% for FICi-0, FICi-1, and FICi-2 growth endpoints, respectively for EUCAST method, indicating synergy ($P<0.05$). For CLSI method, FICis were lower than 0.5 for 67%, 100% and 89% for FICi-0, FICi-1, and FICi-2 growth endpoints, respectively. For all three groups of isolates, FICi-1 had the lowest FICi, which ranged from 0.06 to 0.76 for CLSI and 0.05 to 0.25 for EUCAST. Comparing FICis significant differences between EUCAST and CLSI methodologies were observed at the 10% growth endpoint ($P=0.0001$), with isolates harboring M220 mutations (**Figure 1**). However, FICi-0 but not FICi-1 and FICi-2 were statistically significant different between the two methodologies with EUCAST resulting in lower FICi-1s indicating stronger synergy.

Conclusions: The combination of POS+CAS was synergistic against *A. fumigatus* isolates with both the CLSI and the EUCAST reference methodologies although CLSI detected synergy for more isolates particularly at 25% and 50% growth endpoints. EUCAST found more and stronger synergistic interactions at 10% growth inhibition endpoint particularly for isolates harboring M220 mutations. Synergism is probably due to residual activity of POS in these isolates.

Keywords: Caspofungin, Posaconazole, *Aspergillus fumigatus*, checkerboard, CLSI, EUCAST

Words: 474/500

Figure 1. EUCAST and CLSI $FICi_{min}$s determined at 10%, 25%, and 50% growth for each set of azole susceptible isolates with no mutations in the cyp51A gene (WT) and azole-resistant isolates with TR34/L98H (TR), M220 (M220), G substitutions (G/W), and no mutation (isogenic). Significant differences between EUCAST and CLSI methodologies (P=0.0001) were observed at the 10% growth endpoint, with isolates bearing the M220 mutations showing greater differences than the other groups.



P071

Socially important fungal infections: etiological structure, sensitivity to antifungal drugs.

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Objectives:

Most of the animals surrounding humans are a potential risk as a source of infection with dermatophytosis. The greatest epidemiological danger is posed by companion animals that have the longest and closest contacts with people. Farm animals, fur animals are the main reservoirs of dermatophyte fungi in rural areas.

The main purpose of the study is to determine the incidence of dermatomycosis in animals, to find its main etiological agents and to determine sensitivity to commonly used antifungal substances (drugs).

Materials & Methods:

The object of the study was samples of clinical material taken from domestic and farm animals (samples of wool, scabs, swabs from the ears and skin) with clinical signs of the disease or with suspicion of dermatophytosis.

To identify fungal elements in any material, a luminescent dye, calcofluor white, was used.

Isolation and identification of isolates was carried out by seeding on solid nutrient media: Sabouraud (M063, Sabourand dextrose agar, HiMedia) with the addition of chloramphenicol (FD033, Chloramphenicol Selective Supplement, HiMedia) and Sabouraud with the addition of a selective additive containing cyclohexemide (Dermasel Selective Supplement, Oxoid) , wort agar, Chapek-Doxa, potato-glucose agar.

At the final stage, the features of macro- and micromorphology were compared using the determinants of microscopic fungi (S. de Hoog et al. 2020, 4th edition).

The sensitivity to the three main antifungal substances (ketoconazole, terbinafie and enilconazole) was determined according to the standard, EUCAST e.def. 9.3.1.

The minimum inhibitory concentration (MIC) was taken as the drug concentration that limited the growth of the studied culture by at least 90%.

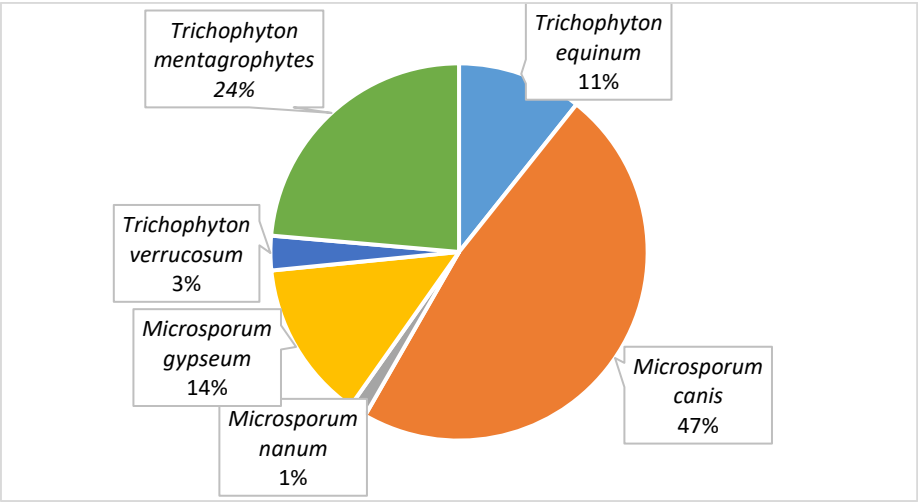
Results:

Sampling was carried out during 2020-2022 from veterinary laboratories and clinics in 27 regions of the Russian Federation.

A total of 851 samples were studied, 298 dermatophyte isolates were isolated, sensitivity to antifungal drugs was determined for more than 100 isolates of dermatophytosis pathogens.

Microscopic fungi belonging to dermatophytes *(*genus *Microsporum and Trichophyton) were isolated from 32.64% of the obtained samples and identified as Microsporum canis (M. c.), Microsporum gypseum (M. g), Microsporum nanum (M. n.), Trichophyton verrucosum (T . v.), Trichophyton mentagrophytes (T. m.), Trichophyton equinum (T. eq).* The percentage and number of the main types of dermatophytes isolated from different regions are shown in the diagram 1.

Diagram 1



Sensitivity was determined by the method of serial dilutions to three antifungal drugs - ketoconazole, enilconazole and terbinafine. The choice of drugs was dictated by information about antifungal drugs registered in the Russian Federation for veterinary use.

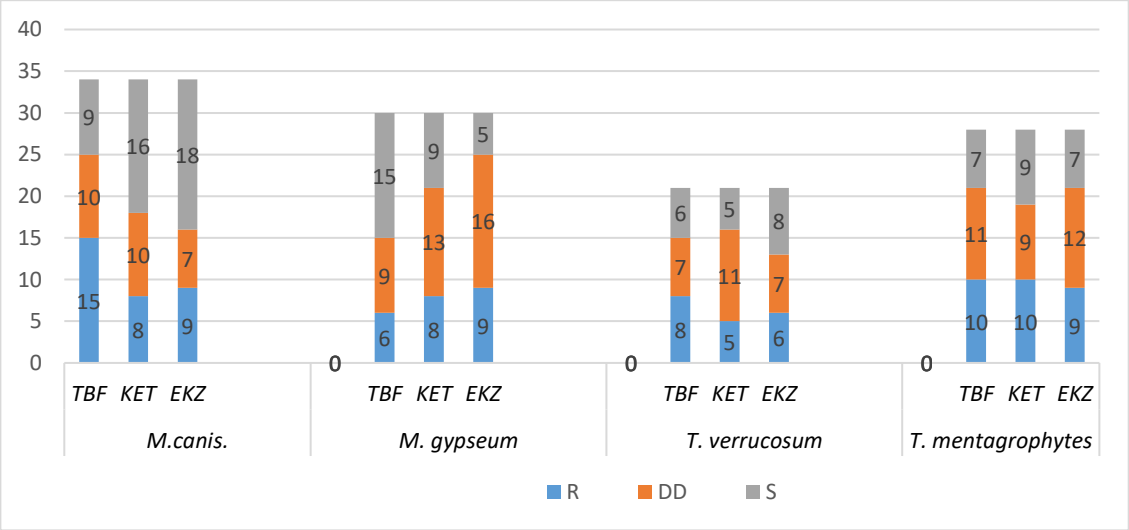
Thiabendazole, enilconazole, miconazole and ketoconazole belong to the same group of drugs, have a similar mechanism of action, which is to inactivate one of the enzymes of the late stage of ergosterol synthesis (lanosterol-14α-demethylase). For this reason, only enilconazole and ketoconazole were used in the present study.

One group of drugs - allylamines - includes terbinafine and naftifine. They have a similar mechanism of action common to the drugs of this group, which is to inactivate squalene epoxidase, an enzyme in the early stage of ergosterol synthesis. For this reason, we limited ourselves to terbinafine in this study.

The number of isolates of *M. canis, M. gypseum, T. verrucosum, T. mentagrophytes* resistant to terbinafine, ketoconazole and enilconazole (imazilil) are shown in diagram 2

Diagram 2

Number of dermatophytes isolates resistant to terbinafine, ketoconazole and enilconazole.



R – resistance, DD – dose dependent, S – sensitive.

Conclusions:

The proportion of *Microsporum canis* isolates resistant to terbinafine reached 44%, to ketoconazole - 24%, to enilconazole - 26%. The proportion of *Microsporum gypseum* isolates resistant to terbinafine reached 20%, to ketoconazole - 30%, to enilconazole - 27%. The proportion of *Trichophyton verrucosum* isolates resistant to terbinafine reached 38%, to ketoconazole - 19%, to

enilconazole - 29%. The proportion of *Trichophyton mentagrophytes* isolates resistant to terbinafine reached 26%, to ketoconazole - 36%, to enilconazole - 32%.

The results of our work clearly demonstrate an increase in the number of resistant isolates of pathogens of "classic" dermatophytosis to antifungal drugs, and the risk of spreading such pathogens has been assessed. The research was supported by RSF (project No. 22-26-00206)

P072

Molecular identification and antifungal susceptibility of clinical and agricultural Fusarium isolates from Korea

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Objectives:
Fusarium is an ubiquitous hyaline mold, some species are known as an important plant pathogen. In human, *Fusarium* causes mostly superficial infections however it can induce fatal invasive fungal infection in immunocompromised host. Antifungal resistance of *Fusarium* varies inter-species but also intra-species, which makes treatment difficult. The aim of this study was to analyse antifungal susceptibility and genotypic characteristics of plant and human *Fusarium* isolates from Korea.

Materials & Methods:
In this study, we focused on *Fusarium solani* species complex (FSSC) and *Fusarium fujikuroi* species complex (FFSC). We collected clinical isolates from patients with culture-proven *Fusarium* infection in a tertiary hospital in Korea. Plant isolates were obtained from the Korean Agricultural Culture Collection of the National Institue of Agricultural Sciences of Korea. Molecular identification of isolates to species level was performed by polymerase chain reaction (PCR) of the internal transcribed spacer (ITS), translation elongation facter 1-alpha (TEF1-α) and benA . We measured the minimum inhibitory concentration (MIC) of the isolates through broth dilution method. Phylogenetic tree was constructed based on ITS of FSSC and FFSC isolates.

Results:
We selected 13 clinical isolates (FSSC n=8; FFSC n=5) and 12 plant isolates (FSSC n=8; FFSC n=4). As echinocandins have no activity to *Fusarium*, we focused on the antifungal susceptibility of voriconazole, posaconazole, isavuconazole and amphotericin B. In FSSC, the MIC_{50} for voriconazole, posaconazole, isavuconazole and amphotericin B were 16 μg/mL, ≥64 μg/mL, 32 μg/mL and 4 μg/mL for clinical isolates and 16 μg/mL, ≥64 μg/mL, 64 μg/mL and 2 μg/mL for plant isolates respectively. For FFSC, MIC_{50} for voriconazole, posaconazole, isavuconazole and amphotericin B were 8 μg/mL, ≥64 μg/mL, 16 μg/mL and 2 μg/mL for clinical isolates and 2 μg/mL, 0.5 μg/mL, 4 μg/mL and 2 μg/mL for plant isolates respectively. Through phylogenetic analysis, FFSC showed monophyletic traits according their subspecies, regardless of source of isolates. However FSSC displayed polyphyletic characteritics, with a tendency to congregate according to origin of isolates.

Conclusions:
Comparison between antifungal agents demonstrate higher MIC in posaconazole and isavuconazole compared to voriconazole and amphotericin B. There was no common trend in antifungal resistance intra-species, inter-species or between clinical and environmental isolates. Through phylogenetic tree, we observed FFSC isolates forming distinct clade according to its subspecies *verticillioides* or *proliferatum*, regardless of sources of isolates. On the other hand, FSSC were dispersed, showing polyphylic traits. Phylogenetic analysis of *F. solani* show a tendency to grouping according clinical or plant isolates. This genotypic distribution could be interpreted by host-associated gene evolution, but also by different

subspecies of *F. solani*. Genotypic identification of *Fusarium* at subspecies level and identification of virulence genes inducing antifungal resistance through molecular approach are needed in future studies.

Table 1. Antifungal susceptibilities of 25 *Fusarium* isolates.

	MIC (μg/mL)					
	clinical isolates			plant isolates		
	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range
Fusarium solani species complex (FSSC)						
voriconazole	16	16	8 ~ 16	16	64	8 ~ 64
posaconazole	≥64	≥64	32 ~ ≥64	≥64	≥64	≥64
isavuconazole	32	64	8 ~ ≥64	64	≥64	16 ~ ≥64
amphotericin B	4	4	1 ~ 32	2	2	1 ~ 2
Fusarium fujikuroi species complex (FFSC)						
voriconazole	8	≥64	2 ~ ≥64	2	4	1 ~ 4
posaconazole	≥64	≥64	0.5 ~ ≥64	0.5	≥64	0.5 ~ ≥64
isavuconazole	16	64	2 ~ 64	4	16	2 ~ 16
amphotericin B	2	4	1 ~ 4	2	4	1 ~ 4

Abbreviations: MIC, minimum inhibitory concentration.

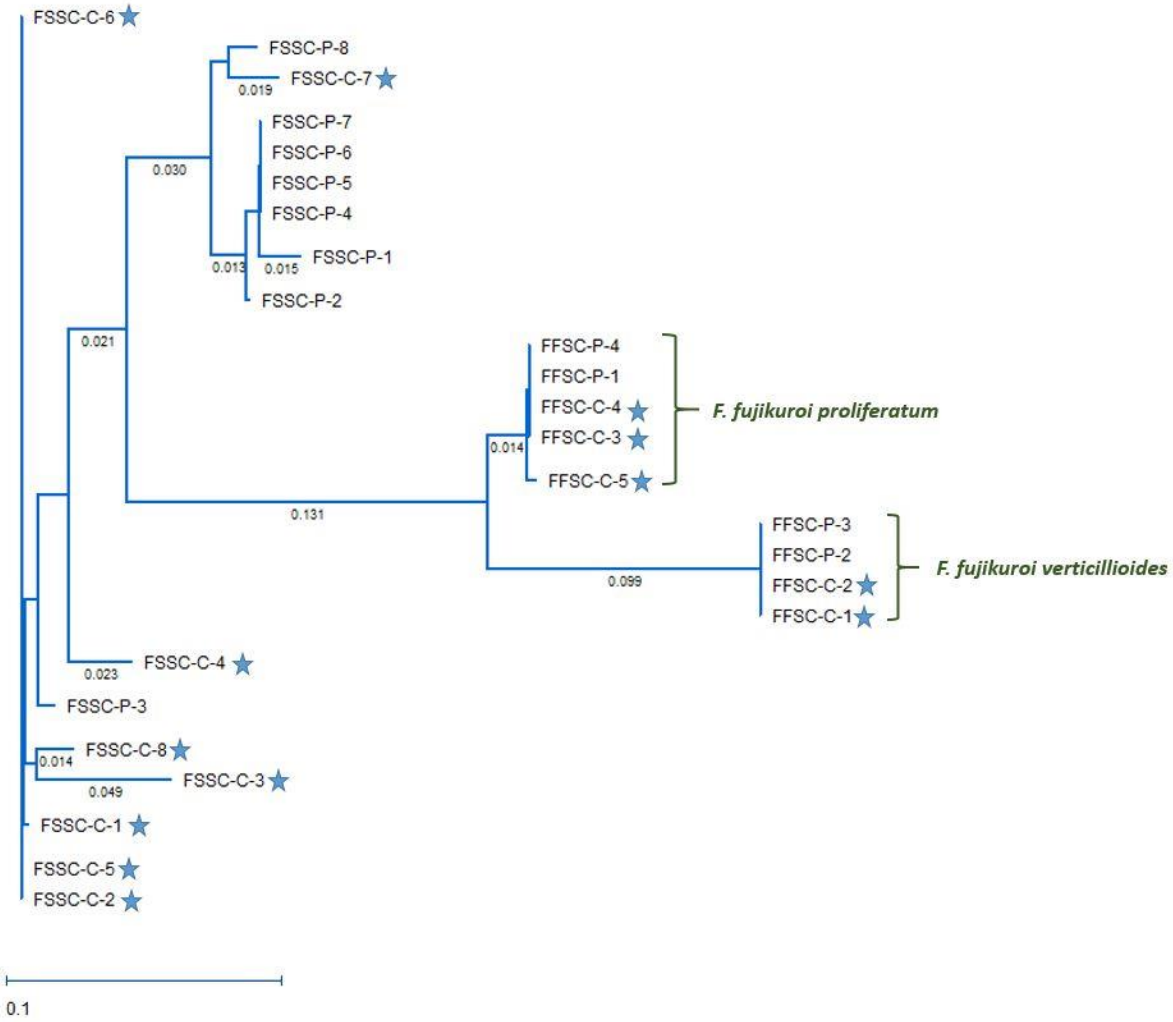

Figure 1. Maximum-likelihood phylogenetic tree created from ITS sequences of 25 *Fusarium* isolates. Species and source of isolates (FFSC, F. fujikuroi species complex; FSSC, F. solani species complex; C, clinical; P, plant) are described as isolates code number. Clinical isolates were marked with a star for visualization.

P073

First study of susceptibility to antifungals of Aspergillus spp isolates from human cases in Uruguay.

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Objectives:
To know the susceptibility to antifungals in clinical isolates of *Aspergillus spp* in Uruguay.
Materials & Methods:
A study of the susceptibility to antifungals of *Aspergillus spp* isolates from the strain collection of the Mycology Laboratory of the Department of Parasitology and Mycology of the Faculty of Medicine, University of the Republic, was carried out. Isolates obtained between July 31, 2018 and July 31, 2023, from clinical cases of aspergillosis were included. Once the strains are included in the collection, they are assigned a correlative number which is not associated with identifying data of the people they come from.
The isolates were initially identified by conventional methods such as the micromorphological study from the isolates in *Sabouraud* agar at 28 ◦C. Identification at the species level was performed by mass spectrometry (MALDITOF) from isolates on glucose *Sabouraud* agar incubated at 28 ◦C for 7 days. The spectrometer was calibrated using *Escherichia coli* protein extract, recommended by the manufacturer (Bruker Bacterial Test Standard). We consider a score ≥ 2 as valid for identification at the species level. The study of susceptibility to antifungals was carried out using a diffusion disk and the broth microdilution method, using CLSI documents M38-A38, M619, M574 and M59 for the interpretation of the results of the minimum inhibition concentration (MIC) for each isolate. The synergy test between terbinafine and the azoles tested by disk diffusion in agar was performed.
Results:
The laboratory receives an average of 250 respiratory samples annually, out of a total of between 400 and 500 total samples per year. During the period analyzed, we made a total of 39 diagnoses of aspergillosis, of which 25 were through respiratory samples and 14 through serological tests such as simple double diffusion on agar.
Of the respiratory samples, 15 were isolated from immunocompetent individuals and 10 from immunocompromised individuals (HIV positive, hemato-oncologic, systemic autoimmune disease). The most frequently isolated species was *Aspergillus fumigatus*, followed by *Aspergillus flavus* and *Aspergillus niger.* Susceptibility to antifungals was obtained by broth microdilution for Amphotericin B, voriconazole, itraconazole and terbinafine. The isolates presented MICs less than or equal to 1.0 for amphotericin B, less than or equal to 0.5 for voriconazole, and less than or equal to 1.0 for itraconazole, both for the agar disk diffusion assay and corroborated by microdilution in broth. Likewise, synergy was found in some strains between terbinafine and the azoles tested.
Conclusions:
The clinical isolates studied in the present study of *Aspergillus spp* from clinical cases in Uruguay do not show resistance to the antifungals tested. There is a demonstrated *in vitro* synergy between terbinafine and the azoels studied in the Uruguayan isolates of *Aspergillus spp.*

P075

Characterising fungi from diabetic foot ulcer

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Objectives:
A chronic wound such as diabetic foot ulcer (DFU) is associated with increased risk of bacterial infection and poor prognosis which could lead to amputation. However, much less is known about fungi as the endogenous microbiota and their association with chronic wound healing. The objective of this study is to characterise the fungal aetiology from DFU's with respect to their burden, and to further phenotypically characterise them.
Materials & Methods:
A total of 297 DFU swabs from different clinically defined grades and stages were assessed. Fungal culture from swabs was performed using Sabouraud dextrose agar supplemented with chloramphenicol, and CHROMagar™ for *Malassezia* species. Identification of yeast and mould isolates were carried out by MALDI-TOF and microscopic examination respectively. DNA was also extracted simultaneously from the swabs and the presence of fungi detected using qPCR of the ITS gene. Individual fungal isolates were then assessed for capacity to form biofilms and antifungal sensitivity profiles were performed against conventional antifungals for both planktonic and sessile forms.
Results:
Conventional culture from the microbiology yielded 7.89 % positivity from DFU swabs, whereas enhanced fungal culture showed 22.7% positivity. The predominant isolates of *Candida* species included *C. parapsilosis* (53.3 %), *C. glabrata* (20 %) and *C. albicans* (13.3 %). Molecular detection identified 29.6 % of the 22.7 % mycology culture showed. Notably, *C. parapsilosis* appeared in all three clinical stages of DFU infection. Crystal violet assay showed varying capacity of the isolates to form biofilms, which were also tolerant to conventional antifungals.
Conclusions:
Overall, this study strengthens the idea that fungi exist alongside bacteria as polymicrobial interkingdom populations in DFU's. This study has also raised important questions on possible clinical implication of wound healing associated with these biofilms in DFU. Together, these data support the notion that antifungal therapy should be considered for treatment of DFU infection.

P076

Influence of culture media in Candida parapsilosis in vitro biofilms

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Objectives: to evaluate the influence of four commonly used culture media on the growth, morphology, and antifungal susceptibility of *C. parapsilosis in vitro* biofilms.
Materials & Methods: RPMI glucose supplemented (RPMId), TSB, BHI, and YPD media were employed. Quantification of biomass using Crystal violet was performed to characterize the growth and antifungal susceptibility (Amphotericin B and Caspofungin) of *C. parapsilosis in vitro* biofilms grown on a silicon substrate in the four tested culture media. Additionally, morphological characterization of the biofilms was conducted under three different conditions (air-liquid interphase zone, bottom zone and continuous media flow) by confocal imaging. The length-to-wide ratio and area of cells were measured by ImageJ software.
Results: Culture media had an impact on *C. parapsilosis* growth and filamentation. It was found that biofilms formed in YPD medium (2% glucose availability) exhibited the highest biomass and lower pseudohyphae content (~3%). In contrast, pseudohyphal growth was particularly enhanced when grown with RPMId medium and in biofilm formed under harsh conditions (low oxygen availability and shear stress). Furthermore, the antifungal susceptibility of biofilms varied according to the culture media used for their formation, with TSB-formed biofilms being the most susceptible.
Conclusions: This study provides a comprehensive understanding of the impact of culture media on key biological characteristics of *C. parapsilosis*, emphasizing the importance of considering pathogen behavior under diverse conditions when designing research protocols and developing effective antifungal therapeutic strategies.

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P077

Transcriptional profiling of Candida auris biofilms following farnesol or tyrosol exposure

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Objectives: *Candida auris* is frequently associated with biofilm-related invasive infections. The resistant profile of these sessile communities necessitates innovative therapeutic options, where quorum-sensing may be a potential alternative target. Farnesol and tyrosol are two well-konwn fungal quorum-sensing molecules with antifungal effects especially at supraphysiological concentrations. This work revealed the molecular events of the response to farnesol or tyrosol in *C. auris* biofilm and demonstrated the transcriptome patterns associated with the observed effect produced by these two fungal quorum-sensing molecules.
Materials & Methods: To date there has been no high-throughput comparative molecular analysis regarding the background of farnesol- or tyrosol- related effects against *C. auris* biofilms. In this work, we performed genome-wide transcript profiling with *C. auris* sessile communities following 75 µM farnesol or 15 mM tyrosol exposure using total transcriptome sequencing (RNA-Seq). Beside of molecular examinations, metal content measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in biofilms. The metal contents of the samples were normalized by dry cell mass.
Results: The analysis highlighted that the number of up-regulated genes (a minimum 1.5-fold increase) was 686 and 138 for tyrosol and farnesol, respectively, while 662 and 199 genes were down-regulated (a minimum 1.5-fold decrease) for tyrosol and farnesol, respectively. The overlap between tyrosol- and farnesol-responsive genes was considerable (101 and 116 overlapping up-regulated and down-regulated genes, respectively). Genes involved in biofilm development-related events, glycolysis, ergosterol biosynthesis, fatty acid oxidation, iron metabolism, and autophagy were primarily influenced. Farnesol caused an 89.9%, 73.8%, and 32.6% decrease in the calcium, magnesium, and iron content, respectively, whereas tyrosol resulted an 82.6%, 76.6%, and 81.2% reduction in the calcium, magnesium, and iron content compared to the untreated control cells, respectively.
Conclusions: In summary, our data give a novel insight into the genome-wide transcriptome changes caused by farnesol and tyrosol exposure in the metal content of biofilms, metabolic regulation, and membrane-related alterations. However, further mutant-based *in vitro* and *in vivo* investigations are needed to fully understand the complete mechanism of these two quorum-sensing molecules in the *C. auris* sessile community.

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P078

Activity of cell-free culture supernatants of Lacticasebacillus casei against Candida albicans biofilms

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Mycoses caused by *Candida* are the most common and clinically relevant fungal infections. This is mainly due to biofilm production. In recent years, the use of probiotic bacteria such as *Lactiplantibacillus* has been proposed as an alternative treatment and the properties of cell-free culture supernatants have gained interest.
Objective: The aim of this study was to evaluate the effect of some compounds from the cell free supernatant of *Lacticaseibacillus casei* against *Candida* biofilm formation both during the initial adhesion phase and on the mature 24 h biofilm.
Materials & Methods: Two phenolic acids (gallic and ferulic acid), one vitamin (riboflavin) and the lactic acid were tested. Concentrations of 256 µg/mL, 512 µg/mL and 1024 µg/mL of each substance were analyzed against four biofilm-forming *Candida* isolates, including the reference strain of *Candida albicans* SC5314, two isolates of *C. albicans* (UPV 12-298 and UPV 15-157), and one of *Candida dubliniensis* (UPV 11-366). The supernatant of *Lacticaseibacillus casei* cultured in BHI medium (neutralized and filtered or unneutralized and unfiltered) was also tested against the *Candida* biofilms. During biofilm formation, the compounds and the supernatant were added to a final inoculum of 1×10^6 *Candida* cells/ml in microtiter plates. In addition, *Candida* biofilms preformed for 24 h were exposed to the supernatant and compounds. After 24 h incubation at 37 °C, metabolic activity (by reduction of XTT tetrazolium salt to formazan) and biomass (by 0.4% crystal violet) were measured at 492 nm and 600mn, respectively.
Results: Overall, the activity of both the compounds and *L. casei* supernatant differed depending on the phase of the biofilm formation. The supernatant acted mainly on adhesion both in terms of metabolic activity and biomass, achieving a 50% or more reduction of growth, while the activity against preformed biofilms was lower according to their biomass. The neutralized supernatant showed antifungal activity, demonstrating that the antifungal effect was not only related to the acidification of the medium. In the case of gallic acid and riboflavin at the highest concentration, they significantly reduced the adhesion and metabolic activity of mature biofilms. In addition, ferulic acid reduced the metabolic activity of preformed biofilms of *C. albicans* SC5314 at the highest concentration. Finally, lactic acid had no significant effect on *Candida* biofilms either on biomass or metabolic activity, at the concentrations tested.
Conclusion: The effect of *L. casei* supernatant, gallic acid and riboflavin could be studied as a promising alternative to the use of current antifungals agents to treat biofilm-associated infections, thus avoiding the use of high concentrations or recurrence and recalcitrance to classical treatment.

P079

Synergistic activity of amphotericin B with posaconazole against Trichosporon asahii biofilms

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Background and Objectives: *Trichosporon asahii* is an emerging opportunistic pathogen able to cause life-threatening disseminated trichosporonosis in immunocompromised patients, especially in patients with underlying hematological malignancies, extensive burns and solid tumors. A predisposing factor for infection is the presence of prosthetic medical devices which could become substrates for microbial adhesion and subsequent biofilm growth. *Trichosporon* species are able to form biofilms (BF), virulent structures with enhanced antimicrobial resistance, compromising antifungal therapy. Little is known about drug susceptibility profiles against *Trichosporon* biofilms. The objective of this study was to assess the *in vitro* antifungal activity of amphotericin B (AMB) and posaconazole (PSC) alone or in combination against *T. asahii* BF.

Materials & Methods: Clinical isolates of *T. asahii* (n=7) from blood, trauma, skin lesion and bronchial secretions of adult patients were grown in RPMI at 10^6 cfu/mL at 37℃ for 48h. BF formation was assessed in 96-well microtiter plates by 1% safranin staining and evaluated spectrophotometrically at 490 nm. Two-fold dilutions of AMB and PSC at a concentration range from 2 mg/L to 256 mg/L were incubated with 48h-BF for 24h (n=6). The combinational activity of AMB (0.125-64mg/L) with PSC (2-128mg/L) against 48h-BF at 37℃ for 24h was determined using an 8x12 checkerboard microdilution method (n=6). The MIC50 for BF was determined as the minimum concentration that caused ≥50% BF damage compared to controls. BF damage was assessed by the XTT reduction assay. Drug interactions were analyzed using Bliss independence model. The combination effect was defined as synergistic, antagonistic or indifferent when the observed BF damage was significantly higher, lower or equal to the expected damage, respectively.

Results: Four isolates were strong biofilm producers (SBF, OD: 0.9-1.6) while three isolates formed weak biofilms (WBF, OD: 0.2-0.6). SBF MIC50 of AMB and PSC, alone, was 4mg/L (median: 2-8 mg/L) and 128 mg/L (median: 32-256 mg/L), respectively, as compared to 0.03mg/L and 0.125 mg/L (median: 0.06-1) for WBF. Synergistic effects were observed for SBF at 0.125-1 mg/L of AMB combined with 4-32 mg/L of PSC: mean ΔE value of significant interactions: 24% (range: 16% to 31%) with mean SE: 3% (range: 1% to 6%). No antagonistic effects were exhibited. Indifferent interactions were observed when AMB was combined with PSC against WBF *T. asahii*.

Conclusions: *T. asahii* strong biofilm producers are resistant to each drug alone. However, the interaction of AMB with PSC makes both drugs to be effective at lower concentrations against mature biofilms. This finding may have important implications in the treatment of biofilm-related *Trichosporon* infections.

P080

Luliconazole shows a signifficant effect against planktonic growth and biofilm formation in Lomentospora prolificans and Scedosporium spp.

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Patients infected with rare opportunistic pathogenic moulds such as *Scedosporium* spp. and *Lomentospora prolificans* are becoming a more common sight in hospital settings. Resistance to most antifungal drugs and high mortality rates are the defining characteristics of these organisms. That is why developing alternative treatments has become crucial. In this study we present the *in vitro* and *in vivo* activity of the imidazole luliconazole (LLCZ) against both *Scedosporium apiospermum* (including its teleomorph: *Pseudallescheria boydii)* and *L. prolificans*.
37 isolates (*L. prolificans* (n=31), *Scedosporium apiospermum/ Pseudallescheria boydii* (n=6)) were tested in total to determine the minimum inhibitory concentration (MIC) of LLCZ, according to EUCAST. Furthermore, the *in vitro* antifungal activity of LLCZ was studied over 48 h through an XTT growth kinetics assay. The capability of LLCZ to inhibit fungal biofilms was tested via two separate assays (crystal violet and XTT assay). Biofilms in different developmental stages were treated with both sub-MIC and supra-MIC LLCZ concentrations. Furthermore, a *Galleria mellonella* infection model was used for *in vivo* treatment assays.
LLCZ showed a MIC_{90} of 0.25 µg/ml for all tested organisms. Planktonic growth was inhibited continuously between 6 and 48 h after incubation. This inhibitory effect was characterised as fungistatic and concentration dependant. LLCZ had the most significant effect against biofilm formation in pre-adhesion stages but was also active in late stage adhesion. Supra-MIC concentrations showed no significant effect against late stage biofilm formation, compared to the isolate-specific MIC concentrations. *In vivo* LLCZ increased the survival of the larvae by 40 % and approximatively 20 % for *L. prolificans* and *Scedosporium* spp. respectively.
This is the first study demonstrating LLCZ activity against *Lomentospora prolificans in vitro* and *in vivo*. Furthermore it is the first study showing the anti-biofilm effect of LLCZ in *Scedosporium* spp.

P081

Dynamic of Secondary Metabolites production during Aspergillus fumigatus Biofilm Development

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Objectives: Mycelial development as a biofilm structure, as well as the secondary metabolism activation, resulting in the release of low molecular weight molecules (secondary metabolites), are some of the already described strategies used by the filamentous fungi *Aspergillus fumigatus* to adapt to hostile environment and survive. Dynamic production of these metabolites during the biofilm development could be decisive to understand the evolution of the Aspergillus infection and determine treatment strategies. We aim to describe two main secondary metabolites (gliotoxin and bis(methylthio) gliotoxin) dynamic production, in a collection of phenotypically different *A. fumigatus* strains in a controlled biofilm model. The role of genes responsible (expression) for gliotoxin synthesis by qPCR were also investigated for selected strains.

Materials & Methods: The biofilm structure was developed in RPMI 1640 medium supplemented with 2% glucose (RPMI-G), and inoculum size of 1x104 and 1x105 cfu/ml per strain. Biofilms were allowed to grow at 37°C. At fixed time points biofilm development was stopped and chemical and molecular analysis were performed. Quantification of diffusible secondary metabolites from supernatant was determined by a reversed-phase chromatographic method using an ACQUITY UPLC® instrument, after two steps chloroform extraction. Transcriptomic analysis of genes involved in gliotoxin synthesis was developed after biofilm hyphae collected using physical methods, RNA extracted from them with trizol, synthesis of complementary DNA (cDNA) and comparative analysis of genes expression by real-time quantitative PCR.

Results: We found different dynamic production of gliotoxin and bis(methylthio)gliotoxin during A. fumigatus biofilm development. The release of these secondary metabolites follows a time and strain dependent pattern. Gliotoxin was mainly detected in early phases, whereas bis(methylthio) gliotoxin appeared in a more advanced stage of development. Transcriptional analysis of genes involved in gliotoxin synthesis, revealed differences by strains, according to temporal dynamics of the GT production. In the shortest sampling times, the synthetic mechanisms, encoded by Gli K, GliP, GliT, Glil, GliC or GliG genes, appear overexpressed in the strain with the highest GT production, At the same time, these genes appear silenced in the less GT-producer strain. Additionally, the regulatory mechanism, encoded by GliZ gene, appears overexpressed before the sustained increase in the release of gliotoxin.

Conclusions: Secondary metabolism activation occurs during the *A. fumigatus* biofilm maduration, and determines the release of low molecular weight molecules, such as gliotoxin. The production of this secondary metabolite follows a strain- and time-dependent pattern, which correlates with the degree of expression of regulatory/synthetic genes involved in its synthesis. The expression of the GliZ regulatory gene conditions the expression of genes involved in the synthetic process and, ultimately, the level of gliotoxin production at each time. This regulator could become a possible target for the modulation of secondary metabolism and the response of the fungus to hostile conditions such as treatment or the host's immune response

P082

Investigation of the Relationship Between HOG1 Gene and Biofilm Formation in the Emerging Pathogen Candida auris

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Objectives:
Candida auris is an emerging pathogenic fungus infecting mainly hospital patients. In addition, *C. auris* is inherently highly resistant to commonly used antifungals, leading to its high mortality rate. Though it has been found that *C. auris* does not have the virulence traits of *C. albicans* such as morphogenetic switching, secreted invasive enzymes, and dense biofilm formation, its prominent characteristic in withstanding osmotic stress and further form thriving biofilm on the skin has not yet been elaborated. To study the relationship between resistance against osmotic stress and biofilm formation mechanism on *C. auris*, the influence of osmotic stresses on biofilm formation of *C. auris* was investigated and the clarification of the relationship with *HOG1* gene was also examined.

Materials & Methods:
C. albicans SC5314, *C. auris* MYA-5001, MYA-5002, and *C. glabrata* CBS138 were used in this study. MTT assay was performed to measure the viability of the biofilm formed by *Candida* spp. To observe the biofilm of *Candida* strains, biofilm samples were fixed with glutaraldehyde and continued to post-fix with 1% OsO_4. These prepared samples of biofilm were then observed using SEM (Phenom ProX).

Results:
From the MTT assay, both *C. auris* strains formed less dense biofilm than *C. albicans* strain but these biofilms were significantly higher than *C. glabrata*. In the observation by SEM, the biofilm formed by *C. albicans* was constituted mostly by filamentous hyphae while the other three strains seemed to not change in cell morphology (Figure 1). To evaluate the biofilm formation under osmotic stress, KCl, NaCl and sorbitol were added before biofilm formation. With increasing concentrations of these compounds, biofilm viability has been shown to be significantly reduced for all four strains. Although the cation salts strongly influenced the biofilm formation of *C. glabrata* rather than sorbitol, this different effect among the three compounds was not shown in *C. auris* biofilm formation.

Conclusions:
The reduction of *C. auris* biofilm formation was observed under increased osmotic stress, like that of *C. albicans* and *C. glabrata*. Especially, sorbitol showed a strong influence on *C. auris* strains, compared to *C. glabrata* strain. Since *HOG1* gene is known to be related to the osmotic stress response of *Candida* species, the disruption of *HOG1* gene in these *C. auris* strains is examined in this study to evaluate the relationship between the osmotic stress and the biofilm formation of *C. auris*.

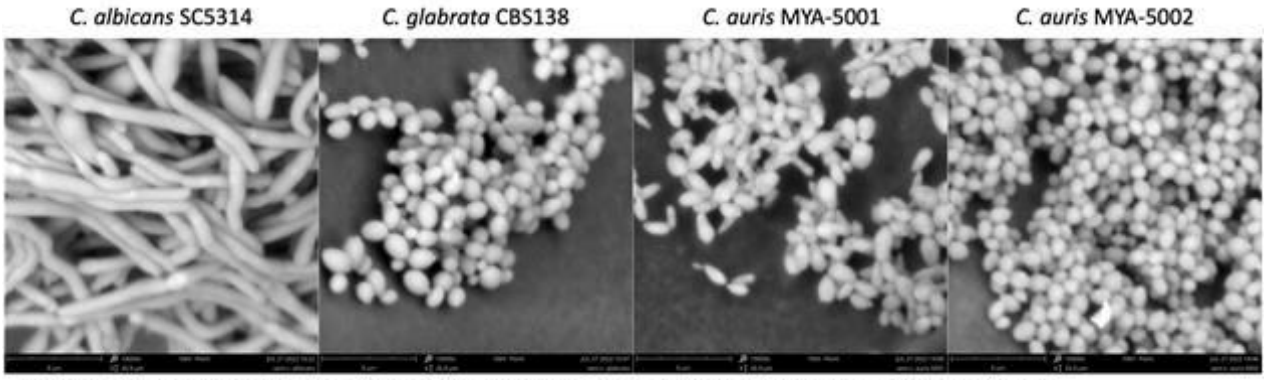

Figure 1. Structure of the biofilm of *Candida spp.* They were observed with 10,000X Magnification by SEM.

P083

Using FTIR Spectroscopy to investigate the formation of a mixed species biofilm

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Objectives:
Characterising the development of biofilms has remained a constant challenge for the last 50 years as most methods of biofilm analysis disrupt the biofilm architecture making the resultant data interpretation difficult. We have utilised Fourier Transform Infra-Red (FTIR) spectroscopy, which is a label-free, non-destructive approach to monitoring biofilm progression. FTIR to study the development of a mixed species biofilm without disrupting the biofilm architecture. S. epidermidis (RP62A) was grown onto calcium fluoride slides for periods of 30 minutes to 96 hours, before semi-drying samples for analysis. We report the discovery of a chemical marker to distinguish between planktonic and biofilm samples. The appearance of new proteins in biofilm samples of varying maturity is exemplified in the spectroscopic data, highlighting the potential of FTIR for identifying the presence and developmental stage of a single biofilm.

Materials & Methods:
S. epidermidis (RP62A) and C albicans were grown onto calcium fluoride slides for, 24 hours and FTIR measurements were taken at 4 and 24 hours after semi-drying to standardise analysis. A desktop Summit PRO FTIR spectrometer (Nicolet, Thermo Scientific, UK) with iD1 transmission sampling apparatus was used for all analysis. All data was collected using OMNIC Paradigm™ software (Thermo Scientific, UK). Data acquisition was performed at 4cm-1 resolution, accumulating 64 scans over a spectral range of 4000-800 cm-1

Results
A signal could be obtained at both time points and the spectral changes that we have previously reported in single species S epidermidis biofilms were present in both species.At 4 hr the mixed species FTIR spectra shares more features with the *S. epidermidis* single strain but by 24 hours the *C. albicans* features predominates

Conclusions:
We have demonstrated that FTIR spectroscopy can be used in mixed species biofilms. The changes in the amide I and II regions that we have previously described in a single species biofilm are present in both species however *S. epidermidis* predominates at first as it proliferates rapidly at the beginning, then as the *C. albicans* grows, the larger cells dominate the signal.

P085

Candidemia and Therapeutic Success in ICUs: A 3-Year Sectional Look

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Objective: Invasive fungal infections (IFIs) demonstrate an increasing incidence with high mortalities. Currently expanding condition of antifungal resistance (AFR) unfortunately supports this high mortality status and accordingly limits therapeutic options. Societies such as ESCMID/ECMM published various guidelines to standardize management and treatment protocols along with fighting against AFR. However, lack of awareness of such efforts, being hesitant to such protocols, rarely encountering condition of IFIs and accordingly lack of experience, strong variability of fungi in their antifungal susceptibility patterns, controversial status in de-escalation/escalation procedures have created a “semi-compatibility” to published guidelines. The aim of this sectional study is to observe the approach to fungemia cases in our tertiary hospital, and to uncover the outcomes of these cases in our study period.

Materials and Methods: Fungemia cases of Balıkesir Atatürk City Hospital adult intensive care units (ICUs) between Jan 2017 - Jan 2020 were included to the study. Etiologic agents, treatments, demographic data, catheterisation status and outcomes were retrospectively investigated. All positive blood culture (BC) vials were Gram stained and subcultured onto 5% sheep blood agar, eosin-methylene blue agar, chocolate agar, sabouraud dextrose agar (RTA Laboratories, Kocaeli, Türkiye). Plates were incubated at 35-37 °C in a 5% CO_2 atmosphere for at least 48 hours. Germ tube testing, Phoenix™ 100 automated system (Becton Dickinson, MA, USA) and Cornmeal Tween 80 agar (GBL Laboratories, Kocaeli, Türkiye) were utilized for identifications. Only the first fungal positive samples or the first isolates in different episodes of the same patients were included.

Results: A total of 108 fungemia cases were detected. 71.5% of patients had central-line catheter and 32.4% (n=35) of patients diagnosed as catheter-associated fungemia. The most frequently isolated organism was *Candida albicans* complex (n=46), followed by *Candida parapsilosis* complex (n=41), *Candida glabrata* complex (n=10), *Candida tropicalis* (n=5) and other *Candida* spp. (n=6). Over 70% of patients had at least one cardiovascular disease (CVD) (hypertension, atherosclerotic diseases) and 14% had a malignancy. 26.9% of patients were unfortunately already lost (n=29) by the time of gram staining notification of BCs. In 8.3% (n=9) and 18.5% (n=20) of patients, clinical cure were achieved by antifungal treatment and catheter removal, respectively (mortality 73.1%). Fluconazole-received 26 patients (16 *C. albicans*

complex, 10 non-*albicans Candida* cases) and caspofungin-received 20 patients (9 *C. albicans* complex, 11 non-*albicans Candida* cases) were also died due to fungemia.

Conclusions: Both ESCMID/ECMM and The Sanford guides recommend primary therapy with echinocandins in candidemia cases, however, the dispute of de-escalation/escalation still remains. In our study, patient losses were emergingly high in patients with both treatments. This situation suggests that the major factor causing mortality might be current clinical status of patients and ineffective timing of notification which cannot provide advantage of early and rapid antifungal treatment. In catheter-associated fungemia cases, catheter removal created a notable effect. It is obvious that rapid methods for fungemia diagnosis has crucial role in prognosis. Diagnostic/detection methods from directly BC vials including molecular analysis might be an effective way, which its efficiency has to be scientifically and financially supported.

Table 1: Results of *C. albicans* complex fungemia cases

Species	Age	Underlying Disease	Status	ICU Length of Stay (Days)	Treatment	Notes
C.albicans complex (n=46)	<35 (1), 35-55 (6), 55-69 (7), >70 (32)	>85% CVD	Death	51	Fluconazole	Fluconazole (n=16), Caspofungin (n=9)
			Death	19	Fluconazole	
			Death	39	Fluconazole	
			Death	85	Fluconazole	
			Death	62	Fluconazole	
			Death	12	Fluconazole	
			Death	63	Fluconazole	
			Death	40	Fluconazole	
			Death	10	Fluconazole	
			Death	15	Fluconazole	
			Death	32	Fluconazole	
			Death	40	Fluconazole	
			Death	19	Fluconazole	
			Death	9	Fluconazole	
			Death	23	Fluconazole	
			Death	41	Fluconazole	
			Death	13	Caspofungin	
			Death	4	Caspofungin	
			Death	35	Caspofungin	
			Death	27	Caspofungin	
			Death	17	Caspofungin	
			Death	16	Caspofungin	
			Death	21	Caspofungin	
			Death	33	Caspofungin	
			Death	14	Caspofungin	
			Death	32	None	Postmortem Diagnosis (n=10)
			Death	6	None	
			Death	18	None	
			Death	3	None	
			Death	52	None	
			Death	5	None	
			Death	3	None	
			Death	7	None	
			Death	29	None	
			Death	40	None	
			Discharged	69	None	Catheter Removal (n=8)
			Discharged	17	None	
			Discharged	11	None	
			Discharged	48	None	
			Discharged	31	None	
			Discharged	42	None	
			Discharged	18	None	
			Discharged	36	None	
			Discharged	34	Fluconazole	traffic accident
			Discharged	87	Caspofungin	CRD
			Discharged	10	Fluconazole	COPD

CVD: Cardiovascular Disease; CRD: Chronic Renal Disease;
COPD: chronic obstructive pulmonary disease

Table 2: Results of non-*albicans Candida* fungemia cases

Species	Age	Underlying Disease	Status	ICU Length of Stay (Days)	Treatment	Notes
C.parapsilosis complex (n=41), *C.glabrata* complex (n=10), *C.tropicalis* (n=5), *Candida* spp. (6)	<35 (7); 35-55 (7); 55-69 (8); >70 (40)	>65% CVD, 10% Neurological Diseases, ~25% Malignancy	Death	148	Fluconazole	Fluconazole (n=10), Caspofungin (n=11)
			Death	63	Fluconazole	
			Death	172	Fluconazole	
			Death	52	Fluconazole	
			Death	31	Fluconazole	
			Death	51	Fluconazole	
			Death	19	Fluconazole	
			Death	39	Fluconazole	
			Death	85	Fluconazole	
			Death	62	Fluconazole	
			Death	23	Caspofungin	
			Death	41	Caspofungin	
			Death	13	Caspofungin	
			Death	4	Caspofungin	
			Death	35	Caspofungin	
			Death	27	Caspofungin	
			Death	17	Caspofungin	
			Death	16	Caspofungin	
			Death	21	Caspofungin	
			Death	33	Caspofungin	
			Death	14	Caspofungin	
			Death	32	None	Postmortem Diagnosis (n=19)
			Death	6	None	
			Death	18	None	
			Death	69	None	
			Death	52	None	
			Death	31	None	
			Death	18	None	
			Death	7	None	
			Death	29	None	
			Death	40	None	
			Death	3	None	
			Death	17	None	
			Death	11	None	
			Death	48	None	
			Death	5	None	
			Death	5	None	
			Death	3	None	
			Death	36	None	
			Death	20	None	
			Death	71	Voriconazole	C.parapsilosis (2), C.glabrata (1)
			Death	18	Amphotericin B	
			Death	20	Amphotericin B	
			Discharged	34	None	Catheter Removal (n=12)
			Discharged	63	None	
			Discharged	41	None	
			Discharged	22	None	
			Discharged	42	None	
			Discharged	57	Caspofungin	
			Discharged	15	None	
			Discharged	42	None	
			Discharged	19	None	
			Discharged	33	None	
			Discharged	80	None	
			Discharged	14	None	
			Discharged	53	Fluconazole	All discharged to palliative care
			Discharged	12	Fluconazole	
			Discharged	23	Fluconazole	
			Discharged	14	Fluconazole	
			Discharged	46	Caspofungin	
			Discharged	42	Caspofungin	

CVD: Cardiovascular Disease

P086

Post-tuberculosis pulmonary aspergillosis - Three distinct cases from a single center

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Objectives:
The precise burden of post-tuberculosis pulmonary aspergillosis is unknown in many countries including in Germany. We have recently observed an increasing number of cases in our center. The burden in countries like Germany may increase due to improved diagnostic tests, an aging population and growing number of migrants from countries with high tuberculosis burden.
Here, we would like to present three distinct cases of immunocompetent individuals with confirmed pulmonary aspergillosis and the diagnostic and therapeutic challenges in the course of managing these patients:

Materials & Methods:

Results:

1. A 65 yrs old male with a history of pulmonary tuberculosis 8 years ago, chronic obstructive pulmonary disease, smoking and previously treated chronic pulmonary aspergillosis. On presentation to our intensive care unit with severe respiratory failure and haemoptysis the diagnosis of invasive aspergillosis was confirmed with extremely high levels of galactomannan in blood and bronchial lavage. Cultures revealed growth of azole-resistant *Aspergillus fumigatus.* Treatment included combination therapy with liposomal amphotericin B, caspofungin and posaconazole, followed by Posaconazole and rezafungin in the outpatient setting.
2. A 74 yrs old female with a history of treated renal tuberculosis aged 9 was diagnosed with aspergillus endophthalmitis two years prior to presentation. Voriconazole therapy had been stopped after four weeks due to a rash. Prolonged investigations for a progressive large pulmonary mass confirmed the diagnosis of pulmonary aspergillosis.
3. A 54 yrs old female with no co-morbidities is diagnosed with pulmonary aspergillomas following investigations for haemoptysis and previously treated pulmonary tuberculosis aged 28. She is commenced on half of recommended treatment dose of voriconazole.

Conclusions:
Post-tuberculosis pulmonary aspergillosis requires increasing awareness among physicians to improve diagnosis and management.

P087

Differences in Candidemia Management Between Hub and Spoke Hospitals in a Northeastern Region of Italy (Friuli-Venezia Giulia).

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Objectives:

Our goal was to find any differences in management and mortality of Candidaemia cases, comparing the Hub Hospital (Santa Maria Della Misericordia University Hospital, Udine) and the Spoke Hospitals of our area (Palmanova, Latisana, San Daniele del Friuli, Tolmezzo, Cividale del Friuli, Gemona del Friuli, Physical Medicine and Rehabilitation Institute *'Gervasutta'*, Polyclinic *'Città di Udine'*).

Materials & Methods:

We retrospectively evaluated a series of 372 patients, with positive blood cultures for *Candida spp.* The *Charlson* comorbidity index and presentation symptoms were reported. We analyzed the wards where candidaemia occurred, the amount of patients who had control blood cultures, echocardiography and *fundus oculi* assessment. For every case we evaluated whether the patients were treated with parenteral nutrition, if they were carriers of central venous catheters or other devices and if these were appropriately removed during the candidemia episode. We noted whether there were any prior abdominal surgery as a possible source of candidaemia. Data on the antibiograms and the antifungals used were collected.

Results:

Patients hospitalized in Hub Hospital were younger than those from Spoke ones (U=21218.5, p<0.001) and more often admitted to the Surgical Area (OR=2.658 [1.433, 5.142], p=0.001).
Comparing those from Spoke Hospitals, more patients from Hub ones were: admitted in Intensive Care Units (OR=3.713 [2.144, 6.638], p<0.001), underwent surgery (OR=2.140 [1.359, 3.399], p<0.001), had more than two devices (OR=2.403 [1.133, 5.471], p=0.014.
No statistically significant difference between Hub and spoke hospitals was observed in species of Candida isolated or in resistance profiles. *C. albicans* was the most isolated *Candida* species.
During treatment, Hub Hospital showed higher frequency of imaging assessment (OR=1.766 [1.063, 2.976], p=0.023) and blood culture control (OR=0.206 [0.087, 0.450], p<0.001).

In Spoke Hospitals: more patients had no antifungal treatment (OR=0.338 [0.143, 0.772], p=0.006) or had antifungal treatment different from echinocandins (OR=0.191 [0.104, 0.345], p<0.001); they removed indwelling catheters less frequently (OR=3.961 [1.623, 10.009], p=0.001) and *fundus oculi* was less evaluated (OR=3.521 [2.163, 5.834], p<0.001).
The frequency of deaths was similar between hub and Spoke Hospitals, but the latter showed more deaths with candidiasis as main cause (OR=0.324 [0.086, 1.022], p=0.045). Finally, we observed less patients live at 30-days (OR=2.231 [1.433, 3.494], p<0.001) and at 90-days (OR=2.218 [1.374, 3.616], p<0.001) from Spoke Hospitals.

Conclusions:
Candidemia is associated with mortality rates ranging from 5% to 71% [1]. *Candida* fungemia may have an endogenous or an exogenous origin, and in recent years a growing proportion of episodes of candidemia have been caused by *Candida* species other than albicans [2]. The data we have collected has some limitations: retrospectiveness and the impossibility of recovering all the clinical records of peripheral hospitals. However they suggest the importance of educating all clinicians on the correct management of candidaemia, for example through the diffusion of protocols, especially in spoke hospitals where the presence of the infectious disease consultant is not continuous.

Bibliography:

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	Hospital		
	Hub	Spoke	Comparison
CVC/Midline			
Days for removal	3.3 ±3.02 [0, 16]	4.0 ±5.39 [0, 27]	p=0.416
>2 days	46.2%	47.2%	p>0.999
Punta positive	47.5%	73.9%	p=0.047*
IAP removal	66.7%	50.0%	p=0.627
Fundus oculi	47.9%	20.6%	p<0.001*
Ecocardiografia	35.5%	26.2%	p=0.071
TE	14.7%	18.6%	p=0.609
TT	85.3%	81.4%	p=0.609
Positive	2.6%	7.0%	p=0.350
Therapy duration in days	25.4 ±22.59 [1, 134]	19.7 ±14.79 [1, 99]	p=0.041*

	Hospital		
	Hub	Spoke	Comparison
Patients	212 (57.0%)	160 (43.0%)	
2018	21.2%	17.5%	p=0.429
2019	20.8%	21.2%	p>0.999
2020	14.6%	11.9%	p=0.540
2021	20.8%	29.4%	p=0.067
2022	22.6%	20.0%	p=0.611
Sex (females)	38.7%	45.0%	p=0.243
Age at candidaemia isolation	70.1 ±16.22 [6, 98]	76.3 ±12.86 [30, 96]	p<0.001*
Non-Caucasian	1.4%	-	p=0.263
Area of admission			
Medical Area	60.8%	78.1%	p<0.001*
Surgical Area	24.1%	10.6%	p=0.001*
Anaesthesia and Resuscitation	15.1%	11.2%	p=0.357

P088

Ocular Pythiosis in a Sri Lankan Patient

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Objectives:
Ocular pythiosis is a relatively rare condition that affects previously healthy individuals. This is the second most common form of human pythiosis and its nonspecific clinical presentation makes the diagnosis of ocular pythiosis difficult. Ocular pythiosis is a sight-threatening condition that is associated with significant morbidity evidenced by enucleation or nearly evisceration.
Materials & Methods:
Here we report the clinical case of ocular pythiosis in a healthy adult male.
Results:
A 50-year-old otherwise healthy farmer presented with redness and pain along with a whitish patch in his left eye. His condition was unresponsive to topical antibiotics. A 2mm × 2mm × 0.1 mm size superficial corneal ulcer with a number of satellite lesions was observed in the examination. Early corneal scrapings for bacterial and fungal cultures became negative. Later, fungal filaments were observed in the direct smear of a corneal button biopsy, and empirical antifungal therapy including guttate (G) voriconazole, G. Amphotericin B, and natamycin (topical) was started. Five days later, the cultures grew irregular, off-white, submerged colonies on sabouraud dextrose agar. The lactophenol cotton blue mount of the colony appeared hyaline, infrequently septate, broad filaments with right-angled branches along with an “end-plate”- like structure and numerous internal vesicles. Two slide cultures were unsuccessful and yielded only sterile filaments. Oogonium was observed in a culture inoculated in sterile water with submerged sterilized plant leave. Pythium species were identified based on phenotypic characteristics. Oral itraconazole was added to his existing therapy yet three penetrating keratoplasty were done due to ulcer recurrence. Then corneoscleral graft was performed with the replacement of the whole cornea with a donor graft. Although the patient has poor vision, his globe was preserved.
Conclusion:
Ocular pythiosis occurs as keratitis or corneal ulcers with or without endophthalmitis. An oomycete that ubiquitously inhabits the aquatic environment is the causative agent. The poor awareness of medical professionals and scarcity of diagnostic facilities lead to the underdiagnoses of most of cases. A false-negative result would be obtained by a tiny corneal scrapings specimen for fungal investigations and repeated sampling will enhance the yield. Sporangia development is not permitted in routine media and inoculation in water plant leaf culture induces the production of sporangia. This

condition is refractory to medical treatment alone and a high recurrence rate is observed even with medical treatment in conjunction with surgery.

The nonspecific clinical presentation of ocular pythiosis makes the clinical diagnosis difficult. Laboratory diagnosis requires special techniques and ocular pythiosis may be underdiagnosed in routine laboratory services. Management of ocular pythiosis is challenging and it has a high recurrence rate.

P089

Successful treatment of invasive pulmonary trichosporonosis with isavuconazole in a COVID-19 positive patient with hematologic malignancies

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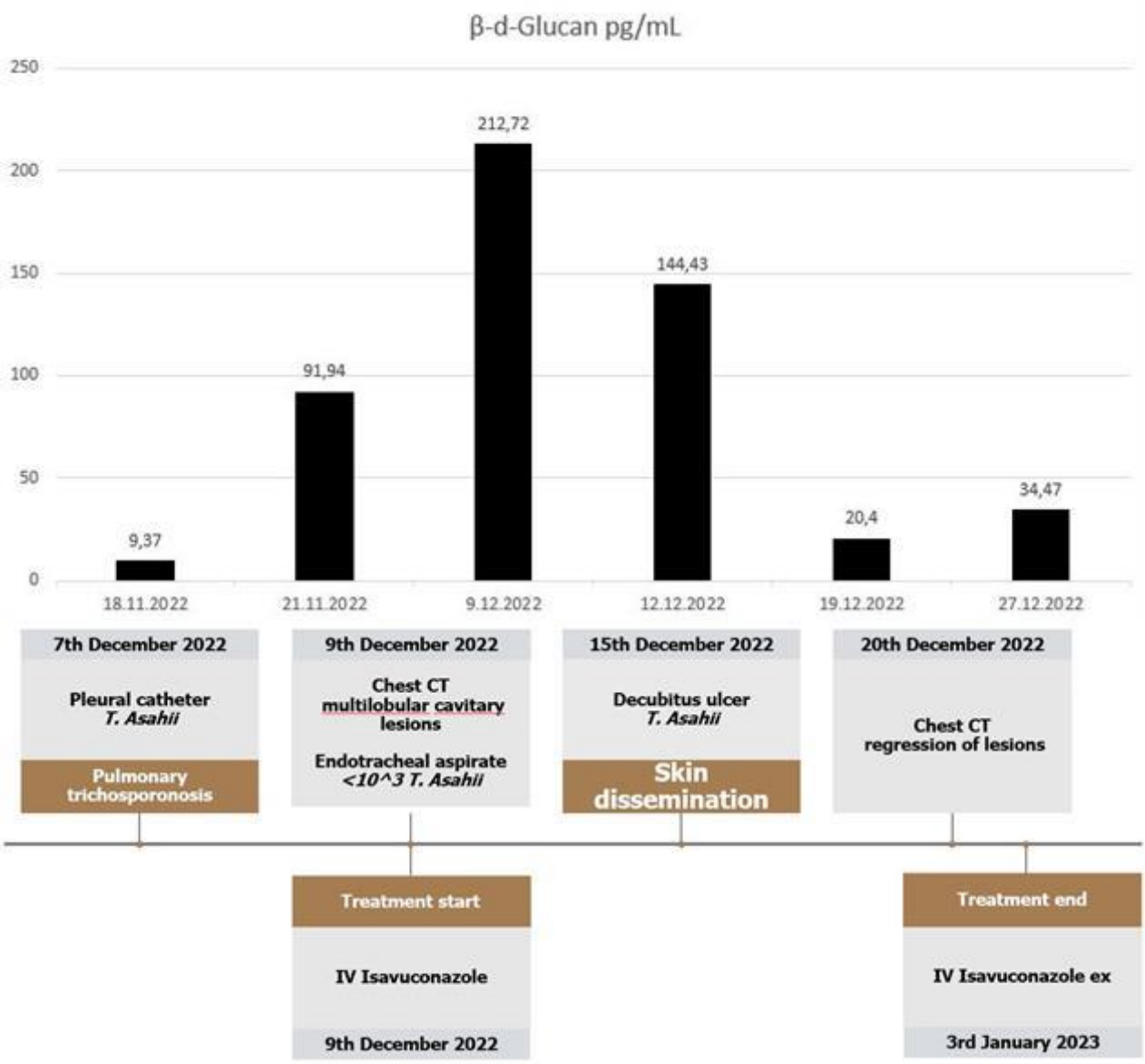
Objectives:
The aim of this abstract is to report a successful treatment of *Trichosporon asahii* pulmonary infection with isavuconazole in a patient suffering from severe COVID-19 pneumonia with prior hematological malignancies.

Materials & Methods:
A 65-year-old patient with *T. asahii* invasive pulmonary infection in December 2022 at the Univerity Hospital Centre Zagreb is presented. Laboratory testing was conducted in Division for Mycology **,** Department of Clinical microbiology, infection prevention and control, UHC Zagreb. Clinical information was obtained from medical records.

Results:
A 65-year-old male patient in remission of B-cell lymphoma was admitted to COVID-19 intensive care unit in November 2022 following transport from another hospital due to deterioration in patient's vital parameters. Initial presentation of patient was with a severe bilateral pneumonia, high C-reactive protein and procalcitonin values, low blood oxygen levels and tachycardia. Patient had been treated with broad spectrum antibiotics due to bacterial pneumonia and had been given micafungin for candidemia, remdesivir and corticosteroids for COVID-19 treatment. Patient was mechanically ventillated for 14 days upon admission, had an almost complete regression of bilateral infiltrates, and was put on high flow nasal catheter for oxygen support (HFNO, 60L/30% FiO2). After 7 days of HFNO, on the night of 6th December, patient started to feel winded and his oxygenation had dropped below adequate levels and was put on mechanical ventilation. Pleural catheter sent to microbiological laboratory had revealed *Trichosporon asahii* as a potential culprit of patient's health deterioration. Subsequent endotracheal aspirate culture discovered *T. asahii* as well, and a chest CT had revealed new multilobular cavitary lesions. After sampling the serum for β-D-glucan assay, patient was promptly switched from micafungin to isavuconazole therapy. We had decided against initiation of voriconazole, fearing its hepatobilliary adverse effects. Laboratory testing had proven low MIC values for both isavuconasole (0,125 mg/L) and voriconazole (0,06 mg/L), while amphotericin B and echinocandins had much higher values (8 mg/L and over 2 mg/l respectively). β-D-glucan assay sampled on 9th December was markedly increased, and after initiation of isavuconazole therapy showed a steady decline in values on 12th and 19th December. Patient experienced skin dissemination of *T. asahii* in form of decubitus ulcer positive culture on 15th December. By 20th December, chest CT showed an almost complete regression in lesions and patient had been feeling a lot better, with a step-down from mechanical ventilation to HFNO to normal nasal catheter oxygenation. By January, patient had suffiecient oxygenation without support, had two consecutive negative β-D-glucan assays and no sign of *T. asahii* in tested cultures, and isavuconazole therapy was discontinued.

Conclusion:
This report suggests that isavuconazole can be potentially used in monotherapy to treat invasive pulmonary infections due to *T. asahii* in non-neutropenic patients. Prior hematologic

malignancies and use of echinocandins should raise a suspicion about possible *T. asahii* infection in patients covered by broad spectrum antibiotics. COVID-19 infection could potentially contribute to *T. asahii* infection as well, with common use of corticosteroids for treatment.



P090

The cost of oral anti-fungal treatment for chronic pulmonary aspergillosis in Uganda

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The cost of oral anti-fungal treatment for chronic pulmonary aspergillosis in Uganda

Objectives
Long-term antifungal therapy improves quality of life and probably survival of patients with chronic pulmonary aspergillosis (CPA). Like TB, CPA tends to affect the poorer sections of society. However, both itraconazole and voriconazole are WHO-listed on the Essential Medicine List, but not in Uganda and thus are not available in public health facilities. We estimated the cost of oral treatment for CPA in Uganda.
Materials and Methods
We estimated direct out-of-pocket cost for purchasing oral itraconazole or voriconazole by the patients from private pharmacies in Uganda. We estimated the cost for 6- and 12-months course of treatment and economic burden for the treatment of CPA in Uganda. Non-affordability was defined as a 400mg/day dosing cost of itraconazole or voriconazole >10% of daily wage of lowest paid civil servant and catastrophic cost 6- or 12-month treatment course cost >20% of annual household income.
Results
We included 31 (23 [74.2%] male) participants with CPA, with a median age of 29.5 (IQR: 25 – 51) years. Eight (25.8%) participants had HIV-TB-CPA triple co-infection. Annual income of lowest paid Ugandan civil servant is $608 and minimum wage of $427/year [$1 = 3,650 Ugandan Shillings (UGX). Only 14 (45.2%) participants were employed with a median annual income of $986 (IQR: 658 – 1,644). The average daily cost of generic itraconazole (400mg/day) was $2.7 ($486 in 6 months and $972 annual cost) and for Sporanox is $7.1 ($1,278 in 6 months and $2,556 annual cost). The cost of voriconazole (400mg/day) [all generic] is $38 ($6,840 in 6 months and $13,680 annual cost). Only 8 (25.8%) participants could afford generic itraconazole and none could afford Sporanox. Voriconazole was unaffordable to all study participants. All study participants would experience catastrophic cost from purchasing any form of itraconazole or voriconazole for 6- or 12- months of therapy.
Conclusions
We found that oral treatment of CPA with itraconazole or voriconazole is unaffordable and causes a catastrophic economic burden on patients in Uganda, even for 6 months. This highlights the urgent need for policy interventions to improve access to essential medicines for the treatment of CPA in Uganda. Efforts should be made to increase the availability of itraconazole and voriconazole in public health facilities. The findings of this study have important implications for the management of CPA in Uganda and other low-income countries. Failure to provide affordable access to essential medications can lead to significant health and economic burdens for patients and their families.

Keywords: Itraconazole, affordability, catastrophic cost, Chronic pulmonary aspergillosis

P091

Emulated trials of liposomal amphotericin B treatment duration in patients with pulmonary mucormycosis

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Objectives:

To determine wether patients with pulmonary mucormycosis (PM) should be treated with liposomal amphotericin B (LAmB) for 14 days, 28 days, or more.

Materials & Methods:

All PM cases from six hospitals between 2008 and 2019 were retrospectively reviewed. Cases were defined according to updated EORTC/MSG criteria with the addition of diabetes and trauma as host factors, and positive serum or tissu PCR as mycological evidence. Factors associated with death up to day 180 were determined using Cox modelling. We then emulated two target trials evaluating LAmB duration on survival, using a cloning and censoring approach and inverse probability weighting to adjust for immortal time bias and indication bias: one comparing LAmB treatment for up to 28 days *vs* more than 28 days, and one comparing stopping or continuing LAmB treatment after 14 days, for those still under treatment, with a 7-day grace period.

Results:

A total of 114 cases were recorded, including 40% of disseminated forms. Main underlying conditions were hematological malignancy (49%), allogeneic stem-cell transplantation (21%), and solid organ transplantation (17%). Ninety-three patients (82%) received a first-line therapy with LAmB for a median of 23 [IQR:11-52] days. The median dose was 5 [IQR:5-6.8] mg/kg. LAmB was discontinuated for adverse events in 16/93 patients (17%) after a median of 7 [IQR:4-18] days of treatment. A scheduled surgery was performed in fourteen patients (12%). Sixty patients (53%) were admitted in the intensive care unit. Overall 90-day mortality was 59%.

Diagnosis in the ICU, dyspnea at diagnosis, neutropenia, disseminated form, pleural effusion and ground-glass opacity on CT-scan were associated with a worst outcome in the Cox

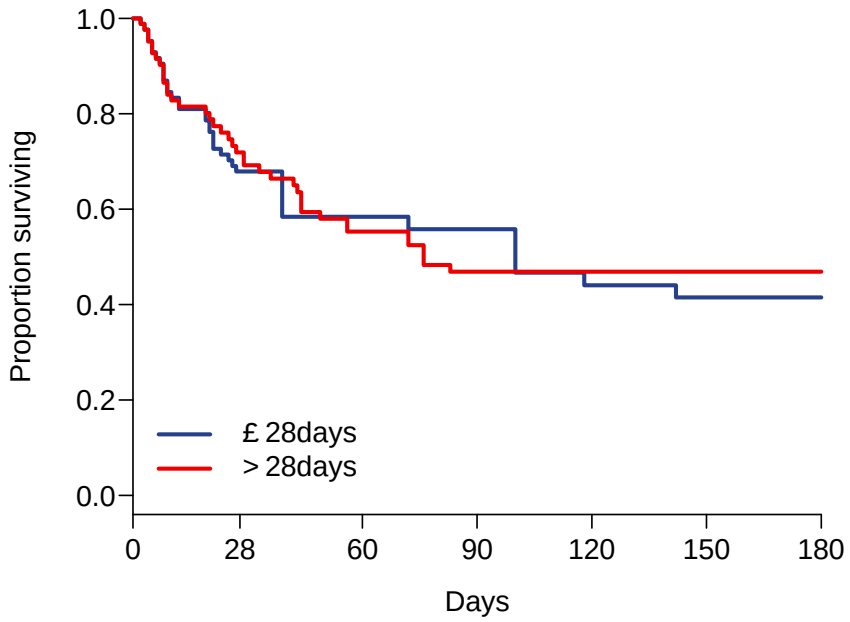
model (Table). 84 patients were eligible and included in the emulated trial comparing LAmB treatment up to 28 days *vs* more than 28 days. There was no difference in survival between the two groups (HR:0.98, IC95%[0.43-5.02]) (Figure). 62 patients were eligible and included in the emulated trial comparing stopping or continuing LAmB treatment after 14 days. There was no difference in survival between the two groups (HR:0.80, IC95%[0.29-1.99]).

Conclusions: No evidence of an average benefit of continuing treatment beyond 14 days in patients with pulmonary mucormycosis was found in emulated trials. Clinical trials evaluating LAmB optimal duration are needed.

Table. Cox model for death up to 180 days.

	Death up to day 180	
Variable	**HR (95% CI)**	***P***
Age at diagnosis (per year)	0.99 (0.98 to 1.01)	0.55
Female	1.48 (0.79 to 2.77)	0.22
Diagnosis in the ICU	2.10 (1.05 to 4.18)	0.035
Dyspnea at diagnosis	2.50 (1.31 to 4.76)	0.005
Neutropenia before or at diagnosis	1.91 (0.94 to 3.86)	0.073
Antifungal treatment since at least 2 days	1.94 (0.97 to 3.87)	0.061
Disseminated bronchopulmonary disease	2.20 (1.20 to 4.04)	0.011
Pleural effusion on CT-scan	1.65 (0.84 to 3.23)	0.15
Ground-glass opacity on CT-scan*		
Main effect	0.14 (0.022 to 0.94)	0.043
× log(Time + 1)	1.99 (1.11 to 3.54)	0.020

Figure. Overall survival up to day 180 according to LAmB treatment duration in the emulated trial.



P092

Clinical and demographic factors affecting trough levels of isavuconazole in critically ill patients with or without COVID-19

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Objectives: The broad-spectrum antifungal isavuconazole is administered to treat invasive aspergillosis and mucormycosis. Isavuconazole plasma concentrations of critically ill ICU patients with or without COVID-19 and invasive fungal infection were determined and factors for subtherapeutic drug levels (<1µg/mL) were evaluated.
Materials & Methods: Isavuconazole plasma levels were measured as part of therapeutic drug monitoring (TDM) in ICUs of a tertiary hospital. Concentrations determined 20-28h after previous dosing were defined as trough (Cmin) levels. A total of 160 Cmin levels of 62 patients with invasive fungal infection were analysed, 30 of which suffering from COVID-19. Patient characteristics included into univariable and multivariable analysis were gender, age, COVID-19 status, body-mass index (BMI), sepsis-related organ failure (SOFA) score, renal replacement therapy (RRT) and extracorporeal membrane oxygenation (ECMO) requirement.
Results: The mean Cmin of isavuconazole in all patients was 1.64 µg/mL (interquartile range 0.83-2.24 µg/mL, total range 0.24-5.67 µg/mL). In total, 34.4% of the Cmin values (corresponding to 46.8% of patients) were below a threshold concentration of 1 µg/mL. Drug concentrations between patients with or without COVID-19 did not differ (p=0.43). In contrast, levels were significantly lower in patients with female sex (p=0.0007), age≤65 years (p=0.002), BMI>25 (p=0.006), SOFA score>12 (p=0.026), RRT (p=0.017), and ECMO requirement (p=0.001).
Conclusions: Isavuconazole plasma levels can be negatively affected by patients' risk factors, supportive renal replacement and ECMO therapy. Future prospective studies analysing the relevance of isavuconazole drug levels in ICU patient outcome are urgently needed.

P093

Clinical characteristics, outcome and factors associated with mortality of mucormycosis: A retrospective study at a tertiary care hospital in Pakistan

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Abstract

Background

Mucormycosis is a rare but potentially life-threatening fungal infection that primarily affects individuals with weakened immune systems. Mucormycosis most commonly affects the sinuses and the lungs but can also spread to other parts of the body. Early diagnosis and aggressive treatment, including antifungal therapy and surgical intervention, are crucial for improving patient outcomes. Limited data exists on the outcomes and factors associated with poor prognosis. The aim of this study was to assess the clinical characteristics, outcome and factors associated with mortality of mucormycosis at a tertiary care hospital in Karachi, Pakistan.

Methods

This retrospective observational study was carried out from January 2018 to December 2022 at the Aga Khan University Hospital, Karachi-Pakistan. Medical records of patients diagnosed with proven and probable mucormycosis during the study period were reviewed. Information on demography, clinical features, risk factors, laboratory and radiological findings, treatment and outcome were extracted and recorded in predesigned performa. Univariate and regression analyses were performed to identify risk factors associated with mortality

Results

A total of 109 mucormycosis patients were reviewed. Mean age was 50.4±20.3 years, and 88 (65.7%) were male. Diabetes mellitus was predominant comorbidity (n=58, 53.2%) and 59.6% (n=65) patients had some form of immunosuppression. 10.1 % (n=11) had current COVID-19 pneumonia. Over half of the patients received steroid therapy (n=59, 54.1%) and 9.2% (n=10) had received recent chemotherapy for underlying malignancy. Most frequent site of infection was pulmonary (n=52, 47.7%), followed by rhino-orbito-cerebral (n=43, 39.4%) and cutaneous mucormycosis (n=10, 9.2%). *Rhizopus* was the most common isolated species (n=68, 62.4%) followed by *Syncephalastrum* species (n=13, 11.9%), *Mucor* species (n=12, 11%) and *Absidia* species (n=6, 5.5%). Neutropenia was noted in 5.5% (n=6) and thrombocytopenia was present in 37.6% (n= 41). Bilateral lung involvement was found in 49 (46.7%) patients. MRI brain was abnormal in 35/37 (94.6%) patients. More than half of the patients received amphotericin B (n=68, 62.4%). More than one-third of the patients received combined medical and surgical treatment (n=44, 40.4%) and 49.9% (n=51) had surgical resections. Mean length of hospital stay was 13.37 ± 11.5 days. Mechanical ventilation (MV) was needed in 38.5 % (n= 42) patients. Overall mortality was 33.9% (n=37) and mortality were significantly higher among patients requiring MV (26/42, 61.9%, p=0.001).. Thrombocytopenia (OR 5.654, 95% CI 1.389 – 23.006, P = 0.016), disseminated disease, (OR 18.730, 95% CI 1.023 – 343.039, P = 0.048), and ARDS (OR 12.365, 95% CI 1.573 – 97.189, P = 0.017) were independent risk factors for mortality.

Conclusion

In our study, nearly one-third (33.9%) of the mucormycosis patients died. The fatalities were common among patients with thrombocytopenia, increased length of hospital stay, and in those who had developed ARDS. Early diagnosis, control of the underlying disease and prompt management may increase the survival rate.

Keywords

Mucormycosis; mortality; clinical characteristics

P094

The clinical characteristics and outcomes of invasive mucormycosis after allogeneic hematopoietic stem cell transplantation

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Objectives: Invasive mucormycosis (IM) is a rare and lethal complication in patients receiving allogeneic stem cell transplantation (allo-HSCT), while there is few reports of IM after allo-HSCT. We aim to summarize the clinical characteristics and outcomes of IM after allo-HSCT.

Materials & Methods: Patients diagnosed with IM after received allo-HSCT in our hospital from January 1, 2010 to Decemer 31, 2021 were retrospectively analyzed. IM was diagnosed according to the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria. Only proven and probable cases were included in analysis.

Results: A total of 50 patients were included in the analysis (table 1), including 21 (42%) proven IM and 29(58%) probable IM. The pathogens detected included *Rhizopus* (n=27, 54%), *Mucor* (n=14, 28%), *Rhizomucor* (n=5, 10%), *Lichtheimia ramosa* (n=4, 8%). The main clinical type was pulmonary mucormycosis (n=36, 72%), rhinocerebral mucormycosis (n=6, 12%), disseminated mucormycosis (n=6,12%), cutaneous mucormycosis (n=2, 4%). The median time of mucormycosis occurrence was 119（9-1979）days after allo-HSCT. The all-cause mortality rate was 82% and IM-attributed mortality was 78%. Fourty-five (90%) patients received antifungal treatment, and 6-weeks survival rate was 20%. Early initiation of antifungal treatment（at least 5 days before diagnosis）was associated with better survival (*P*=0.04).

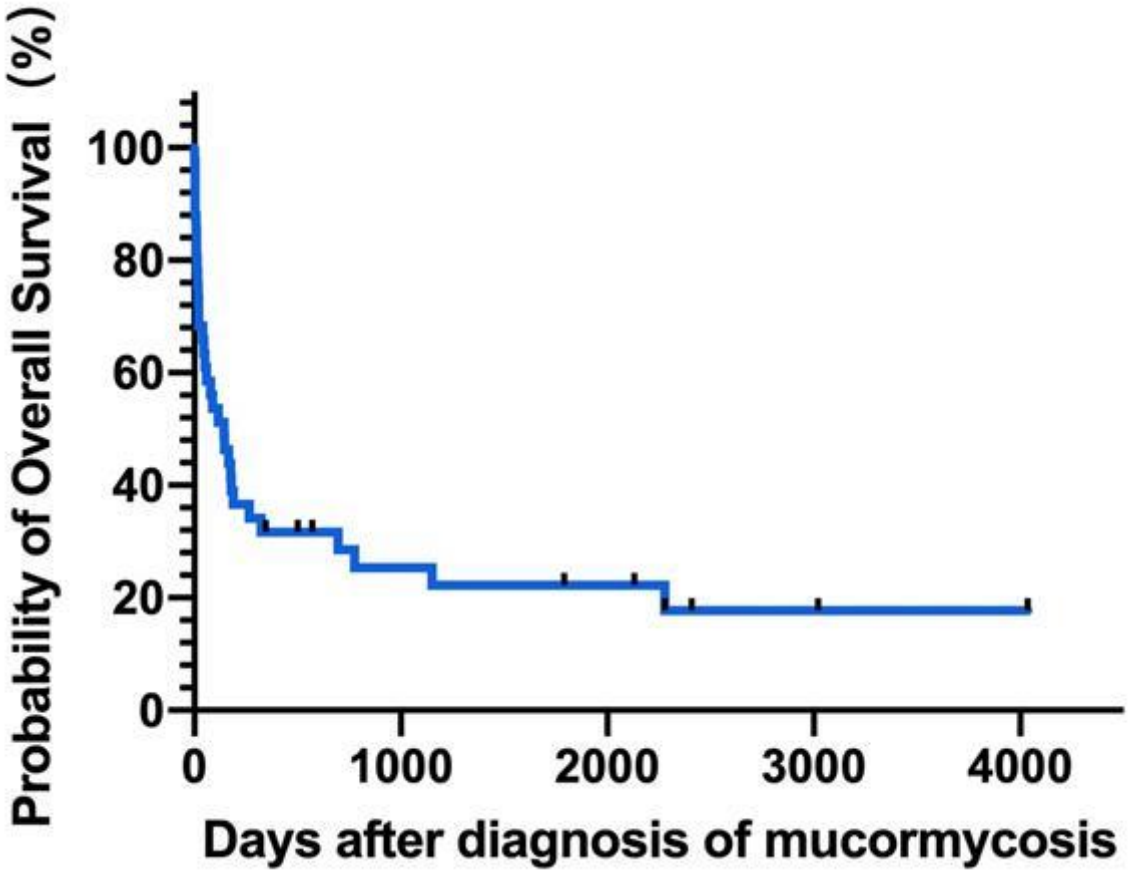
Conclusions: IM after allo-HSCT was mainly caused by *Rhizopus*, and the infection site was mainly in the lung. The prognosis of patients was very poor. Thus, early diagnosis and better management are needed to improve the prognosis of IM after allo-HSCT.

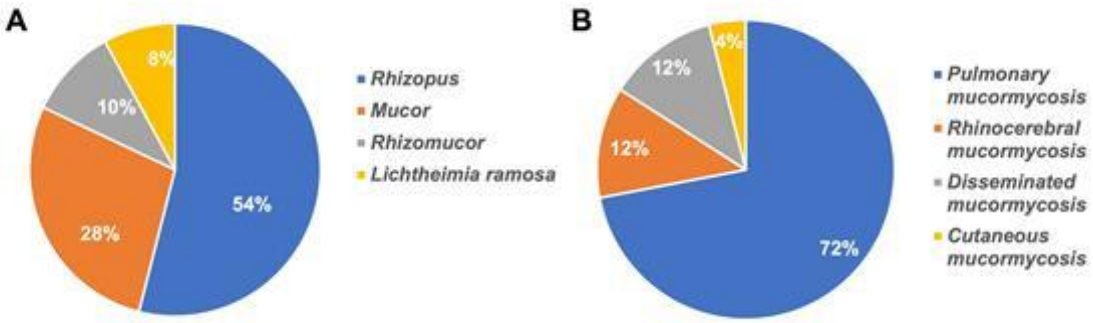
Table1. Baseline characteristics of patients with mucormycosis after allo-HSCT

Patient characteristic	N=50（%）	Death=41（82%）	Survival=9（18%）	*p* Value
Gender				0.429
Male	39（78.0）	33（80.5）	6（66.7）	

Female	11（22.0）	8（19.5）	3（33.3）	
Median Age	35.5（10-66）	39（21-47）	29(23.5-48)	0.471
Disease				0.868
AML	20（40.0）	16（39.0）	4（44.4）	
ALL	12（24.0）	9（22.0）	3（33.3）	
MDS	10（20.0）	8（19.5）	2（22.2）	
CML	3（6.0）	3（7.3）	0（0.0）	
SAA	3（6.0）	3（7.3）	0（0.0）	
lymphoma	2（4.0）	2（4.9）	0（0.0）	
Disease status before HSCT(CR)	30（60.0）	24（58.5）	6（66.7）	0.917
Prognostic stratification				0.966
Intermediate	18（36.0）	15（36.6）	3（33.3）	
Adverse	32（64.0）	26（63.4）	6（66.7）	
Donor type				0.466
Haploidentical donor	46（92.0）	37（90.2）	9（100.0）	
Matched sibling donor	3（6.0）	3（7.3）	0（0.0）	
Unrelated donor	1（2.0）	1（2.4）	0（0.0）	
Comorbidity before HSCT	14（28.0）	13（92.9）	1（7.1）	0.183
Previous IFD before HSCT	8（16.0）	5（12.2）	3（33.3）	0.217
Probable	2（25.0）	1（20.0）	1（33.3）	
Possible	6（75.0）	4（80.0）	2（67.7）	
IFD treatment before HSCT				0.207
Voriconazole	6（75.0）	4（80.0）	2（67.7）	
Caspofungin	1（12.5）	0（0.0）	1（33.3）	
Itraconazole + amphotericin liposomes	1（12.5）	1（20.0）	0（0.0）	
IFD response at transplantation	8（100%）	5（62.5）	3（37.5）	0.217
Median MNC	8.50(2.78-25.16)	8.49(7.47-9.51)	8.53(7.56-9.01)	0.862
Median CD34	2.35(0.47-11.32)	2.63(1.54-4.21)	2.23(1.09-3.90)	0.642
ANC engraftment（yes）	49（98.0）	40（97.6）	9（100.0）	0.033
Median time of ANC engraftment（d）	13（9-23）	13(12-17)	13(9.5-15)	0.234
PLT engraftment（yes）	30（60.0）	23（56.1）	7（77.8）	0.009
Median time of PLT engraftment（d）	13（7-26）	14(12-19)	12(8-22)	0.360
CMV viraemia(yes)	38（76.0）	31（75.6）	7（77.8）	0.969
EBV viraemia(yes)	14（28.0）	12（29.3）	2（22.2）	0.713
aGVHD	30（60.0）	25（61.0）	5（55.6）	0.318
aGVHD grade				0.501
1	11（36.6）	10（40.0）	1（20.0）	
2	8（26.6）	5（20.0）	3（60.0）	
3	6（20.0）	5（20.0）	1（20.0）	
4	5（16.6）	5（20.0）	0（0.0）	
Median time of aGVHD（d）	25（11-80）	33(17-37.5)	19(18-40.5)	0.516
cGVHD	16（32%）	11（26.8）	5（55.6）	0.025
cGVHD grade				0.066
Mild	3（18.8）	1（9.1）	2（40.0）	0.467
Moderate	11（68.7）	8（72.7）	3（60.0）	0.064

Severe	2（12.5）	2（18.2）	0（0.0）	0.039
Median time of cGVHD（d）	226.5（108-1825）	242(183-318)	211(163.5-1170)	0.583
Fungal infection prevention program				0.223
Fluconazole	13（26%）	12（29.6）	1（11.1）	
Itraconazole	24（48%）	21（51.2）	3（27.3）	
Caspofungin	6（12%）	5（12.2）	1（11.1）	
Voriconazole	6（12%）	3（7.3）	3（27.3）	
Posaconazole	1（2%）	0（0.0）	1（11.1）	





P095

The first six patients treated with olorofim for refractory fungal infection at our institution: clinical and microbiological perspectives

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Objectives:
Fungal infections are increasingly reported as refractory to existing anti-fungal agents. Olorofim is the first of a new orotomide class of antifungal drugs, and is reported to be active against a number of fungal species resistant to conventional agents. In Australia, olorofim is only available under a compassionate access scheme. We report our experience with six patients treated under this programme, including criteria for obtaining olorofim, clinical presentation, microbiological data and outcomes to date.

Materials & Methods:
A review of patients treated with olorofim was conducted. The risk factors for fungal infection, prior therapies, the infecting organism and indication for olorofim are described and the outcome at a minimum of 5 months follow-up is reported.

Results:
Six adult patients treated at St. Vincent's Hospital Sydney, Australia were approved for compassionate access olorofim. Clinical characteristics are summarised below:

- M:F = 5:1
- Age range 25-66
- Underlying risk factors (patient identification numbers)
    - One orthotic heart transplant recipient (1)
    - Three lung transplant recipients (2-4)
    - One refractory sarcoidosis (5)
    - One normal host (6)

- Fungal pathogen
    - *Lomentospora prolificans* - 4 (1-4)
    - *Scedosporium apiospermum* – 1 (6)
    - *Aspergillus fumigatus* – 1 (5)

- Indication for olorofim
    - Progressive disease despite optimal antifungal therapy (1,2,3,4,6)
    - Documented severe intolerance of all azole antifunagl agents (5)

- Site of infection
    - Lung (1, 3,4,5)
    - Native hip joint (6)
    - Lung and brain (2)
- Adjunctive surgical Intervention
    - Resection of cerebral abscess (2)
    - Lobectomy (5)
- Timing of surgical intervention in relation to commencement of olorofim

- Resection of cerebral abscess but progression of pulmonary lesions (2) – 37 days prior to olorofim
- Resection of pulmonary lesion (5) – 78 days after commencement of olorofim (resected lesion culture and PCR negative: performed as a precaution prior to cardiac transplantation)

Outcome
At a minimum of 5 months follow up all six patients had complete (3) or partial/ongoing (3) clinical resolution. No patients deteriorated and there were no signifant side effects that required dose re- ducation or drug cessation.

Conclusion:
In our case series olorofim was a highly effective agent in the treatment of refractory/resistant fungal disease in a variety of clinical settings and is an important addition to the antifungal armementarium.

P096

MORTALITY IN ELDERLY PATIENTS WITH ASPERGILLOSIS

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Introduction: Traditionally it had been established that invasive pulmonary aspergillosis affects immunocompromised patients (patients with severe neutropenia, hematological diseases, or solid organ transplantation). However, in recent years there has been an increase in the number of infections in patients with a lower degree of immunosuppression, such as those with pneumopathies receiving chronic corticosteroids (mainly COPD), patients with use in the last month of antifungals and older adults with prolonged hospitalizations or stays in intensive care units. It is a difficult pathology to diagnose that requires a high index of suspicion by the physician and with a high mortality rate. The treatment of choice is voriconazole based on studies with low representation in the elderly population.

Objectives: To evaluate the risk factors associated with mortality in elderly patients diagnosed with aspergillosis.

Methods: Prospective study from 2018 to 2023 of patients older than 80 years with a diagnosis of aspergillosis. Demographic variables (age, sex) care (time of admission), laboratory (leukocytes, neutrophils, lymphocytes percentage) and 30-day mortality were collected. Quantitative variables were analyzed with the non-parametric Kruskal-Walli's test (n <30) and qualitative variables with the Chi-square test.

Results: 73 patients. Mean age 88.5 years, 60% male. Mortality 55.32% at 30 days. Hospital stay non-deceased vs deceased group (16.8; 26.6 days, p 0.00353), neutrophil value (7700 12100, p 0.0144) lymphocytes percentage (10.2; 4.46, p 0.0274). Treated with voriconazole (27 patients), isavuconazole (18 patients), untreated (28 patients). Untreated group 30-day mortality 19.2%, isavucanozol group, 19.2, % and voriconazole group 61.5%, p 0.00426 (Table 1).

Group treated with isavuconazole have lower risk of death compared to voriconazole (OR = 0.07, 95% CI [0.00-0.64]), and no treatment compared to voriconazole group (OR = 0.07, 95% CI [0.01.0.45]) (Table 2.)

Conclusions: Half of elderly patients diagnosed with aspergillosis die within 30 days, with longer hospital stays. Principal mortality risk factors are leukocytosis, neutrophilia and lymphopenia. Furthermore, our results suggest that treatment with isavuconazole is associated with a significant decrease in the risk of 30-day mortality compared to treatment with voriconazole.

Table 2. Mortality at 30 days

Mortality at 30 days			
Predictors	*Odds Ratios*	*CI*	*p*
Isavucanozole	0.07	0.00 – 0.64	**0.046**
No treatment	0.07	0.01 – 0.45	**0.012**

Table 1. Characteristics of non-deceased and deceased patients.

	Total N=73	Not deceased (N=47)	Deceased (N=26)	p-value
Age				
Mean (SD)	88.8 (3.65)	88.6 (3.89)	89.4 (3.19)	0.48
Median [Min, Max]	88.0 [80.0, 98.0]	88.0 [80.0, 97.0]	89.0 [85.0, 98.0]	
Sex				
Male	41 (56.2%)	23 (48.9%)	18 (69.2%)	0.154
Female	32 (43.8%)	24 (51.1%)	8 (30.8%)	
Length of hospital stay (days)				
Mean (SD)	20.3 (14.3)	16.8 (12.5)	26.6 (15.3)	0.00353
Median [Min, Max]	15.0 [6.00, 82.0]	13.0 [6.00, 82.0]	26.0 [6.00, 61.0]	
Leukocytes on admission				0.0371
Mean (SD)	11300 (7850)	9720 (4320)	14000 (11400)	
Median [Min, Max]	9990 [3060, 63300]	8540 [3270, 22000]	11500 [3060, 63300]	
Neutrophils on admission				0.0144
Mean (SD)	9260 (6790)	7700 (3570)	12100 (9820)	
Median [Min, Max]	7760 [2190, 52900]	6960 [2350, 19100]	9840 [2190, 52900]	
Lymphocytes % at admission				0.0274
Mean (SD)	8.17 (11.6)	10.2 (13.7)	4.46 (4.16)	
Median [Min, Max]	6.70 [0.0220, 76.6]	6.80 [0.0480, 76.6]	4.60 [0.0220, 14.0]	
Treatment of choice				0.00426
Isavucanozole	18 (24.7%)	11 (23.4%)	16 (61.5%)	
No treatment	28 (38.4%)	13 (27.7%)	5 (19.2%)	
Voriconazole	27 (37.0%)	23 (48.9%)	5 (19.2%)	

P097

Clinical characteristics of patients with biopsy confirmed fungal pulmonary nodules

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Objectives:
Fungal pulmonary nodules have previously been described in patients without overt immunocompromise, in particular *Aspergillus* nodules which are considered to be a manifestation of chronic pulmonary aspergillosis. Fungal nodules in this clinical setting are often incidental findings on X ray or CT imaging which can mimic lung cancer. There is a paucity of data on the clinical characteristics of patients with histologically confirmed fungal pulmonary nodules. Here, we describe clinical features of patients with fungal pulmonary nodules referred to a national referral centre for chronic fungal infections.

Materials & Methods:
We conducted a retrospective review of patients with pulmonary nodules who had evidence of fungal infection on histological examination of a lung nodule tissue sample obtained during either biopsy or lung resection. Patients were identified from the National Aspergillosis Centre (Manchester, UK) patient database. Classification of lung lesions as nodules on CT imaging was confirmed by review of contemporaneous Consultant Radiologist reports generated during routine clinical care. Data were collected for patient demographics, underlying diseases, *Aspergillus* serology and antifungal therapy.

Results:
Thirty-one patients with histologically confirmed fungal nodules were identified, 19 (61%) of whom were male. Mean age was 64 years (range 35-87). Of those for whom relevant data were available, 21/27 (78%) were past or current smokers, 12/26 (46%) had raised serum *Aspergillus fumigatus* IgG, 15/29 (52%) had multiple pumonary nodules at the time of diagnosis, 13/31 (42%) had COPD, 11/31 (35%) had asthma and overall 26/31 (84%) had some form of underlying chronic lung disease. Lung nodule tissue samples were obtained by biopsy in 19 (61%) patients and by lung resection in 12 (39%) patients. In all patients the nodule samples showed hyphae on histological examination which were reported by a histopathologist as being suggestive of *Aspergillus spp.* Of the 19 patients who did not undergo resection of the fungal nodule, 14 received antifungal therapy and 5 out of these 14 (36%) showed increasing maximal diameter of the fungal nodule on follow up imaging. Of the 5 patients who did not undergo resection and who also did not receive antifungal therapy, there was a reduction in maximal diameter of the fungal nodule in 2 patients, stability of the nodule in 2 patients and resolution of the nodule in 1 patient.

Conclusions:
Similar to other manifestations of pulmonary fungal disease in patients without overt immunocompromise, the majority of patients with fungal nodules had underlying chronic lung disease. Histological examination of the nodular tissue was suggestive of *Aspergillus spp* in all cases, however because the samples were taken primarily to rule out malignancy, very few samples were sent for microbiological testing. A majority of patients had serum *Aspergillus fumigatus* IgG within the normal range, which suggests that this test may have relatively low sensitivity when investigating for *Aspergillus* nodules. Further work is required to identify which patients, if any, benefit from antifungal therapy and to see if specific histopathological characteristics can be used to predict which nodules may progress over time.

P098

Clinico-radio-biological description of 54 mucormycosis of the central nervous system: a french national cohort study

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Objectives: Previous studies on central nervous system (CNS) mucormycosis mainly consist of small series lacking precise clinical, microbiological, radiological or molecular data. The aim of this study is to provide data on the clinico-radio-biological characteristics of these CNS infections in France, according to patients' background and route of dissemination of infection, in order to help clinicians with rapid diagnosis and therapeutic decisions.

Methods & Materials: We conducted a retrospective, multicentric, observational study in 28 French hospitals. All patients diagnosed with proven, probable or putative CNS mucormycosis from 2005 to 2020 -according to modified EORTC criteria- were included.

Results: We enrolled 54 patients (32 proven, 18 probable, 4 putative CNS mucormycosis). Median age was 58 [IQR=48-64] and 74% were men. Thirty-one (57%) had a CNS infection due to haematogenous dissemination, while 23 (43%) had rhino-orbito-cerebral mucormycosis (ROCM) (**Table 1**). Main underlying diseases were haematological malignancies (n=31), solid organ transplants (n=9) and diabetes (n=8). Diabetes and the absence of underlying disease were associated with ROCM (p=0.05 and p=0.04). Patients often presented with fever (64%) or impaired consciousness (44%) at diagnosis. However, 21% had normal neurological examination. Important proportions of neutropenia, lymphopenia and hypoalbuminemia were observed at diagnosis (43%, 75% and 76% of patients). Seventeen patients had a lumbar puncture and 8 showed abnormal cerebrospinal fluid (CSF) analyzes (meningitis and/or increased protein levels). In terms of biomarkers, serum polymerase-chain-reaction (PCR) was frequently positive (82%) and half CSF PCR were positive (n=2/4). Brain imaging could be analyzed for 47 patients: 20 had abscess(es), 11 had macrovascular CNS lesions, 8 microvascular CNS lesions, 24 meningitis, 3 ventriculitis, 6 venous thrombosis of the CNS and 6 cavernous sinus infiltration. Haematogenous dissemination was associated with abscesses and microvascular lesions (p=0.01 and p=0.02), while meningitis and optic neuritis were associated with ROCM (p=0.01). Most patients had first-line treatment with Liposomal amphotericin B (L-AmB) (**Table 2**). Overall six-week mortality reached 64% (including 22 patients who died within the first week after diagnosis). In univariate analysis, 6-week mortality was significantly associated with haematogenous dissemination (OR=3.58), haematological malignancy (OR=3.58) and neutropenia at diagnosis (OR=6.43). The use neurosurgery was significantly associated with decreased 6-week mortality, as well as first-line treatment with high-dose L-AmB (≥ 7.5 mg/kg/day). Among 12 patients (23%) who benefited from azole monotherapy after a first-line with L-AmB, 4 patients had progression of mucormycosis, 2 had treatment-related difficulties (toxicity or pharmacokinetic difficulties).

Conclusion: This study emphasizes that CNS mucormycosis is rapidly fatal, especially in case of hematogenous dissemination and neutropenia. Normal clinical examination should not exclude the diagnosis: CSF analysis and brain imaging should be encouraged when invasive mucormycosis is identified or suspected. In confirmed cases, experts recommend high doses of L-AmB and surgical treatment, both being associated with decreased 6-week mortality in our study. On the other hand, switching to azole monotherapy should be considered carefully, as half of such switches were followed by treatment failure in our series.

Table 1
Clinical and biological characteristics of patients at diagnosis, by route of dissemination

	Total (N=54)	Haematogenous dissemination (N=31)	Rhino-orbito-cerebral mucormycosis (ROCM) (N=23)	p-value
Age (years) at diagnosis, median [IQR]	58 [48-64]	58 [48-63]	58 [42-66]	0.60
Women (n(%))	14 (26)	7 (23)	7 (30)	0.52
Main underlying disease, n(%)				
Haematological malignancy *	31 (57)	20 (64)	11 (48)	0.22
Median time (days) from diagnosis of HM to CM	281.5 [90-543]	195 [87-562]	400 [103-543]	
Solid organ transplant	9 (17)	7 (23)	2 (9)	0.18
Median time (days) from SOT to CM	161.5 [27-351.5]	237.5 [154-397]	7.5 [3-12]	
Heart	4	4	0	
Lung	2	2	0	
Kidney	1	1	0	
Liver	2	0	2	
Diabetes mellitus	8 (15)	2 (6)	6 (26)	**0.05**
Autoimmune disease	2 (4)	1 (3)	1 (4)	0.83
Drepanocytosis (treated with HSCT)	1 (2)	1 (3)	0	0.39
Isolated neutropenia	1 (2)	0	1 (4)	0.24
No underlying disease	2 (4)	0	2 (9)	**0.04**
Main predisposing treatments, n(%)				
Chemotherapy <6 months	24 (45)	16 (52)	8 (36)	0.28
Systemic corticotherapy <3 months	18 (33)	11 (35)	7 (30)	0.70
Immuno-suppressors <3 months	18 (33)	9 (29)	9 (39)	0.44
Immunotherapy <6 months (except ibrutinib)	6 (11)	2 (6)	4 (17)	0.21
Ibrutinib <6 months	1 (2)	1 (3)	0	0.39
HSCT	14 (26)	7 (23)	7 (30)	0.52
Median time (days) from HSCT to CM	160 [18-393]	239 [70-416]	44 [5-160]	
GVHD (% of HSCT)	6 (43)	3 (43)	3 (43)	0.70
Clinical signs at diagnosis, n(%)				
Fever (n=50)	32/50 (64)	22/30 (73)	10/20 (50)	0.10
Headache (n=48)	11/48 (23)	5/27 (19)	6/21 (29)	0.42
Impaired consciousness (n=48)	21/48 (44)	16/27 (59)	5/21 (24)	0.02
Seizure (n=48)	1/48 (2)	0	1/21 (5)	0.26
Focal sign (n=48)	9/48 (19)	9/27 (33)	0	**0.003**
Impaired vision (n=48)	12/48 (25)	2/27 (7)	10/21 (48)	**0.001**
Cranial nerve palsy (n=48)	7/48 (15)	2/27 (7)	5/21 (24)	0.11
Normal neurological examination (n=48)	9/48 (19)	5/27 (19)	4/21 (19)	0.96
Extra-cerebral location of infection, n(%)	53 (98)	30 (97)	23 (100)	0.39
Bone	3	2	1	
Sinus	22	0	22	
Orbit	12	0	12	
Lung	33	29	4	
Skin	6	6	0	
Kidney	15	15	0	
Spleen	10	10	0	
Extra-cerebral vessel	1	1	0	
Endocarditis	3	3	0	

Table 2
Medical and surgical treatment, by route of dissemination

	Total (N=54)	Haematogenous dissemination (N=31)	Rhino-orbito-cerebral mucormycosis (ROCM) (N=23)	p-value
First-line medical treatment				
No antifungal treatment (n=53)	5/53 (9)	5/30 (17)	0	**0.04**
Liposomal amphotericin B (L-AmB) (n=53)	45/53 (85)	24/30 (80)	21/23 (91)	0.26
Alone	38	19	19	
In dual therapy	7	5	2	
Posaconazole or Isavuconazole alone (n=54)	3/54 (6)	1/31 (3)	2/23 (9)	0.39
Surgical treatment				
Any site of infection (n=53)	23/53 (43)	5/30 (17)	18/23 (78)	**<0.01**
Neurosurgery (n=53)	8/53 (15)	4/30 (13)	4/23 (17)	0.69

P099

Exploring Management Strategies and Complications in Genitourinary Mucormycosis in Immunocompetent Hosts - A prospective study from Western India

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Introduction & Objectives

Mucormycosis (MM) is a severe fungal infection associated with high morbidity and mortality. It is caused by various species of *Mucorales* and is commonly observed in immunosuppressed individuals. The incidence of mucormycosis has increased during the COVID-19 pandemic. While Rhino-orbital-cerebral (ROCM) is the most common form, urogenital mucormycosis involving the genitourinary system is rare but has been increasingly reported. Isolated renal mucormycosis cases have been documented, but involvement of other parts of the urinary tract is uncommon. This study presents a series of cases of genitourinary MM, focusing on clinical features, diagnostic approaches, treatments, and to analyze the complications associated with urogenital mucormycosis, including drug-related adverse events, multidrug-resistant infections, and disease extension.

Materials & Methods:

This prospective study was conducted at AIIMS Jodhpur between January 2022 and January 2023. All the patients who were suspected to have genitourinary mucormycosis were included in the study. This included patients with fever, chills, flank pain, urinary symptoms (e.g., dysuria, frequency, haematuria), genital or perineal swelling, necrotic ulcers, and systemic signs of infection. A detailed clinical history, examination along with radiological imaging, and microscopic examination of tissue samples was undertaken. Clinical history, examination, radiological imaging, and microscopic examination of tissue samples were performed. Demographic and clinical details, risk factors, treatment received, outcomes, and complications were documented. Patients were followed up for up to 6 months.

Results:

Fourteen cases of genitourinary MM were identified, including 12 cases of renal MM and 2 cases of penile MM. The majority of patients were male (92.8%), with a mean age of 38.07 years ranging from 5 to 64 years. Common symptoms included fever, chills, and flank pain which was present in 78.5% of the cases. Uncontrolled diabetes mellitus (DM) (64.2%) was the most common risk factor. Radiological imaging revealed characteristic findings, such as bulky kidneys with wedge-shaped renal infarcts and thrombus in renal vessels. All patients were confirmed to have mucormycosis based on post-operative biopsy. All the patients were initiated on Inj Liposomal amphotericin B at the dose of 5mg/kg/day for mean duration of 23.9 days (15-38 days), with a mean cumulative dose of 5.37g. Most (78.5%) of the patient received T. Posaconazole loading dose of 300mg q12h followed by 300mg q24h as step down therapy until clinico-radiological clearance. Complications included drug-related adverse events, electrolyte imbalances (100%), postoperative collections with multidrug-resistant organisms (85.7%), acute kidney injury (71.4), and disease extension (78.5%). Among the patients who survived, mean duration of antifungal therapy was 4.5 months. Overall mortality in our study was 42.9%. The median follow-up of surviving patients was 5.5 months (1-20 months). The overall mortality rate was 42.9%.

Conclusion:

	2	3	4	5	6	7	8	9	10	11	12
	+	+	+	+	+	+	+	+	+	+	+
	+	-	-	+	+	+	+	+	+	-	+
	+	+	+	-	-	-	-	+	+	-	-
	+	+	-	+	+	+	+	+	+	+	+
cus *oli* *a* *ae*	*Enterococcus faecium* CRE *E.coli* *Klebsiella oxytoca*	CRE *E.coli* *Klebsiella Citrobacter freundii*	*Burkholderia cepacia*	CRE *E.coli* *Klebseilla pneumoniae* *Enterococcus faecalis*	-	CRE *E.coli* *Pseudomonas aeruginosa*	CRE *Klebsiella Providencia Enterococcus*	CRE *E.coli* *Klebsiella Enterococcus*	CRE *E.coli, Klebseilla Enterococcus faecium Burkholderia contaminans*	-	CRE *Klebsiella pneumonia*
	+	+	+	-	-	+	-	+	+	-	+
	+	+	-	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	-	+	+	+
	-	-	-	-	-	-	-	-	+	-	+
	+	-	-	-	-	-	-	-	+	-	+
	+	+	-	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	+	+	+
	+	-	-	+	-	-	-	-	+	-	+
	+	-	+	-	-	-		-	+	-	+
	+	+	-	-	-	-	-	-	+	-	+
	80	65	21	20	10	30	35	30	30	30	24
	-	-	10	20	-	20	-	-	-	2	1
	Death	Death	Alive	Alive	Death	Alive	Death	Death	Death	Alive	Alive

Urogenital mucormycosis, although rare, presents significant challenges in diagnosis and management. This study provides insights into the clinical features, diagnostic approaches, treatments, and outcomes of urogenital MM cases. The study highlights the importance of early recognition, aggressive surgical interventions, and appropriate antifungal therapy in improving patient outcomes. The high mortality rate underscores the need for comprehensive management strategies to address the extensive disease, postoperative infections, and associated complications.

Case	Age/Sex	Symptoms	Co-morbidities	Radiology	KOH/HPE	Final Diagnosis
1.	62/M	Fever, chills, rigors Flank pain	No	Right renal infarct with thrombus With involvement if ascending colon, hepatic flexure & transverse colon	Broad hyaline aseptate hyphae	Right renal mucormycosis with colonic involvement
2.	75/M	Fever, chills Flank pain	Type 2 DM since 20y	Bulky Left kidney with multiple wedge shaped renal infarcts with inflammation extending to renal vessels, extension to colon, spleen, pancreatic tail & psoas muscle	Broad hyaline aseptate hyphae	Left renal mucormycosis with involvement of colon, iliopsoas
3.	36/M	Fever, chills Pain abdomen	Newly detected Type 2 DM	Bulky right kidney with multiple wedge shaped renal infarcts with inflammation extending to renal vessels, psoas muscle	Broad hyaline aseptate hyphae	Right renal mucormycosis with extension to psoas
4.	64/M	Pain abdomen Obstipation Vomiting Cough with expectoration	No	Bulky right kidney with multiple wedge shaped renal infarcts with inflammation extending to renal vessels, psoas muscle	Broad hyaline aseptate hyphae	Right renal mucormycosis extending to psoas muscle
5.	36/F	High grade fever, chills Flank pain Vomiting Decreased urine output Malena	No	Hepatosplenomegaly Right renal infarct with thrombus With involvement if ascending colon, hepatic flexure & transverse colon	Broad hyaline aseptate hyphae	Right renal mucormycosis with colonic duodenal & iliopsoas involvement
6.	31/M	Fever, chills, Flank pain Vomiting	Newly diagnosed Type 2 DM	Bulky left kidney with surrounding gross inflammatory changes with renal vein thrombosis	Broad hyaline aseptate hyphae	Left renal mucormycosis with extension to colon
7.	31/F	Fever, chills, rigors Flank pain	No	Right renal wedge infarct with thrombus involvement of hepatic flexure, ascending colon, Ileocaecal junction, iliopsoas muscle	Broad hyaline aseptate hyphae	Right renal mucormycosis with involvement of hepatic flexure, right flank abdominal wall & psoas muscle
8.	38/M	Fever, chills Flank pain	Newly diagnosed type 2 DM	Enlarged Right kidney with renal artery & vein thrombosis with inflammation of adnexa	Broad hyaline aseptate hyphae	Left renal mucormycosis with extension into colon, psoas muscle
9.	35/M	Pain abdomen Constipation Vomiting Weight loss	No	Long segment asymmetrical circumferential thickening involving the right colon and terminal ileum with surrounding inflammation & anterior abdominal extension	Broad hyaline aseptate hyphae	Ileocaecal mucormycosis
10.	45/M	Fever, chills Flank pain	No	Enlarged Right kidney with renal artery & vein thrombosis with inflammation of adnexa	Broad hyaline aseptate hyphae	Right renal mucormycosis with involvement of colon & adnexa
11.	55/M	Fever, chills Flank pain Burning micturition	Newly diagnosed type 2 DM	Enlarged left kidney with renal artery & vein thrombosis	Broad hyaline aseptate hyphae	Left renal mucormycosis
12.	35/M	Fever, chills Flank pain	Newly diagnosed Type 2 DM	Left pyelonephritis with non enhancing infarct with left renal artery thrombosis	Broad hyaline aseptate hyphae	Left renal mucormycosis with involvement of colon, liver & psoas muscle
13.	13/M	Pain abdomen Blister over the penis	Newly diagnosed Type 1 DM	-	Broad hyaline aseptate hyphae	Penile Mucormycosis
14.	4/M	Increased frequency Itching Burning micturition Altered Sensorium	Newly diagnosed Type 1 DM	MRI – Soft tissue inflammation with edema with involvement of pelvic soft tissue & pubic rami osteomyelitis	Broad hyaline aseptate hyphae	Penile Mucormycosis

Case	Surgery	Amphotericin B Dose	Cumulative dose	Duration	Posaconazole dose	Duration	Isavuconazole Dose	Duration
1.	Right Nephrectomy with right hemicolectomy with cholecystectomy	5mg/kg 300mg/day	6g	20 days	300mg/day	2 months	200mg/day	6 months
2.	Left nephrectomy, left hemicolectomy	5mg/kg 400mg/day	10g	30 days	300mg/day	2 months	200mg/day	1 month
3.	Right nephrectomy with hemicolectomy	5mg/kg 250mg/day	5g	20 days	300mg/day	1 month	200mg/day	1 month
4.	Right nephrectomy with iliopsoas debridement	5 mg/kg 250mg/day	4g	15 days	300mg/day	6 months	-	-
5.	Right nephrectomy with right hemicolectomy with iliopsoas debridement	5mg/kg 250mg/day	4g	20 days	300mg/day	6 months	-	-
6.	Left renal nephrectomy with hemicolectomy	5mg/kg 250mg/day	5g	20 days	-	-	--	
7.	Right nephrectomy with hemicolectomy with debridement of psoas & flank skin debridement	3mg/kg 300mg/day	5g	20 days	300mg/day	4 months	-	-
8.	Left nephrectomy with hemicolectomy & psoas debridement	5mg/kg 300mg/day	6g	20 days	300mg/day	2 months	-	-
9.	Hemicolectomy	5mg/kg 300mg/day	5g	17 days	300mg/day	1 month	-	-
10.	Right nephrectomy with hemicolectomy	5mg/kg 400mg/day	8g	20 days	300mg/day	1 month	-	-
11.	Simple left nephrectomy	5mg/kg 300mg/day	4.5g	15 days	-	-	200mg/day	1 month
12.	Simple left renal nephrectomy with hemicolectomy	5mg/kg 300mg/day	5g	30 days	-	-	Combination of Inj Amphotericin B + Isavuconazole 200mg/ day	15 days
13.	Complete Penectomy	5mg/kg 130mg/day	3.9g	30	100mg q12h	6 months	-	-
14.	Complete Penectomy	5mg/kg 60mg/day	1.9g	38 days	100mg q12h	6 months	-	-

P100

Epidemiology and Outcome of mucormycosis

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Objectives:
Mucormycosis is a potentially lethal mycosis caused by filamentous fungi of subphylum Mucoromycotina. We reviewed peer-reviewed publications on mucormycosis that aim to advance the understanding of case distribution, diagnosis, and management of mucormycosis.

Materials & Methods:
A systematic literature search was performed using Ovid MEDLINE and EMBASE databases to identify manuscripts describing human mucormycosis according to EORTC/MSG criteria with therapeutic outcomes published from 2000 to 2022. Single case reports, case series of <10 cases, reviews, editorials, letters, conference abstracts, and animal studies were excluded from the search. Articles that provided antifungal names, duration (>1 day), and outcomes (death or cure) were then selected to assess the impact of therapy. Cases series in languages other than English were read and if the abstract justifies translation, a translation was obtained.

Results:
The primary database search identified 2322 articles and 131 articles which fulfilled all the inclusion and exclusion criteria were analyzed. A total of 10,335 patients were described with a mean age range of 12.6-62 years. Males outnumbered females (66%). The majority of the patients were reported from India (n=6113, 60%), followed by North America (n=1686, 17%), Europe (n=1119, 11%), the Middle East (n=645), East Asia (n=300), China (n=219), and Australia (n=29). A few studies describe global (n=224) cases.
Diabetes mellitus was the most frequently observed underlying disease (n=6193, 60%), and steroid use (n=4670, 45%), post-Covid 19 (n=1732, 17%), hematological malignancies (n=1629, 16%), neutropenia (n=676), and HSCT (n=564) were among other underlying conditions. 222 (2.1%) patients had no underlying disease.
Direct smear was positive for 3651 (35%) cases and both histopathology (n=3076, 30%) and culture (n=2766, 27%) methods were regularly employed for the diagnosis. Molecular methods (n=380) and immunofluorescence (n=21) were infrequently used. 1001 patients were on antifungal prophylaxis at the time of diagnosis; voriconazole (n=323) and fluconazole (n=245) were the most frequently used. The dominant clinical form of mucormycosis was rhino-orbital cerebral mucormycosis (ROCM) (n=7159) with pulmonary (n=1062), disseminated (n=605), cutaneous (n=433), gastrointestinal (n=149) and renal mucormycosis (n=70) the next most frequent; other sites were involved in 169 patients.
A total of 5364 patients (individual data:150 patients in 11 articles and group data: 5214 patients in 23 articles) provided satisfactory data on management. Amphotericin B was the most commonly used antifungal (n=3749, 70%). Other antifungal choices were

amphotericin and azole combination (n=843), amphotericin and echinocandins combination (n=25), posoconazole (n=250), echinocandins (n=1), isavuconazole (n=65) and amphotericin followed by azole (n=357). The mortality of patients treated with antifungals was 25.7% compared to 100% mortality in 74 patients not treated with antifungal therapy. The dose and duration of antifungals vary widely. Of the 141 patients individually reported with a survival outcome 38 (27%) died; 38/120 (31.7%) died after surgery and 16/21 (76.2%) died without surgery (P <0.0002), 11 of whom had disseminated mucormycosis.

Conclusions:

Mucormycosis is more frequently reported in Asia than in Europe. ROCM is the commonest clinical form, but clinical presentation varies with an underlying condition. In the absence of active antifungal therapy, usually with surgery, the outcome is devastating.

P101

Safety profile after exposure to different amphotericin B formulations in 1879 patients with invasive fungal infection: a Brazilian observational study

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Objectives: To evaluate the safety profile of different formulations of amphotericin B (AMB) for the treatment of invasive fungal infections (IFI), in the context of hospital practice.
Materials & Methods: Multicenter, comparative and retrospective study, carried out in ten Brazilian tertiary hospitals. Records of patients with possible, probable, and proven IFI exposed for the first time to any AMB formulation - deoxycholate (D-AMB), liposomal (L-AMB) and lipid complex (ABLC), were eligible.
Results: From the total of 1879 patients, 637 (33.9%) had some change in creatinine levels during exposure to AMB, 70 (11%) requiring dialysis. When stratified by formulation, 351 (55.1%) belonged to D-AMB, 59 (9.3%) to L-AMB and 121 (19%) to the ABLC group ($P < 0.0001$). Eighty-nine (4.7%) out of 637 had to interrupt or discontinue treatment in the first 14 days due to renal dysfunction or nephrotoxicity. One hundred and fifteen (59.3%) patients required potassium replacement after AMB-induced hypokalemia: 608 (54.5%) from D-AMB, 120 (10.8%) from L-AMB and 227 (20 .4%) from the ABLC group. Interruption or discontinuation totaled six (0.32%) cases. One thousand and thirty-nine (55.3%) patients received transfusion of blood components soon after starting or while using AMB, 548 (48.1%) from D-AMB, 129 (11.32%) from L-AMB and 241 (21.20%) from the ABLC group. However, only two (0.1%) interruptions due to hematologic toxicity were reported. Altered electrocardiograms were observed in 106 (5.6%) patients during exposure to AMB and 39 (2.1%) after the end of therapy, without any interruption/discontinuation in the first 14 days due to cardiotoxicity. Sixty (17.2%) deaths were also reported during the first two weeks of AMB treatment. None were directly related to the polyene.
Conclusions: Despite having the same drug, commercially available AMB formulations differ in their pharmacological characteristics. Lipid preparations (L-AMB and ABLC) allow the administration of higher doses, varying in toxicity compared to the conventional formulation (D-AMB). Among the lipid formulations, they presented similar safety profiles, with no statistically significant differences regarding nephrotoxicity between them. However, when compared to D-AMB, they did. The correct choice of an AMB preparation is critical to minimize harmful effects and avoid toxicity.

P103

Histoplasmosis: frequent invasive fungal infection in a tertiary level hospital in Medellín, Colombia.

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Objectives: Determining the epidemiological, clinical, diagnostic, and therapeutic characteristics of patients diagnosed with histoplasmosis in a tertiary level hospital in Medellin, Colombia, during the period from January 1, 2014 to June 30, 2017.
Materials & Methods: We conducted an observational, analytical, and retrospective study of patients over 18 years of age having been hospitalized at the Hospital Alma Mater (former IPS Universitaria) Clínica León XIII headquarters between January 01, 2014 and June 30, 2017, in whom a probable or proven infection by *Hystoplasma capsulatum*-had been-diagnosed according to the EORTC/MSG criteria.
Excluded were patients with clinical suspicion, but who had negative urinary antigen test, histopathology, or culture result for *Hystoplasma capsulatum* performed on.
Results: 51 patients with proven or probable histoplasmosis were included; 86% were males, with 84.3% of them in the age range between 18 and 65; 80.4% (n=41) were VIH positive and 97.6% of them had less than 200 CD4+ T cells. Only 43.9% of those patients had been taking antiretroviral therapy; at the moment of histoplasmosis diagnosis, however, none of them were taking antiretroviral treatment; 25.5% of those having tested HIV positive had a co-infection with tuberculosis (TB), and that was the most frequent oportunistic disease.
92.7% (n=41) of HIV patients had progresive disseminated histoplasmosis (PDH), compared with the HIV negative group, among whom 60% had this clinical presentation. Fever was the most common symptom in patients with VIH and DPH (76.5%), followed by weightloss (72.5%). In the imaging studies -tomography-, the most fresquent findings in lungs were: adenopathies/enlarged lymph nodes (58.8%), nodules (52.9%), followed by hepato/splenomegaly (45.1%). In VIH negative patients with PDH, weightloss (70%) was the most common symptom, followed by fever (50%). In both groups the lung nodular disease is frequent, followed by adenopathies and hepato-splenomegaly. In the laboratory tests, there were abnormal findings in the blood test of 88.6% of patients, and anemia was common in both groups: 68.2% (n=30). Blood tests came out normal for 4.9% of patients with HIV and PDH, while for VIH negative patients with PDH, their blood test was normal in 60% of cases; disturbances in liver function tests were more frequent in patients with HIV and PDH. Mortality in patients with HIV and PDH was 7.3%, contrasting with 20% in HIV negative patients with PDH .
From 51 (100%) patients with histoplasmosis, 86.3% (n=44) had PDH, and 86.4% of them were HIV positive. From the total number of patients with PDH, 15.7% required ICU hospitalitation, and 9.1% of them died.
Conclusions: Histoplasmosis is an endemic mycosis frequent in Colombia -as evidenced in our case series featured in this study- and in our clinical trial group it was present mainly in male patients with HIV, among whom progressive disseminated histoplasmosis was the main clinical form. The most common way of presentation was a febrile, constitutional syndrome with adenopathies, hepato/splenomegaly, and pulmonary nodules. In patients with HIV and PDH, TB was the most frequent oportunistic disease, and in HIV negative patients with PDH, mortality was higher.

P104

Fusarium keratitis in Northeast Brazil: a 10-years prospective study

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Objectives: The aim of the present study was to investigate the prevalence of *Fusarium* species and the antifungal susceptibility profiles of the strains recovered from keratitis patients.
Materials & Methods: The study was performed between March 2012-December 2022. Demographic, clinical and epidemiological data included age, sex, occupation, presence of trauma, outcome and antifungal treatment. The clinical samples were collected by corneal scrapings or swabs and subsequently inoculated onto Sabouraud Dextrose Agar supplemented with chloramphenicol. Media were incubated at room temperature (25 to 30 °C) for 10 days. Colonies showing macro- and micromorphology compatible with *Fusarium* (and related genera) were further identified by DNA-sequencing using fragments of the translation elongation factor 1-α (*TEF1*-α) and RNA polymerase II second largest subunit (*RPB2*) genes. To infer phylogenetic relationships, phylogenetic trees were constructed using IQ-TREE v.2.1.2 and MrBayes v.3.2.7a softwares. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards (CLSI) protocol (M38-A2, 2008), using the following antifungal drugs: amphotericin B, ketoconazole and itraconazole.

Results: Patient´s age ranged between 4-69 years old. The majority of individuals were male (n=37; 74 %), while 13 (26%) of them were female. Thirteen patients (26 %) reported previous trauma of the eye, while 10 (20%) denied it, and the information was unknown for 27 (54%) of them. Corneal transplant was performed in 26 patients (52%), while for 24 patients (12 each), the transplant was either not performed or not informed (48%). The patients received the following antifungal drugs regimen: 10 of them (20%)-amphotericin B only. Ten (20%), a combination of amphotericin B and ketoconazole. Seven (14%), a combination of amphotericin B and natamycin. Six of them (12%) received a combination of amphotericin B, natamycin and ketoconazole. Three patients (6%) each received either a combination of ketoconazole and natamycin or antibacterial agents only (totalizing 12%), while treatment information is missing for 10 (20%) of the patients. A total of 50 positive cultures were found. The clinical strains were identified as *Neocosmospora* species complex (n=39; 78%), with 27 *Neocosmospora falciformis* (54 %), 7 *N. keratoplastica* (14 %) and 5 *N. suttoniana* (10 %). Seven (14%) belonged to the *Fusarium fujikuroi* species complex, all of them identified as *F. annulatum*. Four strains (8%) were identified as *F. nirenbergiae* (*Fusarium oxysporum* species complex). The clinical strains showed the following minimal inhibitory concentrations (MICs) range to ketoconazole, itraconazole and amphotericin B (in µg/mL), repectively: *N. falciformis* (0.0625–>16; 0.125–4; 0.5–8). *N. keratoplastica* (0.125–>16; 0.25–8; 0.25–16). *N. suttoniana* (0.125–16; 0.25–8; 4–16). *F. annulatum* (0.25–16; 0.25–4; 0.25–16). *F. nirenbergiae* (1–8; 0.5–8; 1–4).
Conclusions: We found a high diversity of *Fusarium* species and related genera among patients with keratitis, while the clinical strains showed high MICs for most of the antifungal drugs tested.

P105

A case of rhino-orbital mucormycosis: the role of molecular diagnostic on formaldehyde-fixed and paraffin-embedded (FFPE) tissue samples

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Introduction:
Invasive fungal infections (IFIs) are a major threat in immunocompromised patients, characterized by high mortality and morbidity. One of the greatest challenges in mycology is the early diagnosis of IFIs. Conventional diagnostic methods, such as fungal culture, have generally low sensitivity and diagnosis is often obtained at an advanced stage of the disease. Although histologic observation provides proven diagnoses, fast fungal species identification is fundamental to correct therapeutic approach.
In this rhino-orbital mucormycosis case-report molecular diagnostic tools and their application in real life were reported, in order to standardize diagnostic approach in the differential diagnosis of invasive tissue fungal infections.

Materials & methods:
A 49-year-old man with an history of diabetes presented to the emergency department of Piacenza Hospital with jaw pain, edema, impaired vision and paralysis in the left side of the face one month after tooth extraction.
Clinical suspicion was initially investigated by brain MRI.
Laboratory diagnosis was carried out as follows:

- research of the serum fungal biomarkers Beta-D-Glucan (Fujifilm Wako Chemicals) and galactomannan (Vircell);
- culture of both palate and left inferior turbinate tail biopsies on both Columbia agar + 5% sheep blood and Sabouraud agar medium;
- histological tissue examination with Periodic Acid-Schiff stain.

Formaldehyde-fixed and paraffin-embedded (FFPE) biopsies were submitted to DNA extraction by tissue Maxwell-CSC-DNA-FFPE kit (Promega) and the obtained eluate was submitted to a semi-nested PCR for two different genome regions: internal transcribed spacer (ITS) and 18S rDNA Mucorales specific regions (MZ). Sequencing of the amplification reaction products was performed according to Sanger's method.

Results:
Brain MRI shown tissue images suggestive of fungal infection. Both Beta-D-Glucan and Galactomannan antigens on serum were negative.
Microbiological cultures of tissue biopsy were negative for bacteria and fungi.
Histological examination revealed large non-septate mycelial filaments within necrotic and inflammatory material while yeast-like cells were not observed.
Sequencing analysis gave different results depending on the genome region sequenced: ITS analysis showed partial homology with *Candida albicans,* while the sequencing of MZ region showed high homology with *Rhizopus arrhizus* identification.

Conclusions:
Rhino-orbital mucormycosis are one of the most concerned invasive mycoses. Fast surgical and therapeutic approach are needed. Conventional microbiological diagnosis is often irrelevant, as Zygomycetes difficulty to growth. Histological diagnosis is mandatory to exclude neoplastic process, so FFPE tissue are commonly obtained during surgical excision. Despite molecular diagnostic sensitivity, results obtained from FFPE tissue are not yet standardized. Moreover, the choice of the primers has certain limitations that must be considered when setting up the method. In our case-report, the amplification and sequencing of the panfungal ITS primers failed to find the etiological agent of the infection, revealing a possible colonization with *C.albicans*. The choice of primers specific for Zygomycetes enabled the right identification of the invasive mycosis's etiologic agent.
Invasive mycoses diagnostic workflow needs wide-range agents' consideration. Pan fungal molecular target must have to be combined with specific ones, to avoid incorrect results, supported by a multitasking approach.

P106

Comparison of various microbiological methods for diagnosis of invasive pulmonary aspergillosis in hematological patients

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Objectives: Invasive pulmonary aspergillosis (IPA) is more common invasive mycoses among hematological (hem) patients (pts). The objective of the study was to compare the procedures for diagnosis of IPA in hem pts

Materials & Methods: In prospective study were included cases of proven/probable IPA that were diagnosed at National Research Center for Hematology (2000-2022). Episodes of IPA were defined according to EORTC/MSG criteria (2020). A cut-off value of galactomannan (GM) ≥ 1,0 in bronchoalveolar lavage (BAL), ≥ 0.5 in serum and ≥ 0.5 in lung biopsies were considered positive. Culture of Aspergillus were obtained on Sabouraud dextrose agar at 30°C and 37°C for 72 h. Microscopic examinations were performed using Calcofluor white™.

Results: A total of 445 hem pts with IPA were included (11 (2,5%) proven, 434 (97,5%) probable). The main underlying diseases were acute leukemia (57.2%). For diagnosis of IPA were used BALs (84.7%), lung biopsies (2.5%), sputum (5.6%), serum (82.9%). Mycology positive tests were as follows *Aspergillus* spp. in 53.5%, GM in 76.2% (46.6% in serum, 25.5% in BAL, 20.4% serum and BAL, 27.3% in lung biopsies), septate hyphae on microscopic examinations in 30.4% (table 1). GM was more sensitive than culture (76.2% vs 53.5%, p<0.001) and microscopy (76.2% vs 30.4%, p<0.001). *Aspergillus* spp. were obtained from specimens more often than septate hyphae (53.5% vs 30.4%, p<0.001).

Aspergillus spp. were recovered from BAL in 186 (49.3%) of 377 pts, from lung biopsies in 10 (90.9%) of 11 pts (1 pts had gm > 1.0). Microscopy and gm were positive simultaneously in 9 (3.8%) pts. *Aspergillus fumigatus* represented 40.5% (n=94) isolates, followed by *A. flavus* 22.4%(n=52), *A.niger* 16.4% (n=16.4), and 20.7% *Aspergillus* belonged to other species (table 2). Intristically resistance to azoles were detected in 2.2% *Aspergillus* spp. (5 *A.calidoustus*), to amphotericin B – 6.5% (7 *A.nidulans,* 4 *A.terreus,* 3 *A. alliaceus,* 1 *A.ochraceus*).

Gm was more sensitive in serum (46.6%) than in BAL (25.5%), serum and BAL simultaneously (20.4%), lung biopsies (27.3%), p<0.001.

Conclusions: Microscopic examinations, culture and gm are necessary in the diagnosis of IPA. Specimens of choice were BAL (84.7%) and serum (82.9%). GM was more sensitive test (76.2%). A half of BAL were positive for *Aspergillus* spp. A variety of *Aspergillus* spp was revealed, some of them were intristically resistant to amphotericin B (6.5%) and voriconazole (2.2%).

Table 1. Diagnosis of IPA in hem pts

Diagnostic tests	Total n/N (%)
Positive gm	321/421 (76,2)*
• Serum	172/369 (46,6)*
• BAL	96/377 (25,5)
• BAL+serum	50/245 (20,4)
• Lung biopsies	3/11 (27,3)
Culture of *Aspergillus* spp.	221/413 (53,5)*
• BAL	186/377 (49,3)
• Lung biopsies	10/11 (90,9)
• Sputum	25/25
• 1 *Aspergillus* spp.	209/221 (94,6)
• More than 1 *Aspergillus* spp.	12/221 (5,4)
Septate hyphae on microscopic examinations	72/237(30,4)

* - p<0,001.

Table 2. Epidemiology of IPA

***Aspergillus* spp**	**Total 232 N(%)**
Aspegillus fumigatus	94 (40,5)
Aspegillus flavus	52 (22,4)
Aspegillus niger	38 (16,4)
Aspegillus versicolor	12 (5,2)
Aspergillus sydowii	7 (3)
Aspergillus nidulans	7 (3)
Aspergillus calidoustus	5 (2,2)
Aspergillus terreus	4 (1,7)
Aspergillus alliaceus	3 (1,3)
Aspergillus niveus	2 (0,9)
Aspergillus oryzae	2 (0,9)
Aspergillus spp.	2 (0,9)
Aspergillus ochraceus	1 (0,4)
Aspergillus ornatii	1 (0,4)
Aspergillus candidus	1 (0,4)
Aspergillus flavipes	1 (0,4)

P107

Alternative method for identifying candida species in clinical practice

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Conditions such as interdigital candidiasis, onychomycosis and intertrigo are frequent cutaneous mycoses caused by yeasts of the genus *Candida*. Due to the limited number of antifungals available and the known resistance to treatments recommended against different species of *Candida*, identifying these yeasts at the species level is crucial for prescribing the appropriate therapy. In this context, alternatives for the identification of microorganisms are proposed, such as Fourier Transform Infrared Spectroscopy (FTIR).

Objectives: to develop FTIR models, supervised by DNA region sequencing, for the identification of *Candida* species isolated from cutaneous mycoses.

Materials & Methods: For this, 63 *Candida* sp. isolates, from donated clinical skin samples, were identified by sequencing the ITS1-5.8S rDNA-ITS2 region. The sequences were then compared to type strains available on GenBank using the BLAST algorithm.

Results: Of the 63 isolates, 41.3% were identified as *C. parapsilosis; C. albicans (19%); C. tropicalis (14.3%); C. guilhermondii (9.5%); C. glabrata (4.8%); C. krusei and C. dubliniensis (3.2% of each); and C. lipolhytica, C. orthopsilosis and C. metapsilosis* (1.6% of each). Then, the samples were read in ATR-FTIR to obtain structural and compositional information for each species. Due to the variability in the composition of the samples (proteins, sugars and molecules containing aromatic rings) and the average cross-validation error by mutual exclusion (RMSECV) with only 1 latent variable (VL) less than < 0.022, three identification models were developed by FT-IR for the three most prevalent species.

Conclusions: These models allowed classifying the samples as *C. albicans* or not *C. albicans* (1), by the absorption intervals between 2400-1700 cm-1; *C. parapsilosis* or not *C. parapsilosis* (2), with discriminatory characteristics in the 3300-3250 cm-1 range; and *C. tropicalis* or not *C. tropicalis* (3), with almost total spectrum contribution.

P108

Naso-oropharyngeal colonization by Candida species and associated factors in laboratory-confirmed COVID-19 patients in Semnan, Iran

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Objective: The high mortality rates in severely ill COVID-19 patients often are due to super-infections. Invasive candidiasis in these patients is often preceded by the Candida colonization of mucous membrane. Identifying the causative species and distinguish mixed Candida spp. are important to management of infection in these patients. This study aimed to identify naso-oropharyngeal colonized Candida species in laboratory-confirmed COVID-19 patients and relationships between the cycle threshold value, gender, age, occupation, life style, underlying disease and colonization of Candida spp. in Semnan, Iran.

Methods: This descriptive study was performed on 457 early confirmed COVID-19 patients in Semnan, Iran. The patients' COVID-19 disease was confirmed based on the cycle threshold value<40 by COVITECH Multiplex rqPCR kit. The questionnaire used consisted of demographic data, underlying disease, laboratory data of Candida spp. colonization, and cycle threshold value of COVID-19 test. Candida colonization was evaluated by growth on CHROMagar Candida medium and identification of Candida species was performed with restriction fragment length polymorphism–polymerase chain reaction by Msp1 restriction enzyme in ITS regions of rDNA gene. Statistical analysis was carried out using SPSS software. The Chi-square test and t-test were used to determine differences between factors and the association of the groups with each other.

Results: Candida colonization was confirmed in 162 (35.5%) of the 457 COVID-19 patients. Of these, 59.2% (96/162) patients were males; and the highest incidence of Candida colonization was within the age group of 40–49 years. Candida colonization had significant relationship with age and education level in COVID-19 positive patients and no significant relationship was detected between it and the type of job, urban/rural residence, and cigarette smoked daily and various dental prostheses. A significant relationship between Ct≤20 and the presence of diabetes, use of anti-bacterial antibiotics and Candida colonization in the naso-oropharynx were identified (P=001). 6 different Candida species were diagnosed and *C. albicans* was the most common spp. followed by, *C. glabrata*.

Conclusion: This study demonstrated that Candida colonization on naso-oropharynx associated with early COVID-19 disease. For patients with confirmed COVID-19 via direct qRT-PCR, particularly patients with Ct≤20, the risk of Candida colonization rises with age increasing, low level education and housewives and worker jobs. Nevertheless, further prospectively designed studies are warranted to confirm whether COVID-19 is an independent risk factor of Candida colonization on naso-oropharynx.

Key words: Candida colonization, COVID_19, nasopharynx, oropharynx, associated factors

P109

Current state of diagnostic and antifungal treatment opportunities of clinical mycology in Hungary

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Objectives: Fungal infections continue to be a global challenging concern, which is influenced by several factors, especially the limitations in the availability of state-of-the-art diagnostic tools. Therefore, the administration of adequate therapy may be delayed. Knowledge on the availability of diagnostic tools has been published from several countries worldwide; however, the current state of clinical mycology diagnostic opportunities remains poorly studied in other, including Hungary.
Materials & Methods: An online survey was designed including ten main question categories covering various aspects of mycology diagnostic process and therapy related information. Details on institution profile, self-perception on invasive fungal infections, and access to microscopy, culture, serology, antigen detection, molecular testing, and therapeutic drug monitoring for invasive fungal infections were collected in a survey.
Results: As of May 2023, a total of 17 Hungarian institutions responded to the questionnaire. All centers have adult intensive care unit and COVID-19 ward. Haematology, oncology, solid organ transplantation and neonatal intensive care unit are available at 76.5% (n=13), 76.5% (n=13), 29.4% (n=5) and 58.8% (n=10) of asked centers, respectively. Seven, 4 and 6 out of 17 institutes classified the institutional incidence of invasive fungal infections into “very low”, “low” and “mild” categories, respectively. The majority of centers considered *Candida* species (94.1%, n=16) and *Aspergillus* species (82.4%, n=14) as the most prevalent fungal pathogen. Almost half of laboratories have MALDI-TOF based identification platform (47.1%, n=8). All institutions had access to microscopy and culture-based diagnostic (100%, n=17, each) approaches. Moreover the majority of centers had access to antigen detection (70.6%, n=12) and different molecular assays (58.8%, n=10). The majority of the centres surveyed have reported access to yeast and mold-active antifungal agents. At least one triazole was available in 100% (n=17) of the reporting sites [voriconazole (76.5%, n=13) was the most common mould-active azole], whereas 70.6% (n=12) had at least one amphotericin B formulation, and 64.7% (n=11) had at least one echinocandin. 5-flucytosine was available only in 17.6% (n=3) of the participant centers.
Conclusions: In Hungary, the resources available for diagnosis of invasive fungal infections and the antifungal management opportunities depends on the localisation and total bed number of hospitals. Surveys like ours have crucial importance to understand the gaps and limitations towards the fungal infections. Interregional cooperation within Hungary may be a good strategy to overcome the emerging limitations.

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P110

Development of an ELISA assay for Scedosporium/Lomentospora serodiagnosis.

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Objectives
Scedosporium / *Lomentospora* species are ubiquitous molds that may be responsible for deep-seated infections and allergic broncho-pulmonary mycoses (ABPM). Serodiagnosis is currently performed by detection of specific IgG using immunoprecipitation techniques and homemade antigenic extracts. Immunoprecipitation techniques are time-consuming and lack reproducibility. Moreover, commercially available antigenic extracts for these fungi are lacking. Consequently, many centers stop performing this analysis. Therefore, there is a need for an automatable quantitative alternative, such as ELISA. This study aimed at developing an ELISA assay for serodiagnosis of *Scedosporium* / *Lomentospora* species.

Material and Methods
All sera received for *Scedosporium* / *Lomentospora* serodiagnosis from April 2022 to February 2023, and tested using our in-house immunoprecipitation techniques, were further tested with the new ELISA using antigenic extracts from both *S. apiospermum* and *L. prolificans*. Clinical and biological data such as positive culture, total IgE measurement and final diagnosis were collected. Concordance between techniques was calculated. Khi 2 test was applied to investigate correlation between ELISA and culture as well as ELISA result and diagnosis of ABPM due to these fungi.

Results
A total of 28 sera, corresponding to 25 different patients, were tested with both immunoprecipitation techniques and ELISA. We observed a 68% and 64% concordance between immunoprecipitation techniques and ELISA for *S. apiospermum* and *L. prolificans*, respectively. A significant correlation was observed between ELISA results using *S. apiospermum* antigenic extract and cultures (*p* 0.003, Khi2) as well as with the diagnosis of ABPM due to Scedosporium *(p* 0.036, Khi2).

Conclusions
This series included 6 cystic fibrosis patients, and six ABPM to *Scedosporium* were diagnosed (2/6 cystic fibrosis patients). The *Scedosporium* / *Lomentospora* ELISA presented satisfying results, especially for *S. apiospermum.* Further validation, on a larger cohort, is needed before integrating this ELISA in the routine workflow instead of immunoprecipitation techniques.

TIMM
Trends in Medical Mycology

P111

A simple PCR-RFLP method for rapid and accurate identification of Candida auris

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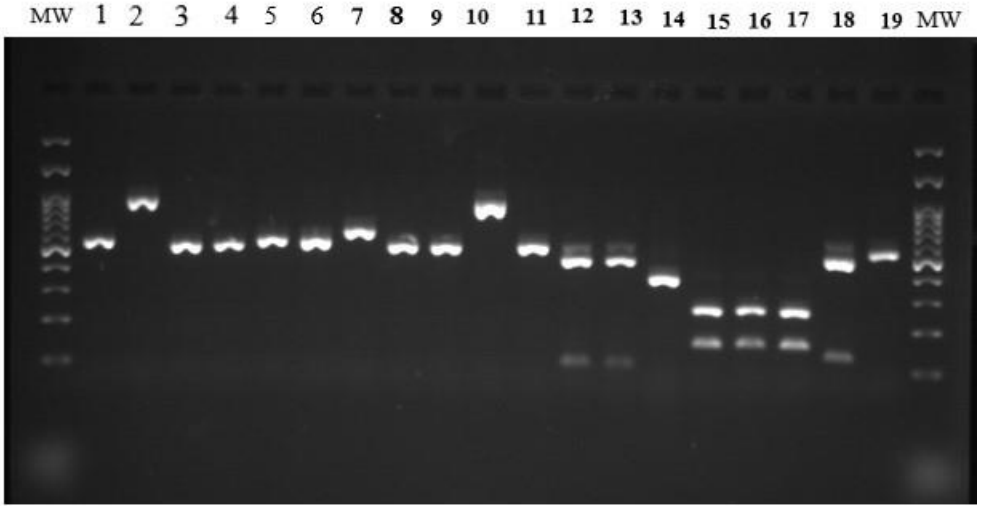
Objectives: In the lack of a simple general identification method for *Candida auris*, the precise prevalence of the yeast has not been established. However, several national and international health organizations have issued warnings about the spread of the yeast. Commercial assays misidentify the yeast, and reference methods of identification, such as PCR-sequencing or MALDI-TOF MS, are not always available. The worrisome rise in cases and the spread of those cases across the globe highlight the necessity of expanding the lab capacity and accessible diagnostic methods. A simple PCR for fungal rDNA amplification followed by restriction-enzyme digests can be utilized to quickly and accurately identify *Candida auris*.
Materials & Methods: The restricted *Bpi*I enzyme was selected after hundreds of ITS1-5.8S-ITS2 nucleotide sequences from medically important yeast species, including *Candida auris* and related species, had been investigated for the presence of specific cutting sites for *Candida auris*. The yeasts' ITS1-5.8S-ITS2 regions were amplified using the ITS1 and ITS4 universal fungal primers. *Bpi*I was used to cut the PCR products before electrophoresis. The yeasts were recognized based on the predicted pattern from the *Insilco* investigation.
Results: The nucleotide sequence of the ITS1-5.8S-ITS2 region in *Candida auris* is a fragment of 401 base pairs, according to *Insilco* analysis and DNA amplification. The *Bpi*I enzyme broke the amplified products into 144 and 257 base pairs fragments, which generated a specific electrophoresis pattern. Fortunately, the enzyme had a distinct pattern and did not result in a cut for other species.
Conclusions: Using the PCR-RFLP method, *Candida auris* can be differentiated from related species such as *Candida haemulonii* in particular. It is not very complicated and is therefore simple to apply. The current approach enables rapid and precise diagnosis of *Candida auris* in clinical laboratories.
Electrophorogram of digested products of yeasts' ITS1-5S-ITS2 region with BpiI enzyme.
Image legend: MW: 100 bp DNA Ladder, 1: *C. albicans*, 2: *C. glabrata*, 3: *C. krusei*, 4: *C. krusei*, 5: *C. dubliniensis*, 6: *C. albicans*, 7: *C. guilliermondii*, 8: *C. parapsiolosis*, 9: *C. parapsiolosis*. 10: *C. glabrata*, 11: *C. tropicalis*, 12: *Cryptococus neoformans*, 13: *Cryptococus neoformans*, 14: *C. haemolunii*. 15-17: *C. auris*, 18: *Rhodotrula rubra*, 19: *Sacharomyces cervisea*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P112

Fungal infections during Covid-19 pandemic in a tertiary care hospital

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Objectives: To determine the occurrence of fungal infections among Covid-19 infected patients during the second wave in a tertiary care hospital.

Materials & Methods : This retrospective study was done in Mycology Laboratory, Department of Microbiology, Lady Hardinge Medical College, New Delhi during the period of second wave of Covid-19 infection. The various clinical specimens received from suspected patients of fungal infections were processed according to standard protocol. For the yeast identification, Chrome agar and Corn Meal Agar along with biochemical tests and sugar assimilation tests were put up while for the molds identification, standard identification tests including LPCB mounts were made from growth on culture and observed under microscopy. For zygomycetes, identification of species was also done by five genus specific PN-700 MucorGenius® real-time PCR in 12 suspected patients.

Results: The fungal infection was present in 64% (51/79) Covid-19 positive patients and 43% (163/381) Covid-19 negative patients) during the year 2021 during the second wave of Covid-19. Among Covid infected patients, the fungal infection mostly observed was Candidiasis (63%) followed by Aspergillosis (15%) and Mucormycosis (6%). The maximum samples positive in Covid-19 patients were urine samples followed by Serum (for Aspergillus Galactomannan). Among the urine and respiratory samples (BAL, Tracheal aspirate, Sputum) in Covid-19 positive patients, maximum positivity of Candida species was seen. Mucormycosis in Covid-19 positive patients was isolated in Nasal samples followed by tissue sample with Rhizopus arrhizus and Rhizopus homothallicus. The 33% samples with BAL Galactomannan positivity showed positive correlation with BAL culture results among total Galactomannan positive patients or Aspergillosis.

Conclusions: There has been an increase in fungal co-infections during the Covid-19 pandemic which is a matter of great concern. There is a need for stringent approach for the early diagnosis and management of invasive fungal infections including aspergillosis, candidiasis, cryptococcosis, and mucormycosis. The predisposing factors like overusage of steroids, diabetes etc should be followed properly. Careful examination and evaluation of patients having Covid-19 is a must for reaching at the underlying fungal disease. More robust prospective and multicenter studies are needed to determine the real impact of SARS-CoV-2 on the incidence of invasive fungal infection.

Table 1: Distribution of Fungal Infection during the COVID-19 second wave period (N=460)

Status	Fungal Infection Present	Fungal Infection Absent	Total	p value	Pearson Chi-Square
Covid-19 positive	51 (64%)	28 (35%)	79 (17%)	0.0004	12.47
Covid-19 negative	163 (43%)	218 (57%)	381 (83%)		
Total	214 (47%)	246 (53%)	460		

Fig.1: Distribution of fungal Infection

Fig.1a: Distribution of total fungal infection from suspected patients (N=460)

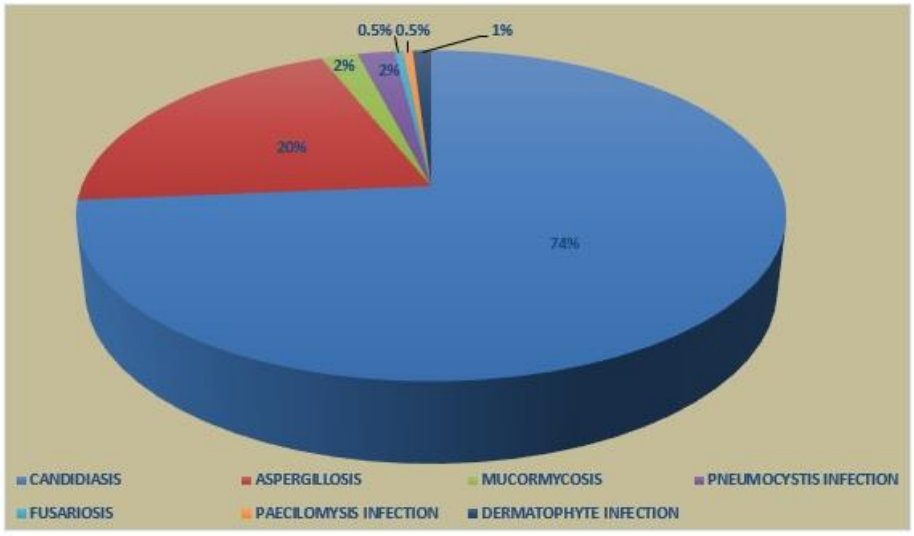
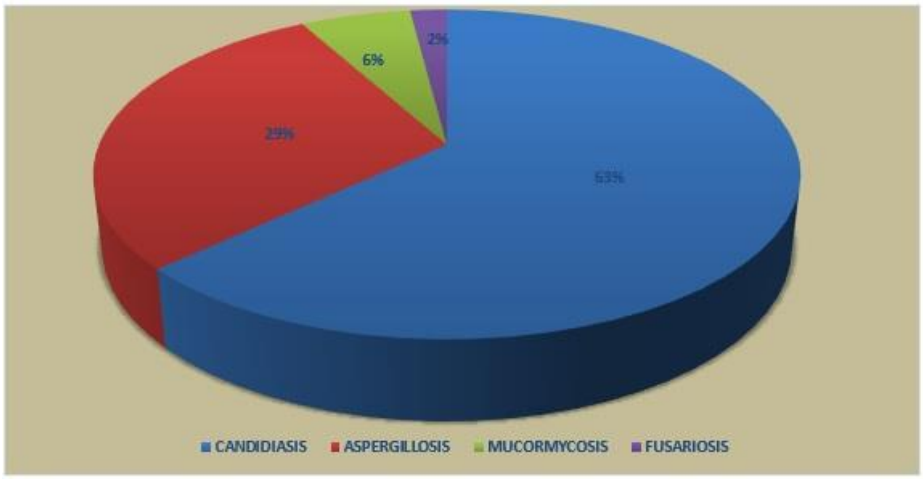


Fig. 1b: Distribution of fungal infection in Covid-19 positive patients (N=79)



P113

Clinical mycology capacity and access to antifungal treatment in Portugal

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The success of the clinical management of invasive fungal infections (IFI) is highly dependent on the availability of suitable tools for early and accurate diagnosis. Unfortunately, the lack of appropriate diagnostic tools still remains one major hurdle contributing to the high mortality associated with these infections. An in-depth analysis of the ability of European institutions to promptly and accurately diagnose IFI was previously conducted to identify limitations and aspects to improve. As a follow-up, we evaluated the specific case of Portugal, for which, to our knowledge, there are no reports describing the national mycological diagnostic capacity and access to antifungal treatments.

Data from 16 Portuguese medical institutions were collected via an online electronic case report covering different parameters, including the type of institution, IFI incidence, target patients, diagnostic methods and reagents, and available therapeutics. Out of the 16 participating institutions, 2 (12.5%) reported a high incidence of IFI, 3 (18.8%) mild, 6 (37.5%) low, and 5 (31.3%) very low. *Candida* spp. was indicated as one of the most relevant fungal pathogens by all the institutions (n=16, 100%), followed by *Aspergillus* spp. (n=12, 75%), *Cryptococcus* spp. (n=5; 31.3%), *Fusarium* spp. (n=2, 12.5%) and Mucorales (n=1, 6.3%). All the institutions had access to culture and microscopy, 14 (87.5%) to serology, and 15 (93.8%) to molecular tests and antigen-detection assays. Among the 15 institutions with available antigen testing, 12 (75%) reported to have access to *Aspergillus* and *Cryptococcus* antigen detection, 7 (43.8%) to Candida, and 6 (37.5%) to *Histoplasma*. The availability of anti-fungal drugs at these institutions was slightly lower than the reported European average, with 13 (81.3%) having access to amphotericin B and triazoles, and 11 (68.8%) to echinocandins. Therapeutic drug monitoring is however still a very restricted practice in Portugal, with only 1 institution (6.3%) performing therapeutic monitoring of itraconazole and posaconazole, 2 (12.5%) of flucytosine, and 3 of voriconazole (18.8%).

Overall, and despite Portugal is prepared to manage IFI, access to specific diagnostic tools, antifungal drugs, and therapeutic drug monitoring is not generalized to all institutions, which may compromise early diagnosis and the effective treatment of patients.

P114

Using Aspergillus IgG to predict and avoid invasive Aspergillosis pneumonia

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Objectives:
Invasive aspergillosis is the most common mold infection in immunocompromised hosts. The most common infecting species is Aspergillus fumigatus complex, but other species complexes that are common causes of disease include A. flavus, A. terreus, and A. niger. Less common species, such as A. nidulans, A. calidoustus, A. lentulus, and many others, have been reported to cause infection in highly immunosuppressed patients.In theory, diagnosing the IAP have to as soon as possible due to its high mortality rate. So if we can quickly predict the IPA happen by another tool to replace culture, it could avoid patient dead.

Materials & Methods:
In this study, we collected 126 patients' serum to detect IgG (Aspergillus fumigatus) and detected the galactomannan. Otherwise, we also collected the broncho-alveolar lavage for Real-time PCR. After RT-PCR positive, we did the PCR and sequencing to check its result was corrected or not.

Results:
In this study, we found most of the IPA patient's Aspergillus fumigatus IgG were over cut-off value, the sensitive and specific were 96.4% & 93.1% and the Positive predictive value (PPV) and negative predictive value (NPV) were 92.3% & 87.4%. And the sequencing showed 98.4% were Aspergillus fumigatus , 1.6% were A. niger.

Conclusions:
In this study, the Aspergillus fumigatus IgG can predict the possible IPA. Once the IgG title higher than cut-off value, it would became IPA, so in this moment, we can take the antibiotic as a preventive medication.

P115

Evaluation of Candida colonization of oral cavity, anal area, ear canal and urine samples of hospitalized infants and children

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Evaluation of Candida colonization of oral cavity, anal area, ear canal and urine samples of hospitalized infants and children

Background and purpose: Candidiasis often follows colonization. The colonization index in areas of the body is used to follow the patient's condition and an index higher than 0.5 indicates the possibility of infection and requires follow-up and appropriate treatment methods.

Materials and methods: A cross-sectional descriptive study was performed on 200 infants and children hospitalized in Behshahr hospitals. Demographic information and clinical status were recorded in a questionnaire. The obtained samples were cultured in mycological media. Candida species of colonization index ≥ 0.5 were identified by PCR_RFLP method using Msp1 restriction enzyme. Finally, the drug susceptibility pattern of Candida isolated was determined for 5 antifungal drugs.

Results: 43 patients (21.5%) had Candida colonization index ≥ 0.5. Out of 124 Candida isolates, *C. albicans* was the most isolated (74.4%). The index was higher in the oral cavity (69.8%), boys (17.3%) and the age group of 11-16 years (36.5), ICU (34.5%), patients with respiratory problems (10.5%). The association between fever and antibacterial antibiotic use showed a statistically significant relationship with the index but the mean hospital stay, surgical history and catheter use in the two groups did not show a significant difference. Ketoconazole for *C. albicans* had better effects. While *C. glabrata* showed the highest MIC compared to fluconazole and amphotericin B.

Conclusion: The colonization index is a tool to help predict the possible incidence of candidiasis in hospitalized patients, and identifying colonization index ≥ 0.5 in hospitalized patients can prevent infection.

Keywords: Candidiasis colonization index, candidiasis, hospitalized patients, oral cavity, ear, urine, anus, children, neonates

P116

Standardization of Gold Nanoparticles with thiolated DNA for the detection of Candida in blood

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Title:

Standardization of Gold Nanoparticles with thiolated DNA for the detection of *Candida* in blood

Objectives:

1. To develop a standardized protocol for the functionalization of gold nanoparticles (AuNPs) with thiolated DNA probes.
2. To evaluate the sensitivity and specificity of the developed AuNP-DNA conjugates for the detection of *Candida* in blood samples.
3. To compare the performance of the developed AuNP-DNA conjugates with existing diagnostic methods for *Candida* bloodstream infections.

Materials & Methods:

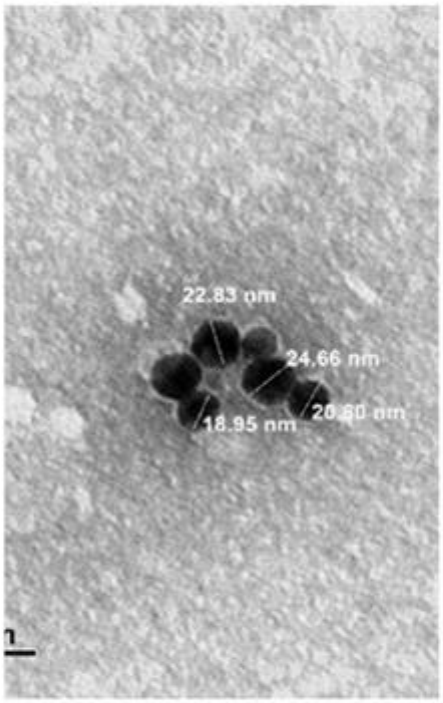
1. Thiolated DNA Probe: The thiolated DNA probe was obtained from sigma to target specific *Candida* DNA sequences.
2. Characterization of gold nanoparticles: Citrate-stabilized AuNP 20nm size was obtained. The size and shape were characterized using transmission electron microscopy (TEM) (Pic:1)
3. Functionalization of AuNPs with thiolated DNA probes: The standardization of the protocol will be developed for the attachment of thiolated DNA probes to the surface of AuNPs through thiol-gold interactions. The optimization of reaction conditions, such as pH, incubation time, and reagent concentrations, will be performed.
4. Evaluation of sensitivity and specificity: Spiked blood samples containing known concentrations of *Candida* DNA will be prepared. The spiked samples will undergo DNA extraction, followed by hybridization with the functionalized AuNPs. The color change and quantitative measurements of the AuNPs will be used to assess the sensitivity and specificity of the detection assay.

Results:

The study will establish a standardized and optimized protocol for the functionalization of AuNPs with thiolated DNA probes, ensuring reliable and reproducible attachment of the probes and providing a solid foundation for the subsequent detection of *Candida* in blood samples.

Conclusions:

We have made significant progress towards the development of a protocol. To further enhance the reliability and purity of the DNA probes, we have planned to incorporate a desalting step using a Sep-Pak column for DNA purification. Although the Sep-Pak column has been ordered, it has not yet arrived at the time of concluding this study. The inclusion of this desalting step aims to remove impurities and potential interfering substances, ensuring the integrity and purity of the DNA probes. Once the Sep-Pak column is available, we will perform the purification step and evaluate its impact on the overall assay performance.



Pic1:TEM image of Gold nanoparticles(NIMHANS, Bangalore)

P117

A new machine learning-based approach to classifying patients with invasive fungal disease

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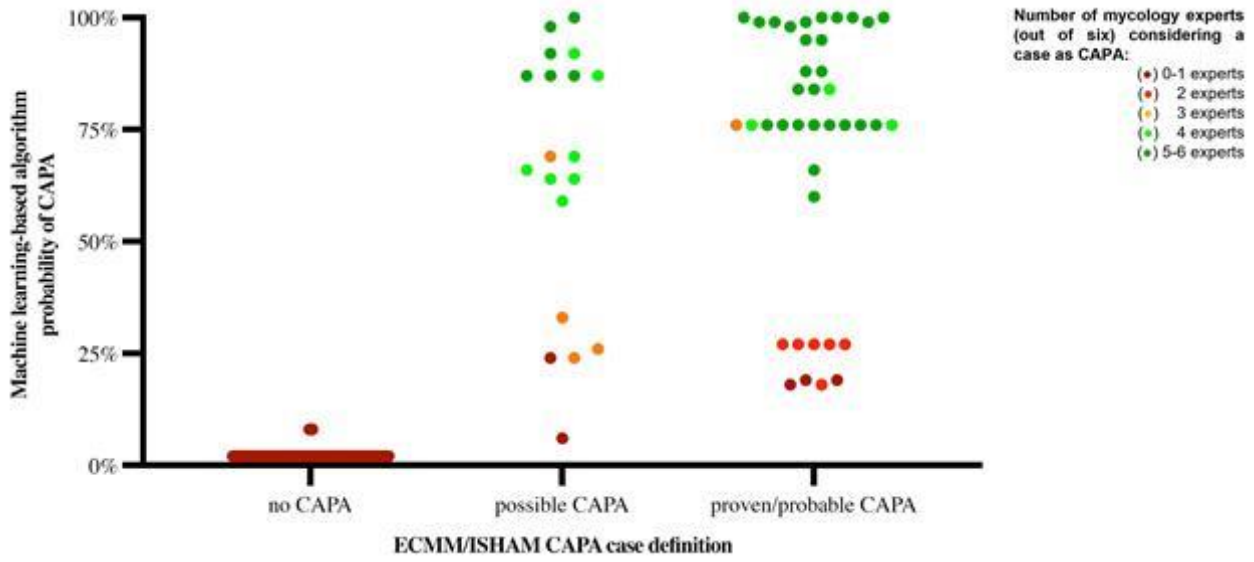
Objectives: Over the past decades new risk groups have emerged for developing invasive fungal disease (IFD), including severe viral pneumonia, such as influenza and COVID-19. It has been difficult to classify these patients based on existing IFD case definitions (e.g., EORTC/MSGERC and *Asp*ICU) due to absence of host factors and specific radiological findings, resulting in new case definitions for influenza-associated pulmonary aspergillosis and COVID-19-associated pulmonary aspergillosis (CAPA). These case definitions rely on fungal biomarkers as host factors and radiological findings may be absent or non-specific. The increasing number of case definitions and reliance on fungal biomarkers prompted us to investigate if a machine learning approach would be applicable to classify patients with IFDs.
Materials & Methods: A data set of 219 critically-ill COVID-19 cases was used, including one proven, 38 probable, 19 possible, and 161 without (sufficient) evidence of CAPA. Six international mycology experts were provided with diagnostic information, antifungal therapy and outcome, and asked to determine if CAPA was present. These results were then used to train a machine learning-based logistic regression model, to determine the relationship between the various *Aspergillus* test profiles and the mycology expert classification. To overcome overfitting, 100-fold cross-validation was performed, to determine the performance and how well the algorithm would generalize to an independent cohort. Finally, the probability of CAPA for each case as calculated by the algorithm was compared with the ECMM/ISHAM classifications.
Results: Our results indicate that experts weigh diagnostic information differently, taking into account the type of sample (e.g., a positive test in bronchoalveolar lavage was more convincing than in non-bronchoscopic lavage), type of test (e.g., a positive galactomannan was more convincing than *Aspergillus* culture), and number of positive and negative tests in their decision making. Furthermore, information on disconcordant test results proved critical to classifying patients with IFD (e.g., a positive *Aspergillus* culture while galactomannan remained negative was considered as colonization). In patients classified as proven/probable/possible CAPA (ECMM/ISHAM), the median probability of CAPA (algorithm) when four or more experts agreed on CAPA was 77% (IQR, 76-94%) compared to 29% (IQR, 26-29%) when two or less experts agreed on CAPA (Figure 1). The results of 100 repetitions of training and testing the model, the median sensitivity was 86.4% and the specificity was 97.0%.
Conclusions: The current ECMM/ISHAM CAPA case definition is easy to interpret and understand, however, it is limited as it provides no information on the relationship between

the different samples and tests, as they are all assessed independently. We developed a machine learning-based algorithm to take into account all diagnostic information, which was able to account for factors that are critical for experts to determine the presence or absence of CAPA. Further exploration of machine learning algorithms through incorporating underlying host factors, immunomodulating therapy, immune status and imaging may provide a uniform approach to classifying IFD in various risk groups. Given the ever-increasing population “at-risk” of IFD, such an endeavor should be considered a worthwhile investment by the mycology community.



P118

Development of a low-cost molecular assay for the diagnosis of Sporothrix brasiliensis

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Sporotrichosis has become an emerging zoonotic infectious disease in the latter years, due to its worldwide impact and high prevalence in Brazil. The species *Sporothrix brasiliensis* has been specifically associated with this severe epidemiological impact. **Objectives**: Considering that quick and easy diagnosis contribute to the control of the situation of this zoonosis, the purpose of the study was to develop and validate a Loop-mediated Isothermal Amplification (LAMP) assay for identification of *S. brasiliensis.* **Materials & Methods:** LAMP reaction was developed using six primers designed (FIP, BIP, F3, B3, LF and LB) based on calmodulin gene sequences, using the software NEB LAMP Primer Design Tool. To validate the reaction, human clinical fungal isolates (n=20) and biopsy samples from left footpad (n=10) from murines (Specific Pathogen Free' BALB/cJ) experimentally infected with *S. brasiliensis* were used. These samples were obtained from the Mycology Lab FAMED-FURG and ISCMPA. The DNA of isolates and clinical samples was extracted with the commercial High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). LAMP reaction was performed following the manufacturer's indications from WarmStart Colorimetric LAMP 2x Master Mix (New England Biolabs). Reactions were carried out at 65°C and time of incubattion ranged between 30 to 40 minutes. The LAMP products were visualized by color change from pink to yellow. To evaluate the specificity of LAMP reaction, DNA from isolates of other fungal species [*Cryptococcus* sp. (n=1), *Rhodotorula* sp. (n=1), *Candida albicans* (n=1), *S. schenckii* (n=2), and *S. globosa* (n=1)] were submitted to LAMP and, in addition, the primer set sequence was hybridized *in silico* with GenBank data using the Basic Local Alignment Search Tool (BLAST) from National Center for Biotechnology Information (NCBI), to verify possible alignments with other fungal species. The limit of DNA detection were evaluated with DNA from isolates and compared by assaying in parallel tenfold serial dilutions, starting with 10 ng/µL and ending with 0.01 fg/µL. *In vivo* samples were obtained according to the Animal Use Ethics (CEUA-FURG P003/2021). **Results**: All fungal isolates of *S. brasiliensis* were positive in the LAMP, and the limit of DNA detection was 1 ng/µL. Other fungal species were all negative. All murine experimental samples showed positivity in the test. The reaction temperature was the same for both types of samples, but the reading time differed slightly, with a standardized 30-minute reading for isolates and a 40-minute reading for clinical samples. **Conclusions**: LAMP diagnosis technique may be a promising alternative to sporotrichosis diagnosis, offering several advantages in terms of specificity, sensitivity, reaction efficiency, and product yield. In addition, LAMP can contribute with high sensitivity, and specificity to epidemiological sporotrichosis studies, in a simple and cost-effective way. Further studies are warranted to validate this technique using clinical samples obtained from both humans and animals. **Acknowledgments**: The authors are grateful *to the Conselho Nacional de Desenvolvimento*

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P119

Development of digital droplet PCR assay to quantify Aspergillus species - Is it useful?

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Introduction/Objectives

Invasive aspergillosis (IA) remains a major complication in patients with haematological malignancies (HM) and post allogeneic haematopoietic stem cell transplantation (HSCT). Early detection of *Aspergillus* infections has the potential to facilitate a more effective management of invasive disease. Digital droplet PCR (ddPCR) has the potential to be more sensitive than real-time PCR (qPCR) for the detection of low DNA concentrations. By dividing the reaction volume in up to 20,000 separated entities/PCR reactions (droplets) and by not negatively impacting the limit of detection (LOD) for multiplex PCRs (as in qPCR), analytical sensitivity can be enhanced.
In this study, we transferred a qPCR assay protocol to detect *Aspergillus spp*. into a digital format, compared LODs, and tested clinical, external quality control (QC) and DNA-spiked samples to evaluate the new assay formats.

Methods

A qPCR assay specific for *Aspergillus spp.* and compliant to FPCRI recommendations was adapted to digital PCR using the Bio-Rad system (QX200). Different cycling protocols and a gradient of the annealing/elongation temperature were tested to determined best performance. Furthermore, additional digests using different restrictions enzymes (RE) were evaluated to increase sensitivity in serum samples. REs were selected to cut rDNA replicons appropriately to generate amplifiable DNA fragments which can be distributed to single droplets. Dilutions of different *Aspergillus spp.* DNA (10-fold dilutions) were directly compared in both PCR systems and LODs were determined. A second assay (mold-independent target) used as DNA extraction and PCR inhibition control assay (IC) in qPCR, but run in separated well (monoplex), was used in ddPCR in a duplex reaction. QC samples consisted of blinded DNA spiked samples and specimens obtained from haematological and cystis fibrosis patients with proven and probable IA.

Results

The existing 3-step qPCR protocol performed best in ddPCR and was optimized to detect different *Aspergillus spp*. Using RE digests showed no improvement of ddPCR in clinical samples. LODs were comparable in both systems for all *Aspergillus spp.* tested. A second mold-independent assay run as duplex in ddPCR facilitated monitoring of PCR inhibition and extraction efficacy in the same sample aliquot without losing sensitivity. DNAs from patient sera being positive in qPCR assays were retested by ddPCR after one freezing/thawing cycle and showed reduced positivity rates in both assays.

Conclusions

ddPCR assays can be less prone to PCR inhibition, more reproducible and direct quantification of fungal load in serum is possible. Duplexing using ddPCR reduces costs and enables quantification of two targets in the same reaction. RE digests was not helpful to increase sensitivity. Further comparative validation in large multi-centre studies is warranted.

P120

Phenotypic characterisation and rapid identification of Candida auris by semi-nested colony PCR

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OBJECTIVES

- To demonstrate phenotypic characteristics of *Candida auris*.
- To standardise a semi-nested colony PCR for rapid identification of *Candida auris*.
- To compare the phenotypic and genotypic characterisation of *Candida auris*.

METHODOLOGY

A total of 69 *Candida* species which grew from blood samples collected over a period of one year were considered for the study. All the *Candida* isolates were subjected to conventional phenotypic methods like gram stain, temperature studies by inoculating in Malt Extract Agar and incubating at 37°C, 40°C, 42°C for 24-48 hours, chlamydospore production was observed by inoculating in Corn Meal Agar with 1% tween 80 & trypan blue, colour production on chromogenic media and carbohydrate fermentation & assimilation characteristics were recorded.

Genotypic identification was performed for all the 69 *Candida* isolates. PCR – RFLP was initially done for the genotypic identification. The isolates which were unidentifiable by this method were further identified by gene sequencing. Also, the semi-nested colony PCR was performed using self-designed *Candida auris* specific primers (ITS 1 as forward primer and the self-designed AURP (5'-AATGCAACGCCACCGCGAAG-3') as reverse primer) for all the 69 isolates. ATCC *Candida albicans* 90028 (negative control) and *Candida auris* sequenced and confirmed with Gen bank ID: MK064197 (positive control) were used for the study.

RESULTS

Among 69 isolates considered for the study, n = 21 was found to be *C. tropicalis*, n = 13 *C. albicans*, n = 5 *C. guilliermondii*, n = 3 *C. glabrata*, n = 14 *C. parapsilosis*, n= 13 *Candida sp.* For the 13 *Candida* isolates, phenotypic characterisation was inconclusive and hence were considered as *Candida sp*. by common phenotypic methods.

Phenotypic Characterization of *C.auris*

Candida auris on gram stain showed budding yeast cells but no pseudo-hyphae were observed, Chlamydospore production was negative. No colour difference was observed on

chromogenic media like TTZ and CHROM agar medium. Carbohydrate fermentation test for *C.auris* fermented 2% Maltose. *C.auris* grew at 25ºC, 37ºC, 40ºC and 42ºC, and was found to be thermotolerant.

Genotypic characterisation of *Candida auris*

PCR-RFLP was performed for all the isolates to confirm speciation in which, n = 21 *C.tropicalis*, n = 13 *C.albicans*, n = 5 *C.guilliermondii*, n = 4 *C.glabrata*, n = 5 *C.parapsilosis*, n= 21 *Candida sp*. Among the 21 *Candida sp*., sent for gene sequencing, n = 3 were *C.famata*, n = 3 *C.orthopsilosis*, n = 12 *C.auris*, n = 1 *C.metapsilosis*, n = 1 *C.duobushaemulonii* and n = 1 *C.haemulonii.*

***Candida auris* specific PCR**

All the 69 *Candida* isolates were subjected to *C.auris* specific semi-nested PCR for identification. 12 out of the 69 *Candida* isolates produced amplicons of size 300bp respectively. The rest 57 isolates did not produce bands (Figure 1). All the 12 isolates confirmed as *C.auris* by gene sequencing, produced DNA amplification bands approx. 300bp. The controls were satisfactory.

CONCLUSION

To conclude, a rapid (2-hour) single step semi nested colony PCR method for differentiating *Candida auris* and *Candida haemulonii* was standardised. This method not only differentiates *Candida auris* from *Candida haemulonii* but also other *Candida* species. This test will be useful in the rapid identification of *Candida auris* in any microbiological laboratory setup.

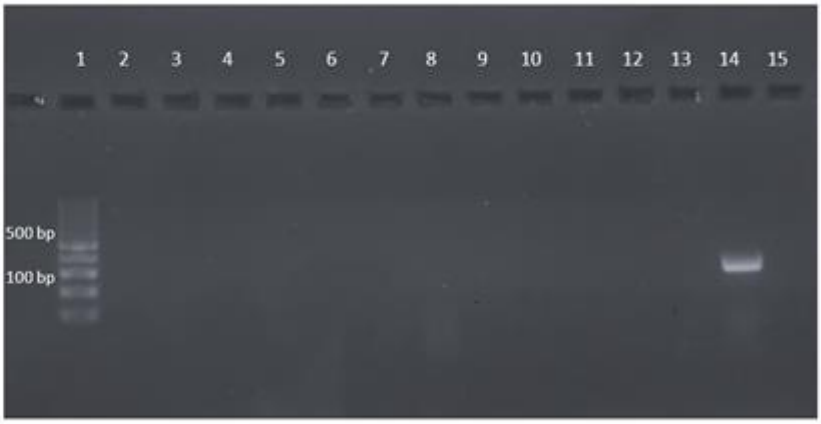

Figure 1: PCR subjected to various *Candida* species (Lane1: DNA ladder, Lane 2: *Candida albicans* ATCC 90028, Lane 3: *C.tropicalis*, Lane 4: *C.parapsilosis*, Lane 5: *C.guilliermondii*, Lane 6: *C.glabrata*, Lane 7: *C.duobushaemulonii*, Lane 8: *C.orthopsilosis*, Lane 9: *C.metapsilosis*, Lane 10: *C.famata*, Lane 11: *C.lusitaniae*, Lane 12: *C.kefyr*, Lane 13: *C.catenulata*, Lane 14: *C.auris*, Lane 15: *C.haemulonii*)

P121

Multicenter Evaluation of the VirClia Galactomannan Assay on Serum from Patients with Hematological Malignancies

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Objectives:

Galactomannan(GM) in serum is used as a biomarker for invasive aspergillosis (IA). The ELISA-based Platelia®test is frequently used for this purpose and has a moderate sensitivity, but good specificity. A drawback is that it requires batching which hampers ad-hoc testing. The VIRCLIA®Galactomannan AG assay can be used on individual serum samples and provides a quantitative result within 1.5h. This assay detects GM in an automated chemiluminescence immunoassay and its performance is comparable to the Platelia®assay on bronchoalveolar lavage fluid(BALf).(1) In this study, we compared the VIRCLIA® with the Platelia® on serum.

Materials & Methods:

Patients undergoing treatment for hematological malignancies or after stem cell transplantation between 2017 and 2022 in 4 tertiary care hematology centers were included when an invasive fungal infection was suspected and serum collected at that time. Informed consent was obtained for the use of residual serum for research purposes. Both tests were performed simultaneously, after the same number of freeze-thaw cycles. In the main analysis, patients were classified according the EORTC/MSGERC 2020 consensus definitions as having proven, probable or possible IA. Proven and probable IA were defined as cases, while possible IA and no IA patients were used as controls. All results were analyzed using the low thresholds of 0.5

ODI for Platelia® and 0.16 ODI for VIRCLIA®. Sensitivity of both tests was compared with McNemar's test for paired proportions. In the secondary analysis, the Platelia® GM result in serum was excluded from the EORTC/MSGERC definitions to avoid incorporation bias which would favor the Platelia®test.

Results:

163 patients were included. Six patients were classified as proven IA(4%), 67(41%) as probable IA, 67(41%) as possible IA and 23(14%) were classified as no IA. Platelia®GM on serum was positive in 32(20%) and VIRCLIA® in 16(10%) samples (figure 1). In comparison, BALf GM(Platelia®) was positive in 64(39%) patients (VIRCLIA® BALf result not available). Using the 0.5 and 0.16 ODI cut-off, a positive agreement of both GM tests on serum was found in 15/32(47%), a negative agreement in 129/131(98%). Cohen's Kappa coefficient was 0.55(moderate agreement). The sensitivity to detect a probable/proven IA was 38%(n=28/73) for the Platelia® compared to 20%(n=15/73) for VIRCLIA® (p=0.004) while specificity was >95% for both tests. When the serum Platelia®GM result was excluded from the definitions, 6 patients had a proven and 63 a probable IA(i.e. based on a positive culture, GM or PCR on BALf as the mycological criterion). The VIRCLIA® and Platelia® detected 13(19%) and 24(35%) of the IA cases (p=0.010). In an exploratory analysis, lowering the VIRCLIA® cut-off to 0.10 increased the sensitivity to 37%(n=27/73) and lowered the specificity to 86%(n=77/90) (figure 1).

Conclusion:

Even in this highly selected patient group, the sensitivity of GM testing on serum collected at the time of IA suspicion was low. While we previously showed that the VIRCLIA® and Platelia®tests performed equally well on BALf, the sensitivity of VIRCLIA® on serum was only similar when the cut-off was lowered to 0.10 but confirmation in an independent study is required. Whether serial samplingwill improve sensitivity remains to be seen.

References

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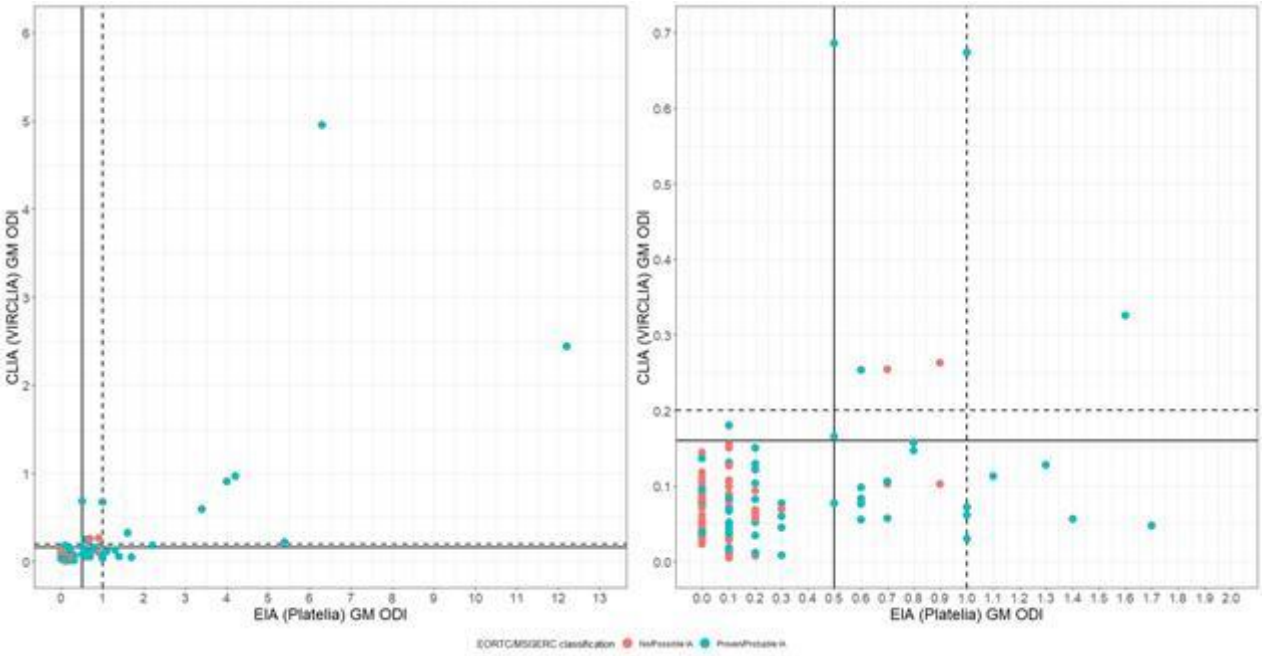

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	ROC AUC (%, 95%CI)
Proven/Probable vs No IA					
GM Platelia ≥1.0	23	100	100	29	78 (69 – 88)
GM Platelia ≥0.5	38	91	93	32	
GM CLIA ≥0.2	14	96	90	26	64 (51 – 76)
GM CLIA ≥0.16	19	96	93	27	
GM CLIA ≥0.10	37	83	87	29	
Proven/Probable vs Possible/No IA					
GM Platelia ≥1.0	23	100	100	62	79 (72 – 86)
GM Platelia ≥0.5	38	96	88	66	
GM CLIA ≥0.2	14	98	83	58	66 (57 – 74)
GM CLIA ≥0.16	19	98	88	60	
GM CLIA ≥0.10	37	86	68	63	
Proven/Probable vs No IA (serum GM excluded)					
GM Platelia ≥1.0	18	100	100	29	77 (66 – 87)
GM Platelia ≥0.5	35	91	92	32	
GM CLIA ≥0.2	14	96	91	27	63 (51 – 76)
GM CLIA ≥0.16	19	96	93	28	
GM CLIA ≥0.10	36	83	86	30	
Proven/Probable vs Possible/No IA (serum GM excluded)					
GM Platelia ≥1.0	19	96	76	62	75 (67 – 83)
GM Platelia ≥0.5	35	91	75	66	
GM CLIA ≥0.2	14	98	83	61	64 (56 – 73)
GM CLIA ≥0.16	19	97	81	61	
GM CLIA ≥0.10	36	84	63	64	
Proven/Probable vs No IA (GM excluded)					
GM Platelia ≥1.0	19	100	100	32	76 (66 – 87)
GM Platelia ≥0.5	35	91	75	66	
GM CLIA ≥0.2	15	96	90	31	61 (47 – 74)
GM CLIA ≥0.16	19	96	92	31	
GM CLIA ≥0.10	34	83	83	33	
Proven/Probable vs Possible/No IA (GM excluded)					
GM Platelia ≥1.0	19	96	76	62	70 (61 – 79)
GM Platelia ≥0.5	31	91	90	34	
GM CLIA ≥0.2	14	98	83	61	59 (50 – 68)
GM CLIA ≥0.16	19	97	81	62	
GM CLIA ≥0.10	36	84	63	64	

Table 1: Performance of the Platelia and VIRCLIA galactomannan assays on serum

P122

Factors influencing the probability for positive beta-D-glucan in patients with candidemia

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Objectives:
To investigate factors that predict a positive beta-D-glucan (BDG) test in patients with candidemia and to assess if a positive BDG result is associated with an increased mortality.

Materials & Methods:
This retrospective study included consecutive patients during 2015 – 2021 with ascomycetous yeasts in blood cultures in the regions of Västra Götaland and Skåne, Sweden, who had a biobanked frozen serum, that had previously been analyzed for BDG with Glucatell (Associates of Cape Cod). Complementary analyses with Wako beta-glucan test (Fujifilm) were performed on all biobanked serum samples. Controls, consiting of patients with a blood culture without growth of fungi, were matched to the cases by age, sex and type of clinic at the day of sampling in a 1:1 fashion. The optimal cut-off defining a positive result for each BDG test was assessed by ROC-curve analysis. Laboratory and medical records were reviewed to obtain data on predefined variables that could be associated with the odds of obtaining a positive BDG result. This was investigated by univariable and multivariable logistic regression analysis. Variables with p <0.2 in univariable analysis were included in the multivariable analyses.

Results:
We included 134 cases with candidemia and 132 controls. Among the cases, 38 patients had a proven or probable deep focus of infection, 21 with intra-abdominal candidiasis (IAC) and 17 with other deep foci. Thirty-eight patients underwent abdominal surgery, 55 were admitted to an intensive care unit at sampling and 23 had septic shock..
ROC curve analysis and calculation of Youden index suggested an optimal cutoff ≥169 pg/mL for Glucatell (sensitivity 0.71 [95% CI 0.62 – 0.78], specificity 0.92 [95% CI 0.87 – 0.93]) and ≥3.231 pg/ml for Wako (sensitivity 0.70 [95% CI 0.62 – 0.67], specificity 0.88 [95% CI 0.81 – 0.93]).
In univariable analysis, abdominal surgery (OR 2.79 [95% CI 1.12 – 8.01]) and a deep focus of infection other than IAC (OR 8.77 [95% CI 1.68 – 162]) significantly increased the odds for obtaining a positive result using Glucatell. Deep focus of infection other than IAC was also statistically significant with similar OR for Wako, while abdominal surgery was not. In multivariable analysis, only deep focus of infection other than IAC remained significant when adjusting for abdominal surgery, age, yeast species, septic shock and time to positive blood culture.
Ninety day mortality of patients with positive BDG using Glucatell was 51%, compared to 26% with negative BDG (p = 0.014). The results were similar using Wako. There was no significant difference at 30 days.
Conclusions:

Patients with candidemia that had a deep focal or disseminated infection other than IAC, were more likely to have a positive BDG result using either Wako or Glucatell analysis. Abdominal surgery was also weakly associated with a positive BDG result. A possible explanation to this could be more extensive infections with higher fungal loads. For variables such as yeast species, IAC and septic shock, the power of the study is too low to exclude possible associations. Patients with a positive BDG had a higher mortality than those with a negative BDG.

P123

Establishment of a novel qPCR based on mitochondrial markers for the detection of eukaryotic pathogens

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Background:

Eukaryotic pathogens such as fungi and parasites have a much lower burden than bacteria and viruses. Moreover, infected tissue often has to be used instead of serum and blood. Amongst the fungal pathogens, mucormycetes are most difficult to diagnose, due to their fragile hyphae that are easily ruptured. To overcome this diagnostic gap and to establish a marker that is particularly suitable for eukaryotic pathogens we establish robust mitochondrial markers for a probe-based pan-Mucorales qPCR assay.

Methods:

The qPCR assay is based on mitochondrial genes and the human mitochondrial 12S rRNA gene as internal control (IC). In a set of experiments, key characteristics of the pan-Mucorales qPCR assay were determined, such as cross-reactivity, specificity, sensitivity, limit of detection (LOD) and amplification efficiency. A total of 150 fungal DNAs were evaluated, including: (a) 51 DNA samples from ascomycetous and basidiomycetous pathogens and (b) 99 DNA samples of mucormycetes. To rule out cross-reactivity with host DNA, human and animal DNA were tested.

The final duplex pan-Mucorales qPCR assay was evaluated on a comprehensive set of human FFPE tissue specimens with confirmed fungal infection and proven/suspected mucormycosis.

Results:

The pan-Mucorales qPCR assay detect over 45 different clinically relevant Mucorales species from pure culture. Cross-reactivity could be ruled out for all tested DNA libraries, except for *Galleria mellonella* that gave a weak signal. A limit of detection (LOD) of 100 fg or 2-3 genome copies was found in the majority of 11 selected mucoralean species. The amplification efficiency ranged from 85.7% to 105.8%. The final duplex pan-Mucorales qPCR assay was able to detect proven mucormycosis in tested human FFPE tissue specimens.

Conclusion:

Mitochondrial genes are promising new markers for the detection of eukaryotic pathogens.

P125

STANDARDISATION OF MULTIPLEX PCR FOR IDENTIFICATION OF DERMATOPHYTES

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OBJECTIVE

To standardise multiplex PCR for early identification of dermatophytes from clinical isolates.

MATERIALS AND METHODS

A total of 44 dermatophyte isolates collected from patients and sent for culture to the mycology laboratory, Department of Microbiology, Sri Ramachandra Medical College and Research Institute were considered for this study. Phenotypic characterization was done by observing colony morphology and growth characteristics on Sabouraud's dextrose agar & Oat Meal Agar, microscopic morphology by LPCB mount, Urease test and hair perforation test. Genotypic characterisation was done by performing a simple multiplex PCR using self- designed primers with appropriate positive and negative controls. The self-designed primers used were, forward primers of *Genera Microsporum* (M2F: 5' -GCACGCCCATTCTTGTCTAC-3'), *Genera Trichophyton* (T3F: 5'- CGGAGGACAGACACCAAGAA -3'), *Genera Epidermophyton* (E1F: 5'-TGTCTACACTACCCGGTTGC - 3') and ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') was used as reverse primer.

An in-house column-based technique was used to extract the fungal DNA.

PCR reaction and amplification protocol were standardized. PCR amplicons were visualized by agarose gel electrophoresis.

RESULT

The multiplex PCR was able to differentiate the three genera of dermatophytes producing an amplicon size of 610 base pairs for genus *Epidermophyton,* 700 base pairs for genus *Microsporum* and 473 base pairs for genus *Trichophyton*. The positive controls also produced similar products of similar base pair size, and the negative control did not produce any band, thus validating our results.

CONCLUSION

Multiplex PCR is comparatively a rapid and cost-effective method for diagnosis of patients suspected with dermatophytosis which complements the conventional diagnostic tools such as microscopy with KOH and culture. This underlines its utility in improving the sensitivity and specificity of the conventional tools as the multiplex PCR detects even a single copy of a specific DNA template. In addition to this it helps to differentiate dermatophytosis from other similar skin conditions and surpasses the 2-4 weeks time taken by the conventional method of diagnosis for the growth and additional time for the sporulation of the organisms in culture.

P126

Performance of single sample β-(1→3)-D-glucan assays: the Fungus (1-3)-β-D-Glucan lateral flow assay, the Fungitell® STAT, and the Fujifilm β-glucan test

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Objectives: β-(1→3)-D-Glucan (BDG) is the most sensitive biomarker for invasive fungal disease. Most data on the performance of BDG were generated using the Fungitell® assay (FA). However, the FA has some disadvantages concerning the workflow if small sample sizes are to be analysed. The most economical way to perform the FA is with 21 patients per run, a sample number that is, however, rarely achieved on a daily basis in most European laboratories. As a consequence, samples are collected and the FA is not performed daily. BDG assays, which can be carried out cost-effectively with one sample or small series, provide a remedy here. Such assays are the new Fungus (1-3)-β-D-Glucan lateral flow assay (LFA, TECOmedical AG), the Fungitell® STAT assay (FA-STAT) and the Fujifilm β-glucan test (GT). The LFA is unique in that it does not require a kinetic measurement like the other tests, and is therefore much easier to perform. In the present study we examined the diagnostic performance of these single sample BDG assays and compare them to the classical FA.

Methods: We performed a case-control study on three patient groups, namely (1) patients with blood culture-proven candidemia, (2) patients with proven and probable invasive aspergillosis and (3) patients with *Pneumocystis jirovecii* pneumonia (PCP). Patients in the aspergillosis group were categorized according to the consensus definitions of Invasive Fungal Disease from the EORTC/MSG. Patients of the PCP group needed to have detection of *Pneumocystis jirovecii* by PCR or microscopy as well as a compatible clinical and radiological presentation. The FA, the LFA, the FA-STAT and the GT were performed according to the manufacturers' recommendations.

Results: The following number of patients were included in the study groups: candidemia group (150 patients with candidemia, 30 and 20 control patients with bacteremia and negative blood cultures, respectively), aspergillosis group (four patients with proven and 43 patients with probable invasive aspergillosis) and PCP group (63 patients with PCP). The sensitivity and specificity in the candidemia group were 82.6% and 68.3% for the FA (cutoff 80 pg/ml), 51.4% and 91.7% for the LFA (cutoff 100 pg/ml), 68.3% and 78.3% for the FA-STAT (cutoff 1.2), and 57.0% and 92.0% for the GT (cutoff 7 pg/ml). With all three assays the mean BDG value was

significantly higher in the candidemia than in the control group (<0.0001). The sensitivity of the FA, FA-STAT and GT in the aspergillosis group was 68.1%, 61.4% and 54.5% and in the PCP group 98.3%, 98.3% and 90.5%, respectively.

Conclusions: The FA is still the most sensitive BDG assay followed by the FA-STAT, the GT and the LFA. All three single sample assays proved to be robust, reliable and easy to use. However, from the point of view of practicality and resource requirements, the LFA is the most suitable, as all that is needed is a pipette, a vortex and a fluorescence reader provided by the company. The LFA will allow any laboratory to measure BDG regardless of the number of samples and equipment.

P127

Reporting on the diagnostic accuracy of a rapid Aspergillus-specific lateral flow device in patients with fungal keratitis.

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Objectives: There is an urgent requirement to develop and implement rapid and simple diagnostics for fungal keratitis (FK) to aid in clinical decision making and to improve patient outcomes. The AspLFD (OLM Diagnostics) *Aspergillus* lateral flow device is approved for use in suspected invasive pulmonary Aspergillosis, however its utility in FK diagnosis has not been studied. Here we evaluate the AspLFD and report on diagnostic accuracy against clinical cases of FK at a tertiary centre in south India.

Materials & Methods:

Approval was obtained from the Institutional Review Board of Aravind Eye Hospital, Madurai, India. 419 patients presenting with suspected microbial keratitis were recruited following informed consent. During routine diagnostic scraping, a minimally invasive corneal swab, and one additional corneal scrape were collected and transferred to aliquots of sample buffer. Routine diagnostic smear microscopy was performed and patients who met inclusion criteria had their additional scrape or swab assessed by AspLFD (n = 198 scrape samples and n = 40 swab samples). The device was visually inspected and scored 20 min after sample addition. Photographs of devices were taken for a non-biased semi-quantitative analysis approach, developed as part of the study. AspLFD results were compared to culture reports (LFD scorers were blinded to culture report at time of scoring).

Results:

35/198 and 17/40 of culture positive samples were *Aspergillus* spp. Ratiometric analysis results for **scrape** samples (95% CI): Sensitivity: 0.89 (0.74 – 0.95); Specificity: 0.95 (0.91 – 0.98); Positive Likelihood-Ratio: 18.05 (9.09 – 35.84); Negative Likelihood-Ratio: 0.12 (0.05 – 0.30); and Accuracy: 0.94 (0.90 – 0.97). Ratiometric analysis, for **swab** samples (95% CI): Sensitivity: 0.94 (0.73 – 1.00); Specificity: 0.83 (0.63 – 0.93); Positive Likelihood-Ratio: 5.41 (2.20 – 13.29); Negative Likelihood-Ratio: 0.07 (0.01 – 0.48); and Accuracy: 0.88 (0.73 – 0.96).

Conclusions: Ratiometric analysis of LFDs for the diagnosis of FK demonstrated high diagnostic accuracy in identifying patients with *Aspergillus* FK direct from corneal scrapes and minimally invasive swabs. This is an important step towards the provision of point-of-care diagnostics for microbial keratitis, and could inform on clinical management strategy.

P128

Comparison of MALDI-ToF MS instruments and databases for identification of uncommon yeasts, Aspergillus and rare filamentous fungi

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Objectives:

While Matrix-Associated Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDI-ToF MS) identification is widely still remains a challenge for the identification of uncommon fungi. The performance of MALDI-ToF-MS depends on the protein extraction method, the instrument, algorithm and database. This study aimed to compare 3 mass spectrometers (MS) [Vitek MS Legacy (bioMérieux, Marcy l'Etoile, France), Vitek MS Prime bioMérieux, and Microflex (Bruker, Billerica, USA)] and 5 databases in order to implement this technique in our routine.

Materials & Methods:

We analyzed isolates of *Aspergillus* species (AS); rare molds (RM) and uncommon yeasts (UY) from our collection stored at -20°C, isolated from 2007 to 2022. All the isolates were previously identified by sequencing the relevant barcoding genes.

A two-steps protein extraction with ethanol then formic acid and acetonitrile was conducted, adapted from Cassagne *et al.* MALDI-ToF identification was performed at day 3 (D3) and day 8 (D8) of growth on 3 MS: VITEK®MS Legacy, VITEK®MS PRIME and Microflex®. The obtained spectra were submitted to their corresponding databases: Knowledge Base (KB) 3.2, KB3.3 and Saramis 4.17 (bioMérieux) and Fungi V5 (Bruker) and MSI2 database (Normand AC *et al.*).

Results:

We analyzed 167 isolates, including 61 AS, 74 RM and 32 UY, representing 33 genus and 93 species. D3 testing gave better results than D8 ($p < 0.001$) and was then used for further analysis (Table 1).

We could not obtain analyzable spectra for 28 strains (16%) using Prime® and for 6 strains (3.4%) using Legacy®. This information was not available for Microflex®.

Regarding UY, the correct identifications ranged from 63% to 84% at the genus level and from 51% to 79% at the species level (Table 1).

Regarding AS, the correct identifications at D3 ranged from 48% to 98% at the genus level and from 18% to 80% at the section level (Table 1).

Regarding RM, the rates ranged from 37% to 81% at the genus level and 11% to 49% at the species level at D3. The increased from 55% to 81% and from 36% to 49% at the genus and species levels respectively using MSI vs. commercial databases.

If the identification was not correct, there was either no possible identification (species not included in the database or confidence score not acceptable) or a misidentification. All databases combined, there was no possible identification at the species level for 32% of the strains using Legacy®, 35% using Prime® and 47% using Microflex®.

Conclusions:

MALDI-ToF seems to be an interesting way to identify fungi. No significant differences in rates of correct identification with Vitek MS Legacy and Prime was observed. The highest scores of correct species identification were reached for yeasts regardless of the instrument. For AS, there were no major differences between bioMerieux's databases and MSI2 at the section level. For RM, MSI2 gave the best results.

Table 1 Correct identification rates (%) at the genus, section or species level at D3

Genus** 0,09	Species/Section** 0,11	Database	Instrument	D3 Genus	D3 Species/Section*	Type
		KB3.2	Legacy	84	77	Yeasts
			Prime	83	79	
		KB3.3	Legacy	84	78	
			Prime	83	79	
		Saramis 4.17	Legacy	67	60	
			Prime	75	71	
		Fungi V5	Microflex	80	51	
		MSI2	Legacy	63	57	
			Prime	67	54	
			Microflex	83	66	
		KB3.2	Legacy	80	80	*Aspergillus*
			Prime	76	75	
		KB3.3	Legacy	85	51	
			Prime	85	55	
		Saramis 4.17	Legacy	70	61	
0,02	0,05		Prime	78	76	
		Fungi V5	Microflex	48	18	
		MSI2	Legacy	98	69	
			Prime	91	60	
			Microflex	92	67	
		KB3.2	Legacy	46	31	Molds
			Prime	37	24	
		KB3.3	Legacy	55	36	
			Prime	46	28	
		Saramis 4.17	Legacy	39	24	
0,001	0,18		Prime	42	22	
		Fungi V5	Microflex	38	11	
		MSI2	Legacy	81	49	
			Prime	75	36	
			Microflex	76	43	

**Fisher's exact test

* Section only for *Aspergillus*

P130

Prospective service evaluation of T2Candida for the diagnosis of Invasive Candidiasis in the ICU: including an audit of antifungal stewardship.

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Objectives: Non-culture based biomarkers are increasingly being used in the diagnosis of invasive candidiasis (IC) as blood culture lacks sensitivity. T2Candida is a novel molecular method combining PCR with magnetic resonance-based technology. A recent meta-analyis reported a pooled sensitivity and specificity of 0.91% (95% CI: 0.88–0.94) and 0.94 (95% CI: 0.93–0.95) using T2candida for the diagnosis of IC (1). Most studies also report high negative predictive values, which may facilitate antifungal stewardship (2, 3). The Royal Free London NHS Foundation Trust is a specialist centre for liver and renal transplants, infectious diseases, and patients with primary immuno-deficiency. The objective of this service development study was to establish the performance of T2Candida for the diagnosis of IC in the ICU and audit how T2Candida impacted antifungal use.

Materials & Methods: T2Candida requesting was restricted to the ICU for patients classified as high risk of IC at fungal MDT/antifungal stewardship rounds. Clinicans were instructed to request blood cultures (BD BacTec) and 1-3-β-D-glucan (Fungitell, Cape Cod) at the time of T2Candida requesting. Proven IC was defined according to EORTC/MSG criteria (4) and probable IC was defined using criteria requiring one host factor, one mycological criteria and one clinical feature, table 1. Performance of T2Candida was calculated for proven/probable disease v's controls**.** The following data was collected:

1. Investigation of Candidemia or deep seated IC at requesting
2. Antifungal therapy at T2Candida requesting a) no antifungal b) prophylaxis c) pre-emptive (BDG) d) targeted (culture source) e) empirical.
3. Impact of T2Candida on antifungal therapy a) discontinue b) initiate/escalate c) prevent administration/escalation d) define duration e) no impact.

Results: 172 T2Candida tests were requested for 113 patients. 4.7% (8/172) tests from 7 patients were excluded due to invalid T2Candida results. Patients were classified as proven IC 2.9% (3/104), probable IC 10.5% (11/104) and controls 86.5% (90/104). T2Candida performance for the diagnosis of proven/probable IC was sensitivity 70% (95% CI: 24.8, 93.3), specificty 98.1% (95% CI: 94.4, 99.6), PPV 70% (95% CI: 41.5, 88.4) and NPV 98.1% (95% CI: 95.1, 99.2). Deep seated IC was being investigated in 61.5% (64/104) and Candidaemia in 38.5% (40/104) of patients.

- At the time of T2Candida requesting antifungal therapy was documented as 27.4% (45/164) empirical, 21.3% (35/164) pre-emptive, 18.9% (31/164) targeted, 15.9% prophylaxis (26/164) and 16.5% (27/164) no antifungals.
- Impact of T2Candida on antifungal therapy was recorded as 23.7% (39/164) define duration, 9.1% (15/164) start/switch, 29.2% (48/164) no impact, 17.1% (28/164) discontinue and 20.7% (34/164) to prevent antifungals being initiated/escalated.

Conclusions: Despite over 80% of patients recieveing an antifungal at the time of testing T2Candida demonstrated a high negative predictive value for the diagnosis of IC. For the majority of patients the T2Candida result impacted on the antifungal administration. In almost 40% of patients antifungal use was either prevented or discontinued. A significant proportion of T2Candida tests had no impact on antifungal therapy. As T2Candida has a considerable cost associated with testing further study is required to establish the optimal use of T2Candida in the ICU setting.

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Host factor	Mycological evidence	Clinical features
Receipt of SOT (<28 days)	Two consecutive positive BDG >80 pg/mL or a single positive BDG >250 pg/mL	Abscesses in liver or spleen (bull's-eye lesions)
Abdominal surgery (<28 days)	Positive culture of *Candida spp.* from a deep site correlating with radiology	Progressive retinal exudates or vitreal opacities
Non-resolving fever >38°C despite antimicrobial therapy >96hrs		Radiological evidence of intra-abdominal collection

P131

Mucorales extracellular polysaccharides: potential targets for the development of new biomarkers?

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Objectives. Fungal extracellular polysaccharides (EPSs) have been shown to exhibit significant biological activities, including immunomodulation, antioxidant, anticancer and antiviral activities. Mucormycosis (MM), an emerging angio-invasive fungal infection, is associated with high rates of morbidity and mortality. Data regarding biochemical characteristics and biological properties of EPSs from Mucorales are scarce. The objectives of this study were to analyse the carbohydrate content and linkage pattern of EPSs from Mucorales and to assess the value of serum anti-EPS antibodies for the diagnosis of MM.

Methods. Two strains of *Lichtheimia corymbifera* (one reference strain IHEM 21658; one clinical isolate) and one clinical isolate of *Rhizopus arrhizus* were used. After 7 days of incubation in yeast nitrogen base media supplemented with 0.5% glucose, the culture broth was precipitated with four volumes of ethanol at 4°C before deproteinisation using the Sevag method to isolate EPSs. The monosaccharide composition of the EPSs was determined by gas-chromatography using a flame-ionisation detector. Structural analysis was performed by nuclear magnetic resonance and gas chromatography-mass spectrometry. Serum samples from 10 healthy subjects, 15 patients with invasive aspergillosis, 10 patients with candidaemia and 12 patients with MM (CHU Lille, France) were collected. Detection of serum anti-Mucorales EPS antibodies was performed using a direct enzyme-linked immunosorbent assay (ELISA).

Results. Fucose, glucose, mannose, galactose, N-acetylglucosamine and glucuronic acid were present in varying proportions depending on the strain. EPSs produced by the reference strain of *L. corymbifera* contained a high average amount of glucose (68%) unlike the clinical strain (2.5%) and *R. arrhizus* (0.5%) strain. Sera from patients with MM exhibited a significant antibody response against Mucorales EPSs. However, antibodies directed against Mucorales EPSs were also present in a subset (25%) of sera collected from patients with invasive aspergillosis, suggesting cross-reactivity between EPSs obtained from Mucorales and *Aspergillus* spp. EPSs from *R. arrhizus* showed less cross-reactivity and were chosen to

immunise mice to develop a monoclonal antibody for further development. After immunisation of mice, sera were tested by ELISA and showed reactivity against several EPSs from *Lichtheimia, Rhizopus, Syncephalastrum* and *Mucor* spp. Interestingly, there was no cross-reactivity with galactomannan from *Aspergillus fumigatus* or mannan from *Candida albicans*.

Conclusion. Inadequate antifungal treatment is recognised as an independent determinant of mortality in patients with MM, demonstrating the need for powerful and complementary tools for the early diagnosis of MM. As EPSs are potential immunogens for anti-Mucorales monoclonal antibody production, they seem to be promising candidates for the development of an antigen detection test for MM.

Key words: Mucormycosis; extracellular polysaccharides; diagnosis

P132

The current state of laboratory mycology and access to antifungal treatment in Argentina

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The current state of laboratory mycology and access to antifungal treatment in Argentina

Background

Immunosuppressed patients, transplant recipients, and those with respiratory diseases are at increased risk for invasive fungal infections (IFI) in Argentina. Despite the national public system guarantees universal access to health care for all citizens, little is known about the quality of available diagnostic and treatment armamentarium for IFI in the country.

Methods

Between June and August 2022, infectious diseases clinicians from each of the 23 provinces and the Autonomous City of Buenos Aires were contacted to describe local access to fungal diagnostic tools and antifungal agents. The information collected included different aspects such as hospital characteristics, patients admitted and wards, access to diagnostic tools, estimated infection incidence and treatment capacity.

Results

Thirty responses were collected from facilities throughout Argentina. Most institutions were governmental (77%). A mycology department was available in 83% of them. Histopathology was available in almost 93% of the sites, while automated methods and galactomannan tests were available in 57%, each; 53% of the sites had access to MALDI-TOF-MS through regional reference laboratories and PCR was present in 20% of the sites. Susceptibility testing was available in 63% of the laboratories. *Candida* spp. (24%), *Cryptococcus* spp. (20%), *Aspergillus* spp. (18%), and *Histoplasma* spp. (16%) were described as the main pathogens. Fluconazole was the only antifungal agent available in all institutions. This was followed by amphotericin B deoxycholate (83%) and itraconazole (80%). If an antifungal agent was not available on site, 60% of the patients could receive adequate antifungal treatment within the first 48 hours upon request.

Conclusions

Although there are no significant differences in access to diagnostic and clinical management of IFI among the Argentinean centres studied, national awareness-raising initiatives led by policymakers could help to improve their general availability.

P133

Direct detection of Candida auris from blood and urine samples and from surveillance swabs using a laboratory-developed real-time PCR Method

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Introduction

Candida auris is an emerging multidrug-resistant yeast which causes invasive candidiasis and health care-associated outbreaks associated with high mortality worldwide. *Candida auris* recovered from environmental surfaces in the rooms of colonized or infected patients has been implicated as one of the major causes of these outbreaks. Rapid identification of *C. auris* is of primary importance for the implementation of public health measures and control the spread of infection. Rapid DNA-based molecular diagnostic tests such as Real-time PCR assays achieve this goal. These assays allow for accurate and rapid screening of *C. auris*. It yields results within 4 hours of sample processing, compared to 4 to 14 days for culture, reducing turnaround time significantly.

Objectives

Our goal is to develop and validate a real-time PCR assay targeting the internal transcribed spacer 2 (ITS2) region of the ribosomal gene, for isolation of *Candida auris* from surveillance swabs, blood, and urine to enable rapid detection of this pathogen.

Methodology

The assay uses commercially available primers and reporter probes. Previously collected 45 isolates of *Candida auris* spiked in blood and urine samples, prospectively collected composite groin/axilla swabs of patients suspected to be colonised with *Candida auris* and swabs from hospital surfaces in the vicinity of these patients were used.

DNA was extracted using the Nucleospin mini kit for DNA (Macherey-Nagel) and real-time PCR assay was performed using the QIAGEN Rotor-Gene Q system. Cycling conditions were 95°C for 20 s, followed by 45 cycles of 95°C for 3 s and 60°C for 30 s. Appropriate positive and negative controls were used. Melting curve analysis was done and CT values determined.

The analytical sensitivity of the real-time PCR assay was determined by performing the assay in serial dilutions of the samples. The analytical specificity of the real-time PCR assay was determined by testing from a panel of other Candida species. The analytical reproducibility, both inter-assay and intra-assay was checked.

Results

This is an ongoing study. Results yet to be updated. The sensitivity and specifity of this assay will be determined and reported.

Conclusion

The *Candida auris* RT-qPCR assay is a fast, direct (without culture), sensitive, and reliable technique for the detection of live *C. auris* cells from surveillance and clinical samples. It could be an invaluable tool in surveillance efforts to control the spread of live *C. auris* in health care environments.

P134

Invasive bone cryptococcosis - A case report

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Objectives: To date, very few cases of cryptococcosis in bone have been reported in literature. We report here a case of cryptococcosis of right proximal tibia.
In the last five years a total 28 of cases of bone cryptococcosis have been reported out of which 8 cases involved lower limbs and pelvis. Literature review since 1977 identified only 5 cases of *cryptococcosis* involving tibia.
Materials & Methods: A 73-year-old male presented with pain and a discharging sinus on the upper part of right leg for 4 months. He was a chain smoker in the past 30 years with a history of cardiovascular event two years back. He had no other known co-morbid or any apparent immunosuppression. He was HIV negative. X-ray of the leg showed an osteolytic lesion on the right proximal tibia. Biopsy and pus was received from the lesion for histopathology and culture. The tissue was deparaffinized and DNA was extracted and PCR of the ITS region was done.
Results: Biopsy of the bone revealed granulation and fibro collagenous tissue with multiple abscesses. There were scattered foci of spherical yeasts within the cytoplasm of the macrophages suggestive of cryptococcosis. Pus culture yielded *Cryptococcus neoformans* with fluconazole MIC of 4 ug/ml and amphotericin MIC of 0.12 ug/ml. Sequencing of the ITS region from formalin fixed paraffin embedded tissue identified the yeasts as *Cryptococcus neoformans* with a 100% match. The patient was started on fluconazole along with surgical debridement and after eight weeks of treatment the lesion has significantly improved. Patient is still on follow-up.
Conclusions: We have presented a case which was diagnosed using combined diagnostics involving molecular, histopathology and microbiology. Such an integrated approach should be adopted in similar situations when there is a diagnostic dilemma.

P135

Rapid Identification of Clinically Relevant Candida Species from Positive Blood Cultures Using a New Molecular Assay

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Objectives: *Candida* ranks as the fourth bloodstream infection (BSI)-causing organism in the United States and as one of ten BSI-causing organisms in Europe. Diagnosis of candidemia relies on blood culture (BC), and a subculture is required for the infecting organism identification. Molecular Mouse (MM) is a newly developed real-time PCR based platform, which allows to perform microbial on-chip identification. We evaluated the Yeast Blood (YBL) chip, combined with an in-house yeast DNA extraction protocol, for direct identification of clinically relevant *Candida* species from positive BC (PBC) samples.

Materials & Methods: We used simulated (*n*=63) and clinical (*n*=43) PBCs for a total of 106 PBC samples included in the study. The simulated PBCs were obtained using well-characterized isolates of *Candida* species, including *C. albicans* (*n*=7), *C. glabrata* (*n*=7), *C. parapsilosis* (*n*=7), *C. tropicalis* (*n*=7), *C. krusei* (*n*=7), *C. dubliniensis* (*n*=7), *C. guilliermondii* (*n*=7), *C. lusitaniae* (*n*=7), and *C. auris* (*n*=7). For each isolate, 0.5-mL suspension in phosphate-buffered saline (equivalent to 500 CFU) was inoculated into a human whole-blood containing BACT/ALERT FA Plus BC bottle. Once signalled positive by the BACT/ALERT VIRTUO BC instrument, each BC sample was processed with an in-house extraction protocol, then the extracted DNA was allowed to react on the YLB chip. The YBL-chip based MM assay identification results were confirmed by those obtained with the MALDI-TOF MS analysis performed on PBC-derived colony yeast isolates.

Results: The MM assay (YBL chip combined with the in-house yeast DNA extraction protocol) allowed to identify correctly 62 (98.4%) of 63 isolates from each *Candida* species with which single BCs were obtained. Only one (1.6%) PBC for *C. lusitaniae* provided an incorrect result. Regarding clinical PBCs, all 43 (100%) of 43 *Candida* isolates that grew from PBC samples were correctly identified, such as *C. albicans* (19 samples), *C. parapsilosis* (13 samples), *C. glabrata* (9 samples), or *C. albicans* and *C. parapsilosis* (2 samples). These results well correlated with sigmoidal or exponential PCR amplification curves amplification curves.

Conclusions: Our findings shows that the new molecular assay combined with a simple DNA-extraction protocol may be a reliable method for testing *Candida* PBC samples. Further experiments will be conducted to define the role of this assay into clinical practice.

P136

Diagnostic performance of the T2Candida panel at Karolinska University Laboratory

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Objectives: The T2Candida panel detects *Candida albicans, C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosis* directly from blood. The objective of this study was to analyse the diagnostic performance of the T2Candida panel for diagnosing candidemia compared to blood cultures (BCs).
Materials & Methods: This was a retrospective study of T2 results after implementation of the T2Candida panel (T2 Biosystems) in the Karolinska University Laboratory. T2 results between May 2021 and March 2023 were included for analysis. Sensitivity, specificity, and positive and negative predictive value (PPV and NPV) were analysed for T2 samples from patients that were sampled only once (from here on referred to as single T2 samples), using results from BCs collected within three days from the T2 samples as reference. Positivity rates was computed for all samples, single T2 samples and T2 samples from repeated samplings (referred to as repeated T2 samples).
To further assess the performance of the method, other relevant microbiological cultures (blood, deep tissues and wounds, drains, grafts and indwelling intravenous catheters), beta-D-glucan and internal transcribed spacer (ITS) DNA results within a week from T2 sampling were evaluated for T2 positive/BC negative samples. Sensitivity, specificity, PPV and NPV was then calculated for single T2 samples using relevant cultures, beta-D-glucan and ITS as reference.
Results: A total of 826 T2 samples were analysed. After exclusion of invalid results, unidentified patients, duplicates and one cerebrospinal fluid sample, 738/826 (89.3 %) T2 samples were included for analysis. Of these, 292/738 (39.6 %) T2 samples were repeated samples and 446/738 (60.4 %) were single T2 samples. The positivity rates for all included T2 samples, repeated T2 samples and single T2 samples were 7.5 %, 9.2 % and 6.3 % respectively.
For 397/446 (89.0 %) of the single T2 samples there was at least one BC collected within three days from the T2 sample. Growth of *C. albicans, C. tropicalis, C. krusei, C. glabrata* or *C. parapsilosis* was detected in 11/397 (2.8 %) patients with single T2 samples. Sensitivity, specificity, PPV and NPV were 81.8 %, 95.1 %, 32.1 % and 99.5 % respectively (Table 1).
A T2 positive/BC negative result was obtained in 19/397 (4.8%) single T2 samples. For 9/19 (47.4 %) of these, the same *Candida* spp. grew in at least one other relevant culture collected within a week from the T2 sample. Beta-D-glucan was measured for 16/19 (84.2 %) patients, and positive in 13/16 (81.3 %). ITS was performed and positive for one graft material sample. For 14/19 (73.7 %) T2 positive/BC negative patients, other cultures, beta-D-glucan or ITS were positive. Considering these results as indication of true candidemia, the sensitivity, specificity and PPV increased to 92.0 %, 98.7 % and 82.1 % respectively (Table 2).
Conclusions: The present study shows that the T2Candida panel has high specificity and NPV compared to BCs. The PPV is low using only BCs as reference but increases substantially when including other relevant microbiological findings as reference.

Table 1: Total number and positivity rates for all included T2 samples and subgroups. Sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) for single T2 samples with blood cultures (BC) within three days from the T2 sample.

	n	%
All T2 samples	738	
Positivity rate	55	7.5 %
Repeated T2 samples	292	
Positivity rate	27	9.2 %
Single T2 samples	446	
Positivity rate	28	6.3 %
Single T2 samples with BC within three days	397	
Positivity rate	28	7.1 %
Sensitivity		81.8 %
Specificity		95.1 %
PPV		32.1 %
NPV		99.5 %

Table 2: Sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) of the T2Candida panel for single T2 samples (n = 397) when using relevant culture results, positive beta-D-glucan and ITS as reference.

Outcome measure	Performance (%)
Sensitivity	92.0 %
Specificity	98.7 %
PPV	82.1 %
NPV	99.5 %

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Development of a multiplex pan-Aspergillus and section Terrei specific qPCR-assay targeting the mitochondrial genome

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Introduction: Bronchopulmonary aspergillosis is a life-threatening infection caused by various aspergilli. The annual global burden of invasive aspergillosis (IA) is estimated as >300,000 cases/year. *Aspergillus fumigatus* is the predominant agent of IA causing 80–90% of the cases, followed by *Aspergillus flavus* or *Aspergillus terreus* depending on local epidemiology. Drug resistant IA is an emerging problem which is driven by acquired azole resistance in *A. fumigatus* and intrinsic polyene resistance in *A. terreus*. Early diagnosis of the causative agent and differentiation between *A. fumigatus* and *A. terreus* is essential for targeted therapy.

Objectives: Most qPCR assays for detecting fungal pathogens focus on conventional genomic DNA targets, being the internal transcribed spacer (ITS) and ribosomal RNA loci. We take a different avenue using mitochondrial genes that have a high abundance in invasive forms such as germlings and hyphae.

Material and methods: We have developed a novel multiplex pan-*Aspergillus* and section *Terrei* specific diagnostic qPCR-assay based on the MIQE-guidelines. *Aspergillus* mitochondrial genome data from 57 species were retrieved and a set of qPCR primers and hydrolysis probes was developed targeting various mitochondrial targets.

Results: The pan-*Aspergillus* assay had a cross reactivity rate of 0–2% when testing 1 ng/µl DNA samples of the non-*Aspergillus* test set (n=135) and human/animal DNA samples (n=5), respectively. A specificity of 100% (n=35) was observed when testing a comprehensive *Aspergillus* test set containing representatives from the five major sections *Flavi*, *Fumigati*, *Nidulantes*, *Nigri*, and *Terrei*. Species-specific amplification efficiency of the pan-*Aspergillus* assay was 88.1–102% and the limit of detection was 10–1,000 femtograms (fg) equal to 0.2–20 genomic copies.

Conclusion: Our results clearly show the potential of mitochondrial diagnostic markers for the detection and differentiation of fungal pathogens.

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Proposal of a strategy using ELISA for the serological diagnosis of farmer lung disease.

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Objectives
Farmer's lung disease (FLD) is a form of hypersensitivity pneumonitis (HP) due to regular exposure to microbial antigens present on moldy hay. Serology is currently performed by precipitation techniques but suppliers are becoming increasingly scarce. These techniques being time-consuming and ageing, there is a need for an automatable quantitative alternative, such as ELISA. This study aimed at developing an effective and automatable strategy for the serological diagnosis of FLD.

Methods
An ELISA-FLD using three proteic purified antigens (PPAg) and three recombinant antigens (r-Ag) targeting three microorganisms (*Saccharopolyspora rectivirgula, Lichtheimia corymbifera* and *Eurotium amstelodami*) was developed.
A 1st retrospective evaluation was performed with sera from 13 FLD and 15 Healthy Exposed Controls (HEC). A 2nd evaluation was performed with recent sera from 29 FLD and 64 Interstitial Lung Disease (ILD) patients.
Concordance with precipitations techniques, Sensitivity (Se), Specificity (Sp) were calculated.

Results
For the 1st retrospective evaluation, the concordance with precipitation techniques was of 75%, the ELISA showed a Se of 100% and a Sp of 93%.
For the 2nd evaluation, the concordance with precipitation techniques was of 70%, the ELISA showed a Se of 93% and a Sp of 80%.

Conclusions
The ELISA-FLD test proposed as screening technique for the serological diagnosis of FLD offers a good sensitivity as well as a good specificity towards sensitization to *S. rectivirgula, L. corymbifera* and *E. amstelodami*. Our strategy includes precipitation techniques for confirmation in case of moderate or positive ELISA-FLD using 6 antigens (*S. rectivirgula, Saccharomonospora viridis, Thermoactinomycetes vulgaris, L. corymbifera, E. amstelodami* and *Wallemia sebi*).

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An evaluation to assess the analytical and clinical performance of a Candida auris Real-Time PCR Kit

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Objectives: *Candida auris* is a multidrug resistant, emerging agent of invasive fungal infection in the intensive care setting, and has been responsible for serious outbreaks in healthcare institutions. Laboratory identification of *Candida auris* is difficult and can be misidentified as *Candida haemulonii,* as well as other fungal species. Real-time PCR methods for the detection of *C. auris* provide quick results for both colonisation and infection, help to control spread in the clinical setting and, with accurate identification, could lead to more appropriate patient management. Bruker have validated a real-time PCR for *Candida auris*, following IVDR guidelines and ensuring sensitive and specific detection by testing on multiple isolates associated with different global regions.

Materials & Methods: The development of the real-time PCR for the detection of *C. auris* uses specific, targeted primer and probe mastermixes and a variety of controls; positive, negative and extraction controls. This real-time PCR assay is designed in an easy to use format with minimum hands on time and results generated in approximately 1 hour from extraction. Two specific primer and probe sequences were originally designed to target *C. auris*. The first identifies the internal transcribed spacer 2 (ITS) sequence between the 5.8S and 28S rRNA genes and the second identifies the Mating Factor (MFα1) gene; both have been evaluated as part of the analytical validation. Initial analytical studies demonstrated a limit of detection of 20 input copies (ipc) using the standardised Fungiplex assay real-time PCR conditions. Extracted DNA from 28 different strains of *Candida auris* have been tested using these PCR methods, as well as a specificity panel, consisting of species closely related to *C. auris*. All extracts were analysed in triplicate. Further to this, the Fungiplex Candida Auris Real-Time PCR Kit is currently in clinical validation with results to be presented. The clinical validation will compare the detection of this kit with an in house developed method and detection by MALDI-TOF.

Results: 100 % detection of the *Candida auris* strains was achieved when targeting the Mating Factor gene, with no cross-reactivity with other species observed. Results from the ITS region show sporadic detection of some strains and a trend of much higher Ct values, some cross-reactivity is also evident. Results are shown in Table 1 where the number of replicates detected (from a total of 3) are reported, along with the average resultant Ct value.

Conclusions: This new PCR test for the detection of *Candida auris* has shown excellent analytical sensitivity from both plasmid DNA and when extracted directly from a variety of cultured *Candida auris* strains. Superior performance for both sensitivity and specificity was observed when the Mating Factor (MFα1) gene was targeted. This assay is currently in clinical validation with results expected for presentation.

Table 1: Results for detection of *Candida auris* strains and additional species tested for specificity purposes

Sample	MFα1 Gene	ITS Sequence
C. auris 21092	3 / 3 (Ct = 24)	2 / 3 (Ct = 40)
C. auris 10031062 CWZ	3 / 3 (Ct = 24)	2 / 3 (Ct = 41)
C. auris 10031064 CWZ	3 / 3 (Ct = 24)	3 / 3 (Ct = 43)
C. auris 10031063 CWZ	3 / 3 (Ct = 23)	3 / 3 (Ct = 42)
C. auris 10051257 CWZ	3 / 3 (Ct = 24)	3 / 3 (Ct = 42)
C. auris 10051259 CWZ	3 / 3 (Ct = 27)	1 / 3 (Ct = 44)
C. auris 10051262 CWZ	3 / 3 (Ct = 26)	1 / 3 (Ct = 41)
C. auris 10051266 CWZ	3 / 3 (Ct = 23)	2 / 3 (Ct = 42)
C. auris 10051295 CWZ	3 / 3 (Ct = 25)	1 / 3 (Ct = 42)
C. auris 10051297 CWZ	3 / 3 (Ct = 23)	3 / 3 (Ct = 42)
C. auris AR0381 CAU	3 / 3 (Ct = 25)	3 / 3 (Ct = 40)
C. auris AR0382 CAU	3 / 3 (Ct = 24)	3 / 3 (Ct = 40)
C. auris AR0383 CAU	3 / 3 (Ct = 26)	3 / 3 (Ct = 40)
C. auris AR0384 CAU	3 / 3 (Ct = 26)	2 / 3 (Ct = 40)
C. auris AR0385 CAU	3 / 3 (Ct = 24)	3 / 3 (Ct = 19)
C. auris AR0386 CAU	3 / 3 (Ct = 24)	3 / 3 (Ct = 19)
C. auris AR0387 CAU	3 / 3 (Ct = 25)	1 / 3 (Ct = 40)
C. auris AR0388 CAU	3 / 3 (Ct = 27)	3 / 3 (Ct = 40)
C. auris AR0389 CAU	3 / 3 (Ct = 26)	1 / 3 (Ct = 40)
C. auris AR0390 CAU	3 / 3 (Ct = 26)	2 / 3 (Ct = 40)
C. auris 171103_23 TAU	3 / 3 (Ct = 28)	3 / 3 (Ct = 23)
C. auris 171103_24 TAU	3 / 3 (Ct = 28)	3 / 3 (Ct = 23)
C. auris 171103_156 TAU	3 / 3 (Ct = 27)	3 / 3 (Ct = 22)
C. auris 171103_172 TAU	3 / 3 (Ct = 27)	3 / 3 (Ct = 22)
C. auris 171103_197 TAU	3 / 3 (Ct = 27)	3 / 3 (Ct = 21)
C. auris 171103_201 TAU	3 / 3 (Ct = 25)	3 / 3 (Ct = 20)
C. auris 171103_597 TAU	3 / 3 (Ct = 28)	0 / 3
C. auris 171103_598 TAU	3 / 3 (Ct = 28)	0 / 3
C. haemulonii	0 / 9	4 / 9 (Ct = 40)
C. albicans	0 / 9	2 / 9 (Ct = 41)
C. dubliniensis	0 / 9	1 / 9 (Ct = 35)
C. lipolytica	0 / 9	1 / 9 (Ct = 37)
C. parapsilosis	0 / 9	1 / 9 (Ct = 36)
C. tropicalis	0 / 9	1 / 9 (Ct = 40)
S. cerevisiae	0 / 9	1 / 9 (Ct = 37)
C. sake	0 / 3	3 / 3 (Ct = 36)
R. glutinis	0 / 3	3 / 3 (Ct = 39)

P140

Positive lateral flow assay in suspected cryptococcosis. Retrospective study.

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INTRODUCTION

Cryptococcus is a ubiquitous cosmopolitan fungus transmitted through inhalation of spores. The major underlying mechanism involved in developing cryptococcal disease is immunosuppression. Cryptococcal antigen (CrAg) is detectable in blood through lateral flow assay (LFA) weeks to months before onset of meningitis symptoms.

The aim of our study was to determine the rate of positive LFA in the absence of a compatible clinical context or a history of cryptococcal disease and to assess the relationship between this phenomenon with mortality.

METHODS

We retrospectively studied all serum samples received from January 2012 to December 2022 for cryptococcal antigen detection by LFA. Positive LFA tests were retested and quantified by latex agglutination (LA). In order to evaluate the significance of a positive test, we considered as false positive (FP) result when none of the following criteria was met: previous history of cryptococcal disease, compatible signs and symptoms as well as if the patient had received previous specific treatment. We considered as true positive (TP) when two or more criteria was present.

RESULTS

We received serum samples from 718 patients during the study period. From which 44 had at least one positive LFA result (6%). Out of 44 patients, there were 20 true positives (46,5%) and 24 false positives (54,5%) using the previous mentioned criteria. The global rate of TP and FP was respectively 2,8% and 3,3%. The majority of false positives either did not have a positive agglutination test or when there was agglutination, it was at low titres (1/10) or in pure serum. Regarding all-cause mortality, there were a total of 6 deaths, 4 of them in the true positive (TP) group (4/20; 20%) and 2 were in the false positive (2/24; 8%) group. Of the 6 patients who died, 4 of them were HIV positive and the other two were immunosuppressed for other reasons. Three patients died from infectious diseases related to advanced immunosuppression, 1 due to multiorgan failure in a transplanted patient and 2 due to end stage cancer.

CONCLUSIONS

In our study, there was a high rate of apparently FP results. The use of LA to confirm positive cases of LFA was a very useful tool to discriminate the meaning of the LFA result together with the clinical context of the patient. Although, the crude mortality rate was higher in the TP group than the TF cohort (20 vs 8%), the small number of cases prevents from drawing conclusive results.

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Interdigital Candidiasis in patients with type 2 diabetes and molecular identification of species

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Interdigital Candidiasis in patients with type 2 diabetes and molecular identification of species

Abstract

Background and purpose: Interdigital Candidiasis is one of the most common opportunistic infections in diabetic patients. Underlying diseases, medication use, and A1C rate are effective in its occurrence. The aim of this study was determine of the frequency of interdigital Candidiasis and molecular identification of the species.
Materials and methods: In descriptive cross-sectional study, 324 sampled from diabetic patients after recording the questionnaire information. The samples were examined using direct microscopy and culturing method in SC, SCC and CHROMagar Candida. The polymorphism analysis was performed by PCR-RFLP technique. The internal spacer region (ITS) was recruited for PCR amplification of target sequences and Msp1 enzyme was employed to digest PCR amplicons.
Results: Out of 324samples, candidiasis was recovered in20 (6.1%) cases. The majority of patients have hyperlipidemia (31.1%) and heart disease (26.2%) as specific underlying complications. A1C 7.1-9 (75%) were the most common and use of antibiotics (in the last 2 months) was higher in patients. The frequency of nail discoloration in both groups was statistically significant. *C. albicans* (30.8%) and *C. guilliermondii* (15%) were the most common Candida agents that were identified individually or mixed with other species.
Conclusion: The frequency of *C. albicans* in diabetic patients has been confirmed by several studies. Studies have shown that *C. guilliermondii*, *C. glabrata* and *C. tropicalis* have the ability to accompany and bind to C. albicans or other species. Detection of fungal infections in patients and timely treatment, prevents the development of infection, high costs and probably amputation.

Keywords: Candidiasis, Diabetes, interdigital candidiasis, *Candida albicans*, *Candida guilliermondii*

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Serological response to Candida albicans Hyr1 protein for diagnosis of Invasive Candidiasis

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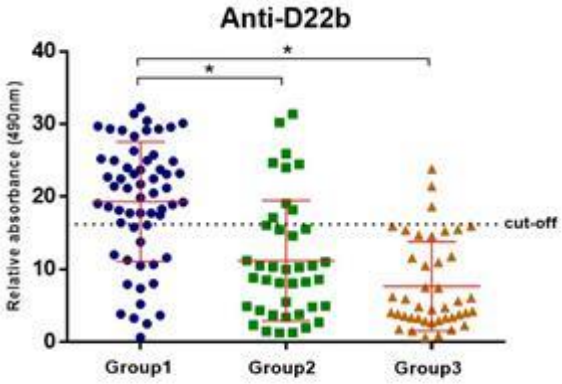
Objectives: Invasive Candidiasis (IC) is a life threatening infection caused by fungi of the genus *Candida*, and risk factors for patients include, among others, immunodeficiency, abdominal surgery, solid organ transplantation, and long stay in the ICU. Blood culture is the current gold standard for IC diagnosis, but its low sensitivity and time required to the result postpone the establishment of a specific antifungal treatment that has been related to a better outcome. *C. albicans* Hyr1p, a GPI-anchored protein that is only expressed by the hyphae of this fungus, was identified as one of the main targets of antibodies developed by IC patients that specifically recognize antigens located on the surface of germ tubes (CAGTA). The aim of this study was to assess the utility of detecting specific antibodies to Hyr1p and to a fragment of this protein (D22b), for the diagnosis of IC.
Materials & Methods: The Hyr1 protein, and a segment located in its middle-to-C-terminal zone (D22b), were obtained as recombinant proteins in *Pichia pastoris*, which performs post-translation modifications such as glycosylation. The sera of 142 patients were evaluated for the presence of IgG antibodies against Hyr1 and D22b by ELISA, and absorbance results were normalized with reference to that of the same control serum, and represented as relative absorbance units (RAU). Patiens were distributed into 3 groups: 1) 60 patients diagnosed with IC caused by *C. albicans* (40), *C. glabrata* (10), or *C. parapsilosis* (10); 2) 40 patients diagnosed with other invasive fungal infections (IFI; *Aspergillus* sp. (18), *Pneumocystis* sp. (14) and others (8)), and 3) 42 patients with no evidence of IFI. The overall diagnostic performance of both assays were assessed by ROC curve analysis, and estimation of sensitivity and specificity.
Results: For both proteins, the mean serological response of IC patients (group 1) was higher than those of control groups 2 and 3 (see Figure), and the differences were statistically significant ($p<0.05$). ROC curves showed a good degree of discrimination with AUC 0.828 and 0.806 for Hyr1 and the fragment, respectively. Sensitivity and specificity for the Hyr1 antibodies assay were 81.7 %. On the other hand, the D22b fragment ELISA resulted less sensitive (73.3%) but more specific (85.4%). With reference to the species of *Candida* causing the disease, no statistically significant differences were found within the IC group.
Conclusions: Detection of antibodies to recombinant Hyr1p and D22b fragment, both obtained in *Pichia*, appear useful for diagnosing Invasive Candidiasis. Both assays are positive for IC patients infected not only by *C. albicans* but also by *C. glabrata* or *C. parapsilosis*. The latter aspect will require further research, as well as protocol adjustments aimed at improving the diagnostic capability of these tests.
Financial support: M. Bregón-Villahoz was recipient of a grant from the UPV/EHU (PIF19/316). GEIFI research team was supported by projects IT913-16 from the Basque Government and GIU21/017 from the University of the Basque Country.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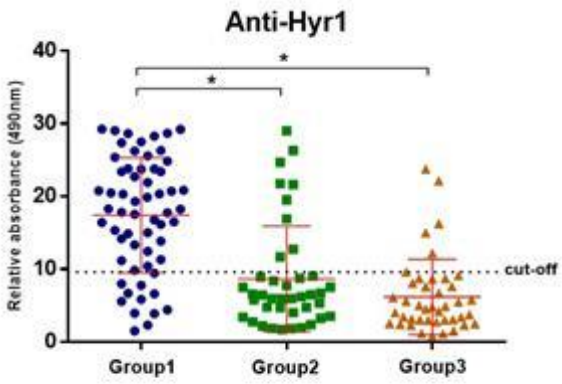
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Relative absorbance (490nm)
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cut-off
Group1
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Comparison of Germ Tube Testing in Different Media: In Search of “Fast and Furious”

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Comparison of Germ Tube Testing in Different Media: In Search of “Fast and Furious”

Objectives: Prevalence and diversity of fungal infections are in a rise, with significant differences in types of etiologic fungal species. Mortalities of invasive fungal infections (IFIs) have been being dwindling for particular species with novel antifungals, however, they remain to be high (>50%). Appropriate and early treatment against IFIs have crucial roles in prognosis and thus, species-level identification has critical importance to lead such treatments. In the last decade, management and treatment guidelines like ESCMID were published particularly focusing on different species. Automated systems (BD Phoenix™, VITEK-2, etc.) and proteomics devices (MALDI-TOF MS) were developed and endorsed to usage instead of conventional methods. On the other hand, it is not possible and also financially practical for every facility to reach such advanced devices. Germ tube testing (GTT) is a rapid, easy-to-perform method in order to identify *Candida albicans* complex, which is the most frequent IFI-causative microorganism. This method can be at first thought as a screening test, but when it is applied with different solutions, different results and performances are obtained. So, it is important to clarify which medium (solution) has optimal results in comparison with gold standard. The aim of this study is to observe GTT performances with different medium solutions.

Materials & Methods: Yeasts isolated from blood cultures between February 2020 to June 2023 in Balıkesir Atatürk City Hospital (Balıkesir, Turkey) were included. GTT was applied to all yeasts (n=350) with fetal bovine serum (FBS) , tryptic soy broth + 15% Glycerol (TSB), sabouraud dextrose broth (SDB), fresh frozen plasma (FFP), cryoprecipitate (CP) and platelet poor plasma (PPP) solutions, and results in 2h, 2.5h, 3h and 4h were compared with BD Phoenix™ (Becton Dickinson, MA, USA) and Cornmeal Tween 80 agar (RTA Laboratories, Kocaeli, Turkey) results. Sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) were calculated with SPSS 22.0 (IBM, NY, USA).

Results: SDB had the lowest sensitivity in all hours (58-67%) alongside with lowest NPVs. FBS and TSB had the highest sensitivity values as 85% and 83% barely in 4th hour,

respectively. FFP showed the highest sensitivity and NPV among all solutions in every hour, but both sensitivity and PPV dropped <95% after 3 hours of incubation. Similar decrease were also observed for CP. PPP barely provided >90% in all categories in 4th hour, while all other solutions except FFP and CP stood under 90%. False positivities due to *Candida tropicalis* started at 3rd hour for all solutions. FFP showed Sn, Sp, PPV and NPV results in 2nd hour as 94%, 100%, 100%, 95%; in 2.5 hour as 94%, 99%, 99%, 96%, respectively.

Conclusions: FFP had the best perfomance in every hour. After 3rd hour, there were significant decreases in specificity alongside with PPV and NPV of all solutions, despite increasing sensitivities. There was not any increase in sensitivity of FFP after 3rd hour, however, specificity, PPV and NPV countinued to decrease. For best results, 2nd hour and 2.5 hour were recommended with FFP solution.

Figure 1: GTT positivities regarding species in 3rd and 4th hours.

Figure 2: Sensitivity, Sprecificity, PPV and NPV of all solutions

Species	P(%) in 3h						P(%) in 4h					
	FBS	TSB	SDB	FFP	CP	PPP	FBS	TSB	SDB	FFP	CP	PPP
C.albicans + *C.africana*	81.9	72.9	65.2	96.7	92.9	87.7	85.2	83.9	67.7	96.7	94.2	90.3
C.dubliniensis	100	100	100	100	100	100	100	100	100	100	100	100
C.parapsilosis complex	None						None					
C.glabrata complex	None						None					
C.tropicalis	85.7	71.4	71.4	78.6	78.6	85.7	100	100	100	100	100	100
Other *Candida* spp.	None						None					
Other Yeasts / Yeast-like	None						None					

P: Germ tube test positive; FBS: Fetal Bovine Serum; CP: Cryoprecipitate; TSB: Tryptic Soy Broth with %5 Glyserol; FFP: Fresh Frozen Plasma; PPP: Platelet Poor Plasma

Candida albicans complex (n=155), *Candida tropicalis* (n=14), Other Yeasts (n=181)												
Media	2h						2.5h					
	P (n)	FP (n)	Sn	Sp	PPV	NPV	P (n)	FP (n)	Sn	Sp	PPV	NPV
FBS	104	0	67%	100%	100%	79%	115	3	74%	98%	97%	82%
TSB	95	0	61%	100%	100%	76%	107	1	69%	99%	99%	80%
SDB	91	0	58%	100%	100%	75%	91	0	58%	100%	100%	75%
FFP	146	0	94%	100%	100%	95%	147	1	94%	99%	99%	96%
CP	138	0	89%	100%	100%	91%	143	2	92%	98%	98%	94%
PPP	132	0	85%	100%	100%	89%	136	2	87%	98%	98%	91%
Media	3h						4h					
	P (n)	FP (n)	Sn	Sp	PPV	NPV	P (n)	FP (n)	Sn	Sp	PPV	NPV
FBS	127	11	81%	94%	92%	86%	132	14	85%	92%	90%	88%
TSB	113	10	72%	94%	91%	81%	130	14	83%	92%	90%	87%
SDB	101	10	65%	94%	90%	77%	105	14	67%	92%	88%	78%
FFP	150	11	96%	94%	93%	97%	150	14	96%	92%	91%	97%
CP	144	11	92%	94%	92%	94%	146	14	94%	92%	91%	95%
PPP	136	12	87%	93%	91%	90%	140	14	90%	92%	90%	92%

P: Germ tube test positive; FP: False Positive (*C.tropicalis*); FBS: Fetal Bovine Serum; CP: Cryoprecipitate; TSB: Tryptic Soy Broth with %5 Glyserol; FFP: Fresh Frozen Plasma; PPP: Platelet Poor Plasma; Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value

P144

Optimization of the recovery of Aspergillus fumigatus from self-collected expectorated sputa of Cystic Fibrosis patients

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Objectives:
Culture recovery of *Aspergillus fumigatus* (AF) from the respiratory tract in patients with cystic fibrosis (CF) and its role in lung function exacerbation is not well-classified, largely due to lack of standardized and optimal laboratory culture protocols. We assessed different pre-treatment processes, incubation conditions, and fungal culture media on home-collected sputa from people with CF to optimize the conditions for the recovery of (AF).

Materials & Methods:
From November 2021 to November 2022, 103 sputum samples from 44 CF patients with a history of culture positive for (AF) were received via overnight FedEx shipping under ambient air conditions. Each specimen (volume range= 0.2-16 ml) was assessed for texture (mucus vs non-mucus) and then equally divided into three portions for unprocessed (no treatment), mucolysis (MUCOSOL™), and ultrasonification (Covaris M220) treatments respectively. After treatment, the specimens were inoculated on three commercially available culture media: Sabouraud dextrose with Gentamicin (SABG), Inhibitory mold with Gentamicin (IMAG), and Czapek-Dox (CD) media for 10 days under the following conditions: 30ºC ambient, 37ºC ambient, 37ºC ambient+5%CO_2, 37ºC+ 1%O_2, 37ºC 1%O_2+5%CO_2. Recovered AF culture isolates were identified by phenotypic characteristics, MALDI-TOF MS, or DNA sequencing. Multinomial logistic regression model was applied for statistical analysis. CLSI antifungal susceptibility testing was also performed.

Results:
Among 44 subjects, AF prevalence was 82%, with 75% specimens (77/103) growing AF. Mucus sputum samples didn't show any more significant growth of AF as compared to non-mucus sputum samples. Likewise, growth of AF was statistically not different after mucolytic or ultrasonificaton treatment as compared to no treatment. However, an increase in specimen volume was statistically associated with more AF recovery ($p<0.05$), showing an increase of 5.4% growth with an increase of 1ml in volume (ml). Furthermore, significant increase of AF growth (24%) was observed under 37ºC +1%O_2 condition but a decrease (20%) under 30ºC ambient condition ($p<0.05$) was noted. Among the three culture media, IMAG had the highest recovery of AF and CD had the lowest ($p<0.05$). Of the 35 AF isolates, 12 (35%) showed reduced susceptibility to azoles (10 Intermediate and 1 resistant to voriconazole; 1 itraconazole MIC >16µg/mL).

Conclusions:
Our preliminary findings suggest that mucolysis or ultrasonication pretreatments do not enhance AF culture recovery. However, sample volume increase is associated with more growth of AF. Incubation of sputum samples on IMAG under 37°C +1%O_2 condition may provide optimal AF growth.

P145

EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF SERUM (1,3)-B-D-GLUCANE ASSAY FOR DIFFERENTIATION BETWEEN PNEUMOCYSTIS PNEUMONIA AND PNEUMOCYSTIS JIROVECII COLONIZATION

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Objectives:
Pneumocytis jirovecii is a ubiquitous fungus, agent of the human pneumocystosis also called *Pneumocystis* pneumonia (PCP). This opportunistic infection is a life-threatening infection that affects immunocompromised patients. The absence of specific clinical and imaging of PCP complicates its diagnosis and makes biological diagnosis necessary. The latter is mainly based on the detection of the fungus in respiratory samples, most often performed by PCR, a highly sensitive technique. However, a positive PCR result cannot be used to distinguish a patient with PCP from a patient colonized by the fungus. Serum (1,3)-β-D-glucan (BDG) assay has been proposed to help in this distinction.
One hundred and two PCR positive patients were included. At the BDG positivity threshold ≥ 80 pg/mL, the sensitivity was 87.9%, the specificity 78.3%, the PPV 65.9%, and the VPN 93.1%. At the BDG threshold of 200 pg/mL, BDG exhibited a sensitivity of 75.8%, a specificity of 87%, a PPV of 73.5% and a VPN of 88.2%. The BDG threshold of 300 pg/mL was also evaluated showing a sensitivity of 72.7%, a specificity of 89.9%, a PPV of 77.4% and a VPN of 87.3%.

Materials & Methods:
A retrospective analysis was performed at the University Hospital of Poitiers (France). Patients with a positive Pneumocystis PCR between June 2020 and July 2022 for which a serum BDG assay had been performed were included. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the BDG assay were calculated according to three different cutoffs (80, 200 and 300 pg/mL).

Results:
One hundred and two PCR positive patients were included. At the BDG positivity threshold ≥ 80 pg/mL, the sensitivity was 87.9%, the specificity 78.3%, the PPV 65.9%, and the VPN 93.1%. At the BDG threshold of 200 pg/mL, BDG exhibited a sensitivity of 75.8%, a specificity of 87%, a PPV of 73.5% and a VPN of 88.2%. The BDG threshold of 300 pg/mL was also evaluated showing a sensitivity of 72.7%, a specificity of 89.9%, a PPV of 77.4% and a VPN of 87.3%.

Conclusions:
For a threshold of 80 pg/mL, the BDG assay presented an excellent ability to exclude PCP when it is negative. In this study, a positive *Pneumocystis* PCR associated to a negative BDG result corresponded in more than 90% of the cases to colonization. In addition, in PCR-positive patients, BDG values ≥ 200 pg/mL were most often associated with PCP and this threshold

permitted to confirm PCP in 75% of the cases. The serum BDG assay is therefore interesting to differentiate colonization and infection, permitting to conclude on colonization when it is negative (<80 pg/mL) and to confirm PCP when it is ≥ 200 pg/mL, with a relatively low risk of treating a patient wrongly in the latter case (25%).

P146

Development and validation of an LC-MS for the measurement of a new antifungal drug olorofim (F901318)

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Background: The dihydroorotate dehydrogenase inhibitor drug olorofim has been developed as an antifungal compound to treat a wide range of moulds . The drug is active against *Aspergillus* (including azole-resistant strains), resistant moulds (e.g., *Lomentospora prolificans*), and dimorphic moulds. The objective was to develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify olorofim in human plasma. This assay was used for the measurement of olorofim in the recently completed Phase II trial (where results were available rapidly after sample collection) and can help to investigate the pharmacokinetics of olorofim in different populations.

Methods: The developed assay was validated according to the EMA Guideline for bioanalytical method validation (2011). Lithium heparin tubes were used for sample collection. 25 µL volumes of plasma were prepared using a protein precipitation method (centrifugation at 18620 ×g for 5 minutes). Deuterated olorofim was used as internal standard. The assay was developed with a calibration range 10-5000 ng/mL. The calibration curve consisted of 8 points with 3 QC samples (30, 300, and 3750 ng/mL). Within- and between-run precision and accuracy, carry over, stability under different conditions (including freeze thaw stability), and recovery were defined. Selectivity and matrix effect were evaluated. The performance of the LC-MS/MS assay in measuring total olorofim concentrations was cross validated using QC samples from another reference laboratory.

Results: The method is able to determine olorofim over a range of 10-5000 ng/mL. Recovery was 92% after correction with internal standard. Retention time of olorofim was 1.22 minutes. Total run time is 3 minutes. Precision and accuracy of all parameters of olorofim were within 0 – 4.30% CV and -3.42-8.00% bias and all well within the acceptance criteria of <15% CV and 15% bias as dictated by the EMA. Olorofim was stable in plasma for at least 3 days at 4°C as well as 20°C and for at least 38 months at -40°C. Furthermore, olorofim was stable for five days in the auto-sampler. Three freeze-thaw cycles did not impact assay performance. No interfering compounds nor a matrix effect was identified.
The assay was successfully used to determine 943 plasma concentrations of trial participants, as well as samples for paediatric patients under compassionate use. Cross-validation results were within 93-100.5% as compared to the reference laboratory between 2019-2022.

Conclusions: The presented method provides fast and accurate measurement of olorofim and was used in the phase II trial for the rapid, real-time measurement of exposure. The developed method is applicable for research of olorofim pharmacokinetics in adults and children.

Total concentrations	**LLOQ(%)**	**QCLow(%)**	**QCMed(%)**	**QCHigh(%)**	**HLOQ(%)**
Within run accuracy (Bias%)	-2.61	2.89	1.31	-3.42	8.00
Between run accuracy (Bias%)	-0.10	1.61	0.38	-0.53	5.21
Within run precision (CV%)	3.03	1.95	2.65	4.30	2.53

Between run precision (CV%)	1.88	1.05	0	2.22	2.46

P147

Utility of chest x-ray scoring in screening chronic pulmonary aspergillosis in patients with history of pulmonary tuberculosis

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Abstract

Objective

Chronic pulmonary aspergillosis (CPA) is one of the most underdiagnosed conditions that is usually seen as a sequelae of destructive lung diseases like pulmonary tuberculosis (PTB), allergic bronchopulmonary aspergillosis, and chronic obstructive pulmonary disease. Because of the high burden of PTB in Pakistan, the number of CPA patients can also be assumed to be high. Patients with CPA are often erred for PTB relapse owing to non-specific symptoms like cough and haemoptysis. Therefore, in developing countries, where Aspergillus specific IgG levels are not freely available, a simplified method to screen CPA needs to be developed. The aim of this study is to evaluate the utility of chest x-ray (CXR) score in efficiently screening of CPA in patients with history of PTB presenting with new hemoptysis and its utility to predict *Aspergillus* serum IgG levels.

Methods

Patients with history of PTB and examined for CPA with serum *Aspergillus fumigatus* IgG (*A. fumigatus* IgG) were investigated. For *A. fumigatus* IgG, results ≥20mg/L was considered positive[1]. A modified scoring system developed by Anna Ralph *et al.* (2010)[2] was used with similar cut-off values (≥71 and ≥40). For the purpose of scoring. CXR was distributed into four quadrants. Infiltrates, fibrosis, nodules, and effusion were regarded as abnormality on CXR. The basis of scoring was magnitude of normalcy versus abnormality witnessed in CXR in each quadrant of the lung. A diffuse lung abnormality like widespread consolidation or bronchiectasis was scored as 100 points (100%). In case of cavitary lesion, further 40 points were added to CXR score. The highest score a CXR can have was 140 points. The pulmonologist, who scored CXR radiograph, were masked from the *A. fumigatus* IgG result.

Results

During study period, a total of 49 adult patients were evaluated. Twenty-nine (59.2%) patients had elevated IgG (≥20 mg/L). Twenty-six patients (53.1%) had CXR score of ≥71 and 40

(81.6%) had CXR score ≥40. With an x-ray score of ≥71 and ≥40, the median *A. fumigatus* IgG levels were significantly higher 90 (IQR 75, 115; *p*-value <0.0001) and 75 (IQR 50, 100; *p*-value <0.0001), respectively. With an x-ray score of ≥71, 80.8% cases were found to be IgG positive (positive predictive value), sensitivity was 72.4%, specificity was 75%, and negative predictive value was 65.2%. With an x-ray score cut-off of ≥40, 67.5% cases were found to be IgG positive (positive predictive value), sensitivity was 93.1%, specificity was 35%, and negative predictive value was 77.8%.

Conclusion

Our findings conclude the efficiency and utility of CXR score in screening CPA in patients with history of PTB. X-ray score of ≥71, has a reasonably good sensitivity, specificity and positive predictive value for a positive *A. fumigatus* IgG and diagnosis of CPA. Whereas a score of < 40 has a good negative predictive value. Such a tool could be an asset in decision-making of assessment of CPA in PTB patients, particularly in resource-impoverished clinical settings. Further studies with large sample size and complete data are advised.

Keywords

Chronic pulmonary aspergillosis; screening; chest x-ray; scoring

P148

Diagnostic Performance of the Commercial Wantai Mp1p Enzyme Immunoassay for Diagnosis of Talaromycosis

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Objectives
Talaromycosis is an invasive mycosis endemic in Southeast Asia and is a leading cause of death in individuals with advanced HIV disease (AHD). Current diagnosis relies on protracted culture methods which take up to 4 weeks, and is detectable only during late-stage infection, leading to delayed treatment and high mortality. We have recently developed an enzyme immunoassay (EIA) detecting Talaromyces marneffei specific Mp1p antigen and have shown that the Mp1p EIA is superior to blood culture in sensitivity and shortens the time-to-diagnosis by up to 16 weeks. Here we report the clinical validation of the first commercial Mp1p EIA test (developed by Wantai in Beijing) in patients with AHD outside of China.
Methods
We employed a case-control study design for assay validation utilizing stored plasma and urine samples from two prospective cohorts of patients with AHD recruited between 2011 and 2019 in Vietnam. Cases were patients with culture-proven talaromycosis from blood or other clinical specimens (N=224). Controls were individuals who screened negative for talaromycosis by culture and did not develop talaromycosis over a six-month follow-up period. We compared the sensitivity, specificity, accuracy (as determined by the area under the receiver operating characteristic [AUROC] curve) of the commercial Wantai Mp1p against our in-house Mp1p EIAs in paired plasma and urine samples, using culture as the reference standard. We also analyzed the performance of both assays compared to blood culture, and their performance when testing plasma and urine in combination versus testing plasma or urine alone.
Results
Cases and controls were similar in age and CD4 count, but controls had higher male prevalence 83.5% versus 67.4% (P = <0.001). Figure 1 showed the optical density distributions between cases and controls and similar AUROC curves for the differentiation powers between cases and controls for the Wantai EIA and the in-house EIA tested on plasma and urine samples. Table 1 showed results of the diagnostic performance of the two assays when testing paired plasma and urine in combination. The Wantai and in-house Mp1p EIAs had similar sensitivities of 96.4% (95% CI: 92.8 - 98.3) and 96.0% (95% CI: 87.1 - 95.2), respectively (P sensitivity = 0.65, McNemar) and similar specificities of 95.5% (95% CI: 91.4 - 97.8) and 92.5% (95% CI: 87.7 - 95.6), respectively (P specificity = 0.52, McNemar). The sensitivities of both assays were significantly higher compared to blood culture positivit, 148 of 222 (66.7%), P < 0.001 by McNemar for both comparisons. In both assays, the sensitivities were significantly higher when testing plasma and urine in combination compared to testing in plasma or urine alone.
Conclusions

The commercially-available Wantai Mp1p EIA has similar diagnostic performance to our in-house Mp1p EIA and is superior to blood culture in sensitivity. The diagnostic sensitivity is significantly higher when testing plasma and urine samples in combination. As the Wantai Mp1p EIA has been standardized and is commercially available, this study supports the clinical implementation of the Wantai Mp1p EIA for rapid diagnosis of talaromycosis in Southeast Asia

Figure 1.

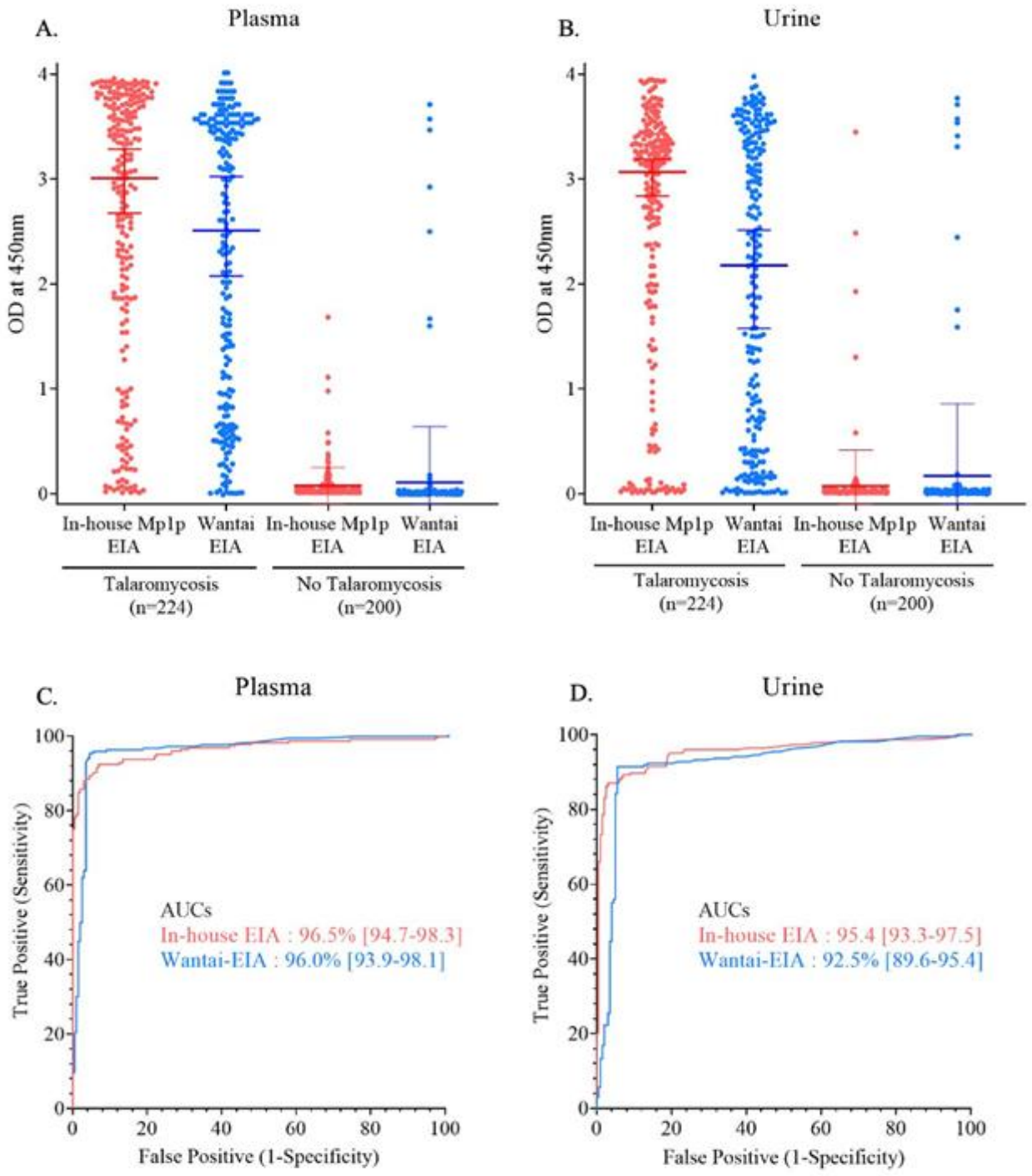

Figure 1A and 1B demonstrate the distribution of the optical density (OD) values of both the assays among the cases and controls on plasma and urine. Figure 1C and 1D demonstrate the receiver operating characteristic (ROC) curves of sensitivity (true positives) against 1-specificity (false positives) of both assays among cases and controls on plasma and urine.

Table 1.

	Talaromycosis cases (N=224)		Non-Talaromycosis controls (N=200)	
Test	**In-house EIA**	**Wantai EIA**	**In-house EIA**	**Wantai EIA**
Mp1p Positive	215 (TP)	216 (TP)	15 (FP)	13 (FP)
Mp1p Negative	9 (FN)	8 (FN)	185 (TN)	187 (TN)

	In-house EIA	Wantai EIA	P-value
Sensitivity	96.0% [95% CI: 87.1 - 95.2]	96.4% [95% CI: 92.8 - 98.3]	0.65
Specificity	92.5% [95% CI: 87.7 - 95.6]	95.5% [95% CI: 91.4 - 97.8]	0.52
PLR	12.8 = .96/(1-.925)	21.4 = .964/(1-.955)	
NLR	0.04 = (1-.96)/.925	0.04 = (1-.964)/.955	

Optical density (OD) cut-off >0.2 (In House EIA); OD cut-off >0.15 (Wantai EIA). Abbreviations: EIA, Enzyme-linked immunoassay; CI, confidence interval; TP, true positive; FP, false positive; FN, false negative; TN, true negative; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

P149

Development of Interferon-Gamma Release Assays for Diagnosing Latent Talaromycosis

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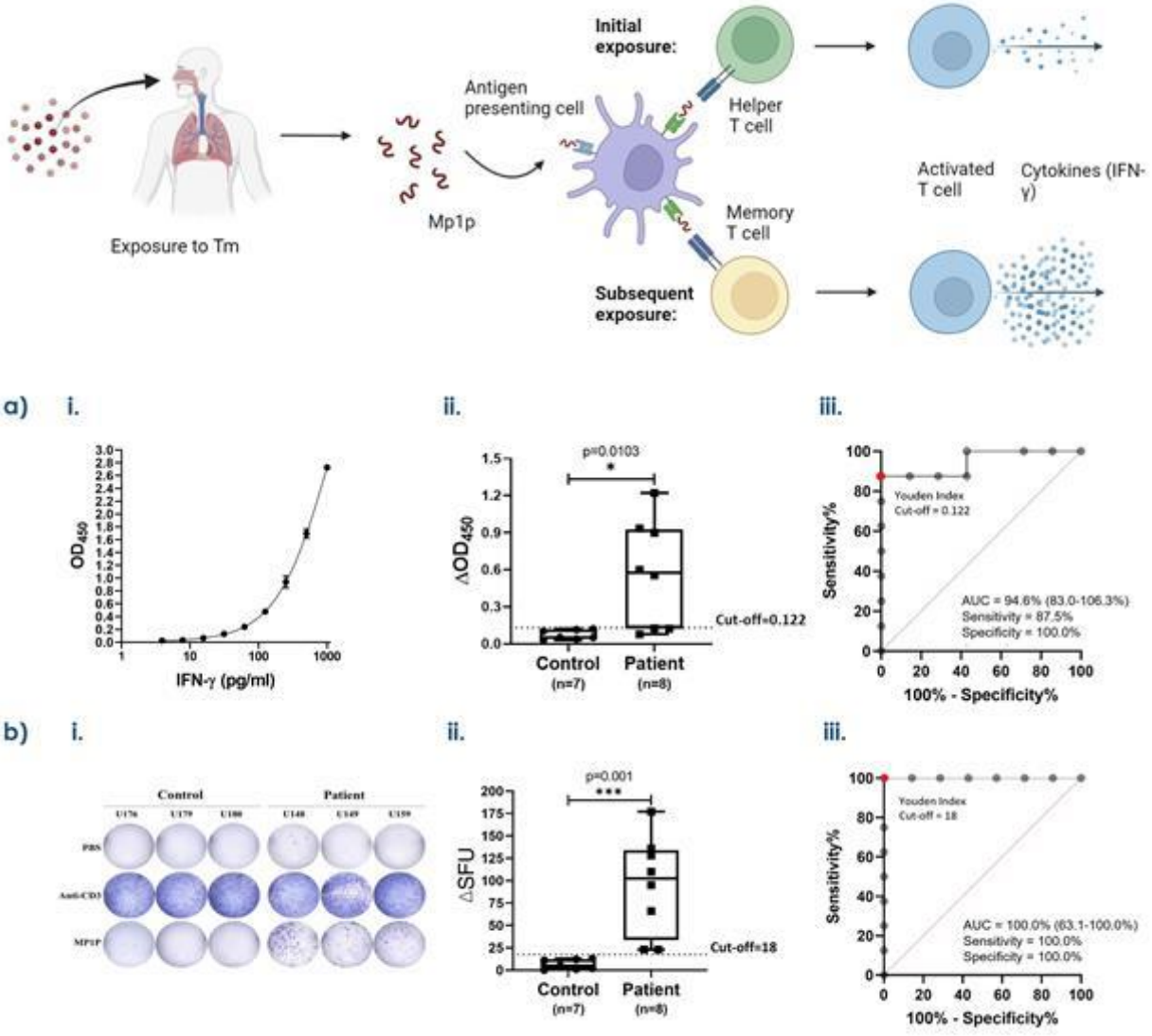
Objectives: *Talaromyces marneffei* (Tm), a thermally dimorphic fungus endemic in Southeast Asia, causes a leading opportunistic infection in patients with advanced HIV disease. The mortality rate despite antifungal therapy is 30%. Existing culture and antigen assays can only detect Tm disease during advanced stages when treatment is less effective. Early detection allows early treatment, which has the potential to interrupt disease progression and reduce mortality. Here, we report the development and pilot clinical assessment of two novel interferon-gamma release assays (IGRAs) to diagnose latent Tm infection.

Materials & Methods: We applied the same immunological principle used by the commercial Quantiferon assay for TB. Tm IGRAs rely on the ability of ex-vivo host memory T cells from individuals who have been exposed to Tm to recall this exposure and release interferon gamma upon in-vitro stimulation with a Tm-specific mannoprotein Mp1p. We employed a pilot case-control study design. Cases (N=8) were HIV-infected patients with a known history of culture-confirmed Tm infection. Controls (N=7) were healthy volunteers who had never traveled to Southeast Asia. We measured interferon gamma release from subjects' Mp1p-stimulated whole blood using the enzyme linked immunosorbent assay (ELISA) and from Mp1p-stimulated peripheral blood mononuclear cells (PBMCs) using the enzyme-linked immunosorbent spot (ELISpot) method. We identified optimal assay conditions for maximum sensitivity and specificity. We used the receiver operating characteristic (ROC) curve and Youden Index to identify preliminary cutoff values (in optical density [OD] for the ELISA and in spot-forming units [SFUs] for the ELISpot), for differentiation between cases and controls and for determining preliminary assay sensitivity and specificity.

Results: For the ELISA-based IGRA, a whole blood volume of 1 mL, a Mp1p concentration of 40 µg/mL, and a 24-hour incubation period between whole blood and Mp1p produced the highest analytical sensitivity and specificity. The difference in the mean OD values between cases (OD_{case} = 0.57) and controls ($OD_{control}$ = 0.07) was 0.49±0.17; this difference was statistically significant, P = 0.01 (unpaired T-test). At the optimal OD cutoff point of 0.12, the ELISA-based IGRA has a preliminary clinical sensitivity of 87.5% (95% CI: 47.4% to

99.7%) and a specificity of 100% (95% CI: 59.0% to 100.0%). For the ELISpot-based IGRA, a cell inoculum of $5x10^5$ PBMCs, a 40 µg/mL concentration of Mp1p, and a 40-hour incubation period between PBMCs and Mp1p produced the highest analytical sensitivity and specificity. The difference in the mean SFUs between cases (SFU_{case} = 94.8, 95% CI: 59.4 to 130.1) and controls ($SFU_{control}$ = 6.3, 95% CI: 2.5 to 10.1) was 88 (95% CI: 43 to 134); this difference was statistically significant, P = 0.001 (unpaired T-test). At the optimal SFU cutoff point of 18, the ELISpot-based IGRA has a preliminary clinical sensitivity of 100.0% (95% CI: 63.1% to 100.0%) and a specificity of 100.0% (95% CI: 59.0% to 100.0%).

Conclusions: This study demonstrates a proof-of-concept that Mp1p can elicit a robust ex-vivo memory T-cell response to Tm. The ELISA-based and ELISpot-based IGRAs have promising clinical performance and should be further optimized and evaluated in larger clinical studies.



P150

Chorus Aspergillus galactomannan Ag assay evaluation for the diagnosis of aspergillosis

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Objectives: The gold standard test for aspergillosis actually relies on the detection of the *Aspergillus* galactomannan antigen by the Platelia (Biorad) assay, based on a sandwich enzyme-linked immunosorbent assay (ELISA) microtiter plate. The development of new diagnostic assays, useful to detect as soon as possible invasive fungal infections (IFI) using non-culture-based biomarkers, is of detrimental importance for the management of patients affected by aspergillosis and for a prompt treatment.
The aim of this study has been the comparison between the Platelia Aspergillus Ag (BioRad) assay and the new immune-enzymatic single test cartridge-based system Diesse Chorus Aspergillus Galactomannan Ag.
Materials & Methods: A total of 112 samples (94 serum and 20 bronchoalveolar lavage), collected from patients with proven or probable invasive aspergillosis were tested in parallel with the Platelia galactomannan assay along with the culture when available. For results evaluation the predefined Chorus cut-off parameters of 1.1 for positive, <0.9 for negative and 0.9 - 1.1 for undetermined values were applied.
Results: Overall, 100% of positive serum samples and 93.7% of positive bal tested by the Chorus assay (Diesse) had a comparable measurement with Platelia (Biorad). Regarding negative samples tested by Chorus assay, 98% of agreement was obtained in comparison to Platelia assay.
Conclusions: Due to its full automatization, the Diesse Chorus Aspergillus Galactomannan Ag test is fast (2.5 hours) and easy to use, moreover it allows to process the samples individually being a valid alternative for the diagnosis of invasive aspergillosis. The comparison between the two tests results suggests that the Chorus assay might be reliably used to detect galactomannan antigen in patients with IFI.

P151

Pneumocystis jirovecii qPCR Ct-values during PJP treatment: A prospective longitudinal follow up study

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Objectives

Pneumocystis jirovecii pneumonia (PJP) is a major complication in immunocompromised individuals with considerable mortality and initiation of empirical treatment outside of office hours is not uncommon. Diagnosis of PJP is based on the combination of a susceptible host, a clinical picture with hypoxia and pulmonary infiltrates, and a positive real-time PCR (qPCR) test of lower respiratory airway samples. QPCR enables a semiquantitative assessment of fungal load and is measured by cycle threshold value (Ct-value), with a low Ct-value indicating a high fungal load consistent with infection and a high Ct-value indicating a low fungal burden consistent with colonization. Successful treatment of PJP has been shown to be associated with conversion to qPCR negativity in respiratory samples but the timely relation between duration of treatment and changes in Ct-value has not been fully determined. The aim of this study was to investigate the increase in Ct-values in sputum during treatment for PJP in patients initially diagnosed with a high fungal load.

Method

This is an ongoing prospective non-intervention study. Patients over 18 years old with a clinical diagnosis of PJP and a positive qPCR (targeting the mt LSU rRNA) in sputum with a CT-value of ≤30 were eligible for inclusion. Follow-up sputum samples were collected for up to eight days after start of treatment of PJP and analyzed with qPCR; a Ct-value ≥ 40 was considered negative. A Gram stain was performed on each follow-up sputum sample to assess if the sample was representative for the lower respiratory tract, defined as a count of > 5 leukocytes per epithelial cell in each examined field at 100x magnification. The data presented here were collected between 2022 and 2023.

Results

By March 2023, 7 of 20 planned patients have been included. Median number of follow-up samples per patient was 3 (range 2-4). Of 21 follow-up samples, 12 (57%) were representative for the lower respiratory tract. A general increase in Ct-values was seen in most follow-up samples regardless of their being representative for the lower respiratory tract or not. The changes in Ct-values for all follow-up samples are shown in Figure 1, with a mean increase in Ct-value per day of 1,4 with a 95% standard deviation (SD) of 0.4. During the up to 8 days follow -up period, three of seven patients reached a Ct-value of ≥ 40, which interpreted as a negative result.

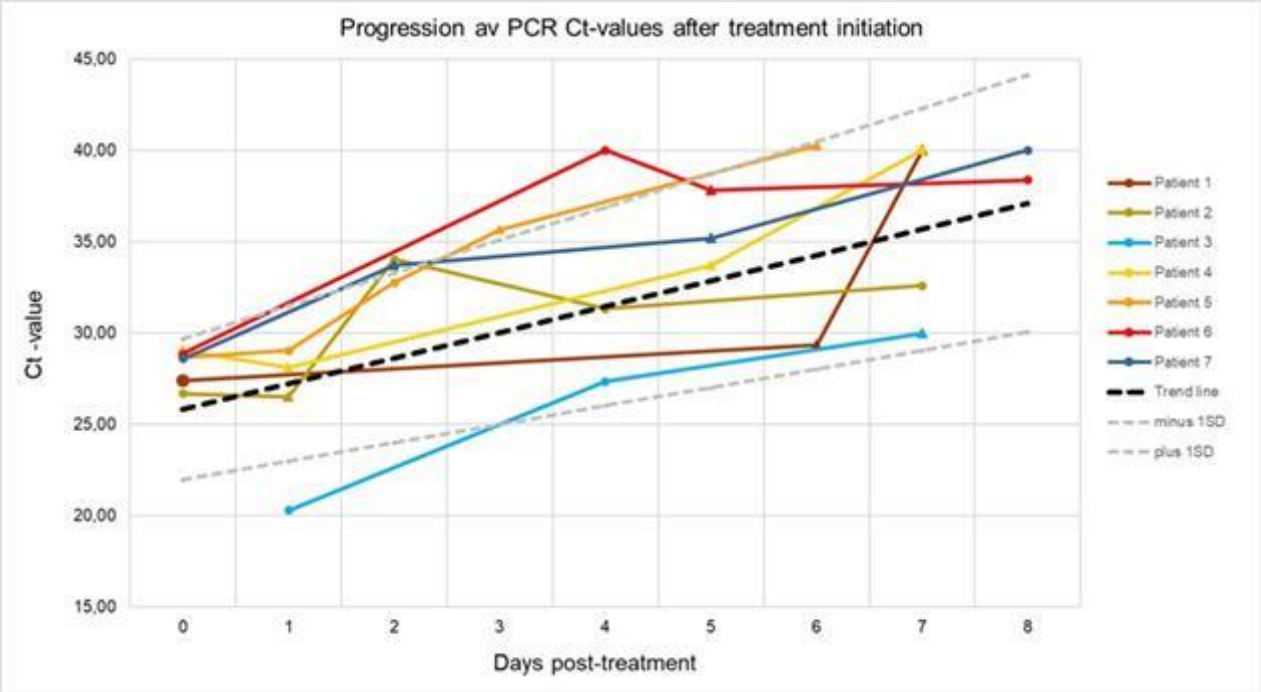
Conclusion

Initiation of PJP treatment increased sputum Ct-values by 1.4 per treatment day which should be considered when evaluating Ct-values in respiratory samples from susceptible patients who have received empirical treatment before sampling. Since the study is ongoing and the result based on only seven patients the results must be interpreted with caution.

Figure 1: Ct-values in sputum samples before and after start of treatment

Day 0: Before or at start of treatment (Pat 3 had first sample performed one day after start of

treatment); Linear fit (+/- 1 SD) as a dashed line. Triangle: representative sample; dot: uncertain representative sample. Ct-value ≥ 40 is negative.



P152

1-3-β-D-glucan and qPCR for the diagnosis of Pneumocystis Pneumonia (PCP): a retrospective audit with optimisation of qPCR and 1-3-β-D-glucan thresholds.

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Performance of qPCR and 1-3-β-D-glucan for the diagnosis of Pneumocystis Pneumonia (PCP): a retrospective audit with focus on optimisation of qPCR and 1-3-β-D-glucan thresholds.

Objectives

Recent studies have reported that a negative 1-3-β-D-glucan cannot be utilised to exclude a diagnosis of Pneumocystis Pneumonia (PCP) in non-HIV patients, in particular patients with haematological malignancies, due to false negativity (1, 2). The recently updated ECIL-9 guidelines for the diagnosis of PCP reflect this by downgrading a negative serum 1-3-β-D-glucan for excluding a diagnosis of PCP from A-II to B-II recommendation (3). The Royal Free London NHS Foundation Trust is a specialist centre for liver and renal transplants, infectious diseases, and patients with primary immuno-deficiency. The aim of this audit was to establish the perfomance of qPCR and 1-3-β-D-glucan and optimise clinical thresholds for the diagosis of PCP in this setting.

Materials & Methods: a retrospective audit of PCP qPCR and 1-3-β-D-glucan testing was performed from 01/03/2016 – 01/04/2023 (testing performed during the COVID-19 pandemic was excluded). Paired results were resticted to those performed within +/- 4 days of each other. qPCR testing was performed from 1-2mL of BAL concentrate or induced sputum, extracted on an EZ1 (DNA tissue kit, Qiagen). Amplification was performed using either Pneumocystis PCR (Fast track Diagnostics, pre 2018) or PneumID (OLM, post 2018) assaysx on the RotorgeneQ 6000 (Qiagen). 1-3-β-D-glucan testing (Fungitell, Cape Cod) was performed as per manufacturers instructions. Both assays targeted the mt-LSU rDNA gene. A clinical gold standard of; clinical diagnosis, hypoxia, radiological features compatible with PCP and response to anti-pneumocystis therapy in patients with underlying immune-suppression was used to classify cases and controls.

Results: In total 184 paired qPCR and 1-3-β-D-glucan results were included. ROC analysis of qPCR results (area= 0.9, $p<0.01$) indicated an optimal threshold of >8.3 x 10^5 copies/mL with a sensitivity of 75% (95% CI: 57.9, 86.9) and a specificty of 100% (95% CI: 67.6, 100). ROC analysis of 1-3-β-D-glucan results (area= 0.82, $p<0.01$) indicated an optimal threshod of >223.8 pg/mL with a sensitivity of 75% (95% CI: 57.9, 86.8), specificity of 79.5% (95% CI:72.3, 85.1), figure 1 b. By utilising the optimised 1-3-β-D-glucan threshold to differentiate between PCP and colonsation for samples with a qPCR result <8.3 x 10^5 copies/mL an overall sensitivity of 90.6% (95% CI: 74.9, 98) and specificity of 99.3% (95% CI: 96.2, 100) was obtained. A very poor correlation (r^2=0.2) between quantitative values of qPCR (copies/mL) and 1-3-β-D-glucan (pg/mL) was observed, figure 1a. Sub analysis of performance will be presented in the full data set.

Conclusions: PCP qPCR is the most accurate method for diagnosis PCP infection. A negative 1-3-β-D-glucan could not be used to rule out PCP. However, the use of 1-3-β-D-glucan results with an optimised threshold would have reduced the uncertainty of low positive PCP PCR results by 31.3% (5/16) in this audit, therefore positive 1-3-β-D-glucan results with an optimised higher threshold can be used to define infection from colonisation in patients with low burdens of *Pneumocystis jirovecii*.

1. Szvalb et al. Serum (1,3)-Beta-d-Glucan has suboptimal performance for the diagnosis of Pneumocystis jirovecii pneumonia in cancer patients and correlates poorly with respiratory burden as measured by quantitative PCR, Journal of Infection, Volume 81, Issue 3, 2020, Pages 443-451
2. Mercier et al. Variable Correlation between Bronchoalveolar Lavage Fluid Fungal Load and Serum-(1,3)-β-d-Glucan in Patients with Pneumocystosis-A Multicenter ECMM Excellence Center Study. J Fungi (Basel). 2020 Dec 1;6(4):327.
3. https://ecil-leukaemia.com/images/resources/2022part2/ECIL9_Update_on_fungal_diagnostics_Revised_Guidelines

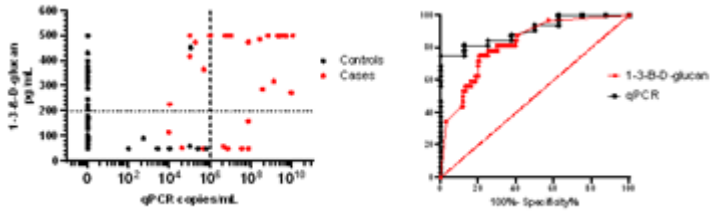

Figure 1a) XY plot of qpCR and 1-3-β-D-glucan results for cases (red) and controls (black) b) ROC analysis of qPCR and 1-3-β-D-glucan results

P153

Candida albicans germ tube antibodies in invasive candidiasis: a comparison of the manual Vircell-IgG-immunofluorescence assay with the fully automated VIRCLIA-IgG-MONOTEST

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Objectives: The incidence of invasive candidiasis is increasing especially in critically ill patients. Besides culture, the detection of fungal antigens and antibodies directed against those antigens are important diagnostic methods. For measurement of *Candida albicans* germ tube antibodies a manual indirect immunofluorescence assay (CAGTA-IFA) is commercially available. However, the processing is time-consuming and the reading subjective. Recently, a fully automated indirect chemiluminescence immunoassay for single sample testing (CAGTA-CLIA-Monotest) has become available. In this study, we aimed to compare the diagnostic performance of both assays in patients with invasive candidiasis.

Methods: We tested sera from 150 patients with blood culture-proven candidemia (study part one) and 689 prospectively collected sera from 346 patients with sepsis and high risk for invasive candidiasis (study part two) with the CAGTA-IFA and the CAGTA-CLIA-Monotest (both Vircell S.L, Spain). The samples for study part 2 were taken from the Candisep study, a prospective multicentre study in German intensive care units.

Results: In study part one, the sensitivity of the CAGTA-IFA and the CAGTA-CLIA-Monotest were 59.9% and 51.0%, respectively, at the manufacturer's recommended cut-off values (titre of ≥1:160 and antibody index >1.1). If the indeterminate results of the CAGTA-CLIA-Monotest were considered positive (cut-off value >0.9) the sensitivity increased to 55.0%. In study part two, 47 patients (14.1%) had invasive candidiasis and 14 of them had candidemia (4.2%). The sensitivity, specificity, positive and negative predictive value were 22.5%, 69.4%, 12.0% and 85.1% for CAGTA-IFA and 21.3%, 78.5%, 13.9% and 86.0% for CAGTA-CLIA-Monotest. In candidemia patients from study part two, the sensitivity, specificity, positive and negative predictive value were 42.7%, 69.4%, 6.4%, and 96.2% for CAGTA-IFA and 35.7%, 78.5%, 7.5% and 96.2% for CAGTA-CLIA-Monotest. In terms of practicability, the CAGTA-CLIA-Monotest proved to be robust and delivered results within one hour with a minimal hands-on-time of 5 minutes.

Conclusions: The CAGTA-CLIA-Monotest is robust and easy to use. However, the diagnostic performance of both assays is limited, especially in patients with invasive candidiasis without candidemia. The CAGTA-IFA is slightly more sensitive than the CAGTA-CLIA-Monotest in patients with candidemia. Conversely, the CAGTA-CLIA monotest is superior in terms of specificity. Overall, both CAGTA assays are a useful complement to other fungal diagnostic tests.

P154

Real-world use of serum (1,3)-β-D-glucan in Candidemia: ECMM Candida III multinational European Observational Cohort Study

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Objectives:
The serum (1,3)-β-D-glucan (BDG) test is a quantitative, laboratory diagnostic parameter frequently used in case of suspected invasive fungal disease. Besides its diagnostic capabilities, particularly its strong negative predictive value, studies have also highlighted its prognostic value, but whether BDG is used in real world for treatment stratification and outcome prediction remains unknown.
With this analysis, we aim to (i) assess the current real-world use of BDG in candidemia patients across Europe and (ii) further elucidate its value as an outcome predictor.

Materials & Methods:
As part of a multicenter observational cohort study, 64 participating hospitals in 20 European countries enrolled adult patients with culture-proven candidemia. Between 1 July 2018 and 31 March 2022, data

were entered into the ECMM Candida Registry (FungiScope CandiReg). The included number of patients in participating hospitals per country was determined by population size.

Results:

In 34 of all 632 (5.4%) included patients serum BDG was measured with a total number of 56 tests, with 40 positive results in 24 patients (defined as > 80pg/ml). The test was documented once in 18 patients (24 days average duration total hospitalization time, mortality 44%, positive BDG test results in 13/15, missing BDG test results in 3 patients), twice in 10 patients (31 days average total hospitalization time, mortality 40%, positive BDG test results in 6/10), while 6 had repeated measurements up to three times (61 days average total hospitalization time, mortality 16%, positive BDG test results in 5/5 patients, missing BDG test result in 1 patient).

Overall mortality was 38% (13/34 patients), 40% in patients where the last BDG test was positive (9/22) versus 38% (3/8) in patients where the last BDG test was negative. Death was attributable to candidemia in 6/13 patients with a fatal outcome, while in the remaining causes of death remained unknown (3/13) or were due to other diseases (4/13). All 6 patients in whom candidemia was attributed as the cause of death were positive for the last measured BDG. In terms of geographic distribution of BDG measurements, 20/34 patients were from the United Kingdom (58.8%), 3 from Sweden, France and Austria (each 8.8%), 2 from Slovenia and Italy (each 5%) and 1 (2.9%) from the Czech Republic. In 94% (32/34), either an infectious disease (41%, 14 patients) or microbiology consult (53%, 18 patients) was performed.

Conclusions:

This analysis shows that serum BDG is rarely used in Europe as a biomarker for treatment stratification and outcome prediction, with strong geographical differences. BDG was positive in all patients where death was attributed to candidemia. Larger studies are needed to determine a potential role of BDG in treatment stratification and outcome prediction in patients with candidemia.

P155

Searching for biomarkers of Invasive Candidiasis: characterization of Candida albicans Hyr1 and potential use for diagnostics

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Objectives:
Characterization of proteins recognized by antibodies which react with *Candida albicans* germ tube surface antigens (CAGTA), in order to test their possible application for the development of diagnostic techniques for invasive candidiasis. In a preliminary study, CAGTA reacted with several clones of a *C. albicans* cDNA lambda-phage expression library, most of them corresponding to different segments of the gene encoding the Hyr1 protein, so we proceeded to their characterization.

Materials & Methods:
The insert sequences of 28 clones of the *C. albicans* cDNA lambda-phage expression library recognized by CAGTA, and corresponding to different segments of the gene coding for Hyr1 protein, were subjected to different analyses: multiple sequence alignment with the Clustal Omega tool, and identification of possible linear epitopes using three algorithms (Kolaskar and Tongaonkar antigenicity scale, Parker hydrophilicity scale and BepiPred 2.0), as well as discontiguous or conformational epitopes with the DiscoTope server. Algorithms were available at the IEDB Analysis Resource web (http://tools.iedb.org). Due to the apparent relevance of conformational epitopes, the *Pichia pastoris* model was chosen for the recombinant Hyr1 protein expression and for a fragment of this protein encoded by clone D22b. Post-translational glycosylation modifications were analyzed with the GPP predictor (Hirst Group de la University of Nottingham, UK; https://comp.chem.nottingham.ac.uk/glyco/).

Results:
The alignment of the Hyr1-carrying clones sequences led us to select clone D22b which contains a segment of about 800 nucleotides of the *HYR1* gene, that was in the common region of most phages encoding Hyr1 fragments.

D22b protein presents potential linear and conformational epitopes, but only fragments of the linear ones overlap between them. On the other hand, DiscoTope showed almost all the phage coding area as a potential conformational epitope.

In order to express the recombinant Hyr1 protein in *P. pastoris*, the encoding gene was localized in *C. albicans* SC5314 revealing major differences between alleles, resulting in a 54 bp size difference between them.

The recombinant proteins produced by *Pichia* showed a 2-to-3-fold increase with reference to their expected protein molecular weight, suggesting the occurrence of post-translational modifications, such as some of the N- and O- glycosylations predicted by the GPP server.

Conclusions:
Candida albicans Hyr1 protein and the internal fragment D22b appear as targets of CAGTA antibodies. Both proteins will be tested by ELISA to evaluate the capacity of the detection of

serum specific antibodies to diagnose Invasive Candidiasis in patients at risk of fungal infections.

The variability between alleles of *HYR1* gene in *C. albicans* SC5314 deserves future studies in this and other strains because it might be related to filamentation, adhesion or virulence capacity of different strains.

Financial support:

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P156

Noninvasive sampling method and PCR for the diagnosis of feline sporotrichosis by Sporothrix brasiliensis

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Sporothrix brasiliensis emerged in Brazil during the late 1990s and has since evolved into a global concern. Outbreaks and hyperendemics have been reported across almost all Brazilian territory and, in recent years, has extended to other countries as well, mainly associated with domestic cats, which are the main transmitters of the fungus and victims of the disease. **Objective**: We aimed to evaluate a polymerase chain reaction (PCR) method for the diagnosis of feline sporotrichosis directly from secretion samples collected using a simple and noninvasive procedure. **Materials and Methods:** This retrospective study included secretion samples collected with swab from 23 domestic cats, which were available in the biorepository of the Mycology Lab (FAMED-FURG) in saline at -20 °C. Out of the total of samples included, 15 were from patients with proven sporothrichosis by *Sporothrix* sp. isolation in culture. The remaining 8 samples were from patients investigated by sporotrichosis, with negative culture results. DNA was extracted from the samples using a commercial High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). We tested a conventional PCR reaction with a species-specific primer for *S. brasiliensis* (Rodrigues et al., 2015) and a final volume of 25 µl, with 3 µl of DNA, 12.5 µl of Gotaq® G2 Master Mix (2×) (Promega Corporation, Wisconsin, USA), and 1 µl (20 pmol/µl) of each *Sbra* primer. Electrophoresis of the PCR products was performed on a 1.2% gel under UV light with a BlueGreen Loading Dye. Negative samples underwent a second round of PCR using the amplicons (3 µL) from the previous PCR under the same conditions. To confirm *S. brasiliensis* amplification, 20% of the positive results were sequenced. The sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), and accuracy of the tests were calculated. **Results:** Out of 15 cases of feline sporotrichosis PCR was positive in 12. None of the cases that tested negative in culture resulted in a positive PCR. Sensitivity, specificity, PPV, NPV of PCR were 80%, 100%, 100%, and 72.7%, respectively, and an accucary rate of 87%. Using the second round of PCR, the sensitivity and accuracy increased to 93.3% (14/15) and 95.6%, respectively. Specifity and PPV remained unchanged. Sequencing of three random positive PCR amplicons confirmed *S. brasiliensis* identification, with 99% homology compared to the GenBank database (Bethesda, MD, USA). **Conclusions**: Our study is pionner in showing high rates of accuracy of noninvasive sampling method evaluated by PCR in the diagnosis of subcutaneous sporotrichosis by *S. brasiliensis* in domestic cats. Since we included only retrospective samples, which were maintained frozen at our biorepository, even better results are expected to be acquired using fresh samples in prospective studies. **Acknowledgments**: The authors are grateful to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* from the Ministry of Education, Brazil (CAPES-BR), to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq-BR) - *Projeto Universal Processo 405653/2021-2,* and to the *Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS-RS).

P158

The current state of laboratory mycology and treatmetn in the Balkans

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Objectives: Diagnostic tools and therapeutic modalities for invasive fungal infections (IFI) are developing continously. However, due to economic heterogeneity they are not widely accesible. Additionally, disease patterns may differ among countries and regions. For that reason, the aim of this study was to assess the current diagnostic capacity and availability of treatments for IFIs for the countries of Balkan region.

Materials & Methods: The data from the following countries were collected: Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Kosovo (as per United Nations Security Council resolution 1244), Moldova, Montenegro, North Macedonia, Serbia, Slovenia, Romania. The survey is accessible online at www.clinicalsurveys.net/uc/IFI_management_capacity. Collected variables included: a) institution profile, b) perceptions on invasive fungal disease in the respective institution, c) microscopy, d) culture and fungal identification, e) serology, f) antigen detection, g) molecular tests and h) therapeutic drug monitoring.

Results: A total of 47 representatives from different relevant medical centers from Balkan responded to survey. The highest number of participants estimated the incidence of IFI as low (18/47, 38.3%) and very low (16/47, 34.0%). The most important pathogens which were reported as causative agents of IFI in our centers were Candida spp.(47/47, 100%), Aspergillus spp. (34/47, 72.3%), and Cryptococcus spp. (20/47, 42.6%). Most of the institutions had access to cultures and microscopy (46/47, 97.9%), as well as the suseptibillity testing technology (44/47, 92.6%). Regarding availability of other diagnostic tests, 80.9% use antigen detection tests (mostly Aspergillus and Candida antiges). However, PCR tests are far less available (in less than a half of centers – Aspergillus PCR in 47.8%, Candida PCR in 34.0%, Pneumocystis PCR in 42.6%, Mucorales PCR in 19.1%). Triazoles were the most available class of antifungals which were prescribed in 95.7% of centers (95.7%), followed by echinocandines in 78.7% (mainly micafungin, 76.6%) and amphotericin B in 68.1% (mainly liposomal formation, 57.4%). Therapeutic drug monitoring was reported only in 14/47 (29.8%) of surveyed centers (vorikonazole in all 14, posaconazole in 10, itraconazole in 9 and flucytosine in 6 centers).

Conclusions: According to the our results, Balkan centers have well-equiped mycology laboratories, with the exception of the mollecular diagnostic tools which are available in less than a half of surveyed centers. When the word is about treatment modalities, triazoles are widely accessible, in contrast to other drugs (echinocandins, amphotericin B, flucytosine, terbinafine) which are far less availbable. The survey is open in order to include the largest number of sites possible.

P159

Clinico-microbiological profile of Chronic Pulmonary Aspergillosis: A descriptive study from a tertiary care hospital in Southern India.

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Objectives:

1. To study the presenting symptoms, co-morbidities, microbiological and clinico-radiological features in a clinically suspected case of chronic pulmonary aspergillosis(CPA).
2. To evaluate the optimal cut-off levels of Aspergillus specific IgG(Asp-IgG) in the diagnosis of CPA.
3. To propose a diagnostic algorithm for CPA in our study population.

Materials & Methods:

A total of 120 patients with a prior history of pulmonary tuberculosis(PTB)/chronic obstructive lung disease(COPD)/other risk factors in clinically suspected cases of CPA were recruited for the study from January 2022-May 2023. The presenting symptoms, co-morbidities, microbiological, clinical and radiological features were recorded and compared with their Asp-IgG levels wherein a cut-off of >12 U/ml was used as positive for *Aspergillus fumigatus* specific IgG. The subjects presenting with haemoptysis were classified as massive if they had coughed more than 200 ml or underwent procedures like bronchial artery embolisation to control the bleeding. The review and scoring of chest x-rays were done using a simple xray scoring system by Priya Ramachandran et al keeping the cut-off score at 40.

Results:

The preliminary results with 21 study participants showed a male:female ratio of 1.625:1 and mean age of 43.7 years. 100% of the patients had a prior history of PTB. Cough with expectoration was the most common symptom observed in 90.4%(19/21) of the cases. Massive haemoptysis was present in 47.6%(10/21) of the cases. 61.9% of the cases had both positive Asp-IgG levels and X-ray cut-off score >40. Cavitation was the most common chest x-ray as well as CT finding at 42.85% and 52.38% respectively. Microbiologically, KOH with calcoflour stain was positive in only 14.28%(3/21) of the cases while only one case was culture positive for *Aspergillus fumigatus.* Serum Galactomannan was sent in only 2 cases(9.5%), which did not correlate with the positive Asp-IgG levels or the X-ray score of >40.

Conclusions:

India has a high burden of TB, and TB is one of the leading causes of mortality in India killing almost 1,000 individuals every day.The incident TB cases are about 2.1 million with the annual incidence of CPA varying between 27,000-0.17 million cases in India. Many patients with CPA live in low and middle income countries and develop CPA following PTB. CPA prevalence is further likely to increase due to decreasing TB mortality. Hence, an early suspicion and diagnosis of CPA, is the need of the hour given it's increasing burden in India. An extensive review of literature resulted in only one such study conducted in Southern India in 2013. CPA is an under-reported entity and it's diagnosis remains a challenge for clinicians. Our preliminary data show that diagnosis is further complicated due to poor sensitivity of mycological culture and how the use of a simple x-ray scoring system and serum Asp-IgG levels can aid in early diagnosis particularly in resource constrained settings. We also plan on evaluating the optimal Asp-IgG cut-off levels by plotting a receiver operating curve and

propose a diagnostic algorithm specific to our study population which shall be presented at the conference.

Table 1: Qualitative analysis of 21 subjects

Character	Number (%)
Total subjects	21
Gender:	
Male	13(61.9%)
Female	8(38.09%)
Asp-IgG levels:	
>12 U/ml(Positive)	13(61.9%)
<12 U/ml(Negative)	8(38.09%)
X-ray score	
>40	13(61.9%)
<40	8(38.09)
History of massive haemoptysis:	
Present	10(47.6%)
Absent	11(52.38)
Patients presenting within:	
2 years of PTB treatment	5(23.8%)
5 years of PTB treatment	13(61.9)

Table 2: Diagnostic measure using a chest x-ray score of 40 as the cut-off

X-ray score	Asp-IgG levels(U/ml)		Total
	>12(%)	<12(%)	
>40	9(69.2%)	4(30.7)	13
<40	4(50%)	4(50%)	8

Sl no.	Age(y)	Gender(M/F)	IgG value	Cough	Haemoptysis	Fever	Wheeze	Wt loss	Dyspnoea(MRC Grade)	COPD	DM	CKD	Xray Score	PTB sequelae	h/o ATT intake
1	32	M	3.33	Y	SMALL	Y							50	4y(2019)	Y
2	41	F	200		SMALL								45	20y	Y
3	43	M	66.5	Y	SMALL						Y		25	1Y	Y
4	45	M	5.8	Y	SMALL								10	1y	Y
5	30	F	42	Y	SMALL								10	7y(2016)	Y
6	55	M	6.48	Y			Y	Y	Y(Gr IV)	Y			35	5Y	Y
7	51	F	7.17	Y		Y			Y(GR II)				70	4y(2019)	Y
8	58	M	5.87	Y	MASSIVE				Y(GR III)				30	6mo, 15d back	Y
9	45	M	187	Y	MASSIVE			Y	Y(GR III)	Y	Y		75	10y	Y
10	50	M	29.7	Y	MASSIVE						Y		20	2Y	Y
11	35	M	7	Y	SMALL		Y						60	6y	Y
12	26	F	173	Y	MASSIVE	Y			Y(GR I)				80	6Y	Y
13	46	F	200	Y	MASSIVE								100	3Y	Y
14	65	M	44	Y		Y			Y(GR II)	Y			55	6y	Y
15	54	M	125	Y	MASSIVE	Y	Y		Y(GR III)	Y			70	3Y	Y
16	32	M	125	Y	MASSIVE								100	5Y	Y
17	52	F	7.33	Y	MASSIVE	Y		Y			Y	Y	65	2y	Y
18	45	M	187	Y		Y	Y			Y			35	20Y	Y
19	28	M	96.8	Y	MASSIVE			Y					50	6Y	Y
20	21	F	3.4	Y	MASSIVE								30	3Y	Y
21	45	F	35		SMALL								45	5Y	Y

P160

The current state of laboratory mycology in Nordic countries

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Introduction: Invasive fungal infections (IFIs) are a particular threat to patients with underlying diseases. Rapid diagnostics are essential for proper treatment.

Methods: Details from Nordic laboratories on institution profile, access to microscopy, culture, serology, antigen detection, molecular testing, and access to antifungal drugs and therapeutic drug monitoring were collected in a survey.

Results: As of May 2023, 33 centers from five countries, Sweden (n=13), Denmark (n=7), Norway (n=8), , Finland (n=4), and Iceland (n=1), answered the questionnaire. The following pathogens could be detected in these laboratories: Candida spp. (97%), Aspergillus spp. (97%) followed by Mucorales spp. (43%) and Fusarium spp. (23%). All institutions had access to microscopy and culture-based diagnostics. In addition, most centers had access to antigen detection and serology (83% for both methods). With regards to molecular methods, 97% had Pneumocystis PCR, 87% had Aspergillus PCR, 70% had Candida PCR and 67% had Mucorales PCR.

Access to antifungals was in general high except for Flucytosine (5-FC) (23%). At least one triazole was available in 86.7 % of the reporting sites, voriconazole (83.3%) most commonly as mould-active azole. 76.7% had at least one amphotericin B formulation, and 86.7% had at least one echinocandin.

Conclusion: The Nordic countries have resources for adequate IFI diagnosis and management. However, some institutions still do not have access to certain diagnostic tools and antifungal drugs. The NSMM board supports/endorses the abstract

P161

An evaluation to assess the clinical performance of a Mucorales IVD Real-Time PCR Kit

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Objectives: The Fungiplex Mucorales IVD Real-Time PCR Kit is a multiplex polymerase chain reaction (PCR) test for the qualitative detection of Mucormycetes DNA (*Actinomucor spp., Apophysomyces spp., Cunninghamella spp., Lichtheimia spp., Mucor spp., Rhizomucor spp., Rhizopus spp., Saksenaea spp.,* and *Syncephalastrum spp*.) extracted from tissue biopsy, bronchoalveolar lavage (BAL) and serum samples. This clinical performance evaluation study was set up to assess the clinical performance of the Fungiplex Mucorales Real-Time PCR Kit and generate sufficient evidence for IVD-CE certification. An overview of the results of the clinical validation study are provided here.

Materials & Methods: Contrived serum samples containing Mucormycetes as stated above were prepared at one clinical evaluation site to take into account low prevalence of Mucorales species. Tissue biopsy and bronchoalveolar lavage (BAL) clinical samples categorised as positive for Mucormycetes in line with the EORTC/MSG definitions for Mucormycosis were collected at a second clinical evaluation site. At both laboratories, samples negative for fungal infection were also included. The samples were then extracted using either the Roche MagNaPure96®, ELITe InGenus® or Biomérieux EASYMAG® platforms.

The Fungiplex Mucorales assay set up includes an Extraction control/ PCR inhibition control to allow confidence in a negative result. Single PCR reactions for each sample were analysed on either the Qiagen Rotor-Gene Q 5plex HRM or the Roche LightCycler 480 II platforms. In total 100 contrived serum samples (PHW, Cardiff) & 91 clinical tissue biopsy or BAL samples (Westerdijk Fungal Biodiversity Institute, Utrecht) were included in this study. Due to lack of diagnostics in this area, an in-house PCR was also used for comparison, as well as the EORTC/MSG criteria.

Results: Table 1 shows the results of the clinical validation study. An overall sensitivity of 94.6% and overall specificity of 99.7% (including well categorised contrived samples and clinical samples) resulted during this clinical performance evaluation study for the Fungiplex Mucorales Real-Time PCR kit. Taking into account the clinical samples alone for tissue biopsy and BAL samples, sensitivity and specificity were reported as 83.4 % and 99.2 %, respectively.

Conclusions: A Mucorales IVD Real-Time PCR assay for the detection of Mucormycetes in patients with Mucormycosis has been validated and shown excellent clinical sensitivity and specificity in multiple sample types. This assay contributes clinically to an underserved area of fungal diagnostics.

Table 1: Sensitivity and Specificity of **Fungiplex Mucorales Real-Time IVD PCR Kit.**

Sample Type	Results	
Contrived Serum Samples (Cardiff)	Sensitivity (%)	98.75 %
	Specificity (%)	100 %
Tissue biopsy samples (Utrecht)	Sensitivity (%)	85.7 %
	Specificity (%)	100 %
BAL samples (Utrecht)	Sensitivity (%)	80.0 %
	Specificity (%)	98.7 %
Total Clinical Samples	Sensitivity (%)	**83.4 %**
	Specificity (%)	**99.2 %**
Results for all sample types	Sensitivity (%)	**94.6 %**
	Specificity (%)	**99.7 %**

P162

Analytical and Clinical Validation of a Pan Fungal PCR Kit

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Objectives: Invasive fungal diseases (IFDs) are a significant cause of morbidity and mortality in patients who are immunocompromised or undergoing intensive therapy. The diagnosis of IFDs is challenging as clinical manifestations are non-specific and standard methods of diagnosis are often slow or have low sensitivity. In recent years, the epidemiology of IFDs has changed; Candida species remain the most common causes of IFD, but the incidence of Aspergillus species and other filamentous fungi, such as Zygomycetes, Fusarium and Scedosporium spp., has increased. Identifying a fungal infection as early as possible is critical for the implementation of appropriate antifungal therapy; early diagnosis can improve treatment outcomes and potentially reduce healthcare costs. This study was set up to evaluate the Fungiplex Universal Real-Time PCR Kit, a pan fungal assay for the detection of fungal DNA which is also provided with Sanger sequencing primers for identification to genus or species level.

Materials & Methods: Analytical and clinical validation studies were planned under new IVDR guidelines. The Fungiplex Universal Kit uses specific, targeted universal ITS primers combined with multiple probes within the mastermix and is also provided with a variety of controls; positive, negative and extraction controls. This assay is designed in an easy to use format with minimum hands on time and results generated in approximately 1 hour from extraction.
The analytical validation study was set up to assess the accuracy and precision of the detection of fungal DNA at detection limits between 50 and 500 copies per PCR reaction. This was achieved using six thermal cycler instruments. Samples tested were specific to the probes included in the assay with starting materials of plasmid DNA and genomic DNA used. The clinical validation study is currently on-going across two different clinical mycology labs in the UK. Enrolled positive tissue samples from patients categorised with histopathologically proven cases of fungal disease according to EORTC criteria are included. Contrived samples prepared in serum and plasma have also been included to account for low prevalence.

Results: The analytical sensitivity of the Fungiplex Univeral Kit was found to be 98.4 % with a 95 % confidence interval of (97.4 – 100 %), see Table 1, while the analytical specificity was reported as 100 % with a 95 % confidence interval of (99.2 – 100%), see Table 2. Clinical validation results are expected for presentation

Conclusions: The Fungiplex Universal Real-Time PCR Kit has been validated analytically and clinically, showing excellent sensitivity and specificity, and covering a wide range of clinically relevant genera. Sanger sequencing primers have been shown to provide additional key information on the fungal DNA detected and due to the broad range of fungal coverage offered by this real-time PCR assay, a negative result could be reliably used to de-escalate treatment and reduce antifungal drug spend.

Table 1: Overall analytical sensitivity for the Fungiplex Universal IVD real-time PCR assay.

Target	All Sample Types	
	Total Tested	Samples Detected
All targets	1032	1015
False Positives (FP)		0
False Negatives (FN)		17
True Positives (TP)		1015
True Negatives (TN)		2064
Assay sensitivity (%)		**98.4 %**
95 % Confidence Interval		**97.4 to 99.0 %**
PPV		100 %
NPV		**99.2 %**
95 % Confidence Interval		**98.7 to 99.5 %**

Table 2: Analytical specificity for the Fungiplex Universal IVD real-time PCR assay.

Target	All Sample Types	
	Total Tested	Samples Detected
All targets	216	174
False Positives (FP)		0
False Negatives (FN)		42
True Positives (TP)		174
True Negatives (TN)		432
Assay specificity (%)		**100.0 %**
95 % Confidence Interval		**99.2-100 %**
PPV		100 %
NPV		**91.2 %**
95 % Confidence Interval		**88.7-99.1 %**

P163

Identification of the most immunoreactive Candida auris antigens through the study of the humoral response to systemic infections in mice

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Objectives:

The delay on making a correct diagnosis and the high antifungal resistance rise mortality rates of *Candida auris* infections up to 60 %. Therefore, identification of new diagnostic markers stands out as a priority in order to deal with this pathogen. Due to that, the aim of this study is to identify new diagnostic markers of infection of *C. auris* by the development of a murine model of disseminated infection and to study the specificity of the detected antigens through the use of sera from mice infected with *C. auris*, *Candida albicans* or *Aspergillus fumigatus*, as well as from patients with *C. auris* infection.

Materials & Methods:

Eight-week old Swiss immunocompetent female mice were intravenously injected with three different infective doses (10^6, 10^7 and $5x10^7$ cells/animal) of non-aggregative and aggregative phenotypes of *C. auris*, as well as with 10^5 cells/animal of *C. albicans*. Total sera for immunoproteomic study as well as brain, lungs, kidneys, spleen and liver for Colony Forming Unit (CFU) counting and histology were extracted 28 days after the injection. The antigenic detection was achieved by two-dimensional western blotting (2D-WB) and the identification of the most immunoreactive spots was done by mass spectrometry (LC-MS/MS). Sera from mice infected with *A. fumigatus* were obtained from a previous study and sera of patients infected by *C. auris* were obtained from the University and Polytechnic La Fe Hospital of Valencia, Spain.

Results:

The highest dose ($5x10^7$ cells/animal) of *C. auris* was determined as the infective dose because several signs of infection (weight loss, head bobbing and inclination, ruffled hair…) were recorded. Even using a higher infective dose, both non-aggregative and aggregative phenotypes of *C. auris* were less virulent than *C. albicans*, as they did not induce mortality and signs of infection were less and weaker. Among both phenotypes, the non-aggregative showed to be more virulent than the aggregative based on the symptoms observed. However, no significant differences were found in the CFU counting, with the exception of the brain, which presented a significantly greater fungal load of the aggregative isolate.

In the immunoproteomic study, 150 and 60 antigenic spots were detected using sera from mice infected with non-aggregative and aggregative isolates, respectively, among the 350 protein spots of *C. auris*. None of these spots were detected by sera from mice infected with *C. albicans* or *A. fumigatus*. Finally, the 200 antigenic spots detected by sera from patients with candidemia caused by *C. auris* gave a similar immunome pattern to that obtained with mice infected by the same yeast, confirming the development of infection on the animals.

Among these antigenic spots, phosphopyruvate hydratase or enolase (Eno1), phosphoglycerate kinase (Pgk), glyceraldehyde 3-phosphate dehydrogenase (Gapdh) and phosphoglycerate mutase (Pgm) were identified by LC-MS/MS as the most immunoreactive antigens and shared between non-aggregative and aggregative isolates.

Conclusions:

This study identified four specific antigenic markers (Eno1, Pgk, Gapdh and Pgm) of *C. auris* that can be useful on the diagnosis or treatment of *C. auris* candidemia caused by both non-aggregative and aggregative growth phenotypes.

P164

Histoplasmosis and cryptococcosis: histopathological pitfalls and relevance of a histomolecular diagnosis based on massive parallel sequencing

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Objectives:
The histopathological features of *Cryptococcus* spp. and *Histoplasma capsulatum* var. *capsulatum* are well described. However, in practice, these features are not always observed. Our objectives were to estimate the frequency of histopathological features classically described for cryptococcosis and histoplasmosis and to evaluate the usefulness of an integrated histomolecular diagnosis.

Materials & Methods:
An analysis of 22 cases of cryptococcosis and histoplasmosis referred for expert review in Lyon (France) from 2012 to 2023 was performed. Sixteen cryptococcoses and six histoplasmoses originated from five different pathology departments. Clinical/biological data were retrospectively collected, including culture and/or panfungal PCR on fresh tissue and/or serum or CSF *Cryptococcus* antigen level.
For each case, the initial diagnosis suggested by the first pathologist could be confirmed, specified, or modified by expert review. The following features were collected: presence/absence of a granulomatous reaction, giant cell, caseous necrosis, clearly visible capsule, "Protozoa-like" features in Hematoxylin-eosin-saffron (HES) staining, pseudohyphae, but also the shape and homogeneous or variable size of the yeasts, intra- and/or extra-cellular location, and alcian blue staining.
When possible, an integrated histomolecular diagnosis was performed using panfungal massive parallel sequencing (MPS), previously developed by our team on formalin-fixed tissues.

Results:
The median age was 53.5 years. An immunosuppressive factor was present for 16/22 (73%) patients. Slide review allowed a diagnostic confirmation, modification, or specification in 72.7% (16/22), 13.6% (3/22), and 13.6% (3/22) of cases, respectively.
Concerning cryptococcosis, a granuloma was present in 13/16 (81.3%) cases, with giant cells in 9/13 (69.2%) cases. Necrosis was observed in 6/16 (37.5%) cases and was caseous in 5/6 (83.3%) cases. On HES, the capsule was not visible (2/16, 12.5%), clearly visible (9/16, 56.3%) or very clearly visible (5/16, 31.3%). The yeast shape was always round (16/16, 100%), with some oval (5/16, 31.3%) and/or dented-looking (10/16, 62.5%) yeasts, and pseudohyphae in 3/16 (18.8%) cases. The yeast size was always variable (16/16, 100%), and the location was always both intracellular and extracellular (16/16, 100%). Capsule was always stained by alcian blue (16/16, 100%); the capsule was thin (7/16, 43.8%) or thick 9/16 (56.3%).
Concerning histoplasmosis, a granuloma was present in 4/6 (66.7%) cases, with giant cells in 2/4 (50%) cases. Necrosis was observed in 5/6 (83.3%) cases and was caseous in 4/5 (80%) cases. On HES, a pseudocapsule was visible in 1/6 (16.7%) cases, and "Protozoa-like" features were observed in 3/6 (50%) cases. The yeast shape was always oval (6/6, 100%), sometimes combined with round (1/6, 16.7%) and/or dented-looking (1/6, 16.7%) yeasts. The yeasts had always homogeneous size (6/6, 100%) and were intracellular and/or extracellular in the necrosis only (16/16, 100%). Alcian blue was always negative (0/6, 0%).

When no identification was possible on fresh tissue (11/22, 50%), integrated histomolecular diagnosis by panfungal MPS on fixed tissue allowed fungal identification in 54.5% (6/11).

Conclusions:

Although morphological criteria are essential for histopathological diagnosis when mycological investigations do not allow the diagnosis, they can be misleading or not always observed. Therefore, an expert review and an integrated histomolecular diagnosis are crucial to improve patient management.

P165

EVALUATION OF PCR-HIGH-RESOLUTION MELT (HRM) ANALYSIS FOR THE DETECTION AND IDENTIFICATION OF MUCORALES FROM CULTURE ISOLATES USING PAN-MUCORALES-SPECIFIC PRIMERS

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INTRODUCTION:

Zygomycosis is a diverse group of increasingly recognized and frequently fatal mycotic diseases caused by members of the class zygomycetes. The prevalence of zygomycosis in India is about 80 times the prevalence in developed countries i.e. approximately 0.14 cases per 1000 population. The identification and diagnosis of zygomycetes are difficult as well as challenging. The Order Mucorales include *Rhizopus, Mucor, Rhizomucor, Apophysomyces, Lictheimia, Cunninghamella, Syncephalastrum,* and *Saksenaea*. Among them, *Rhizopus sp, Mucor sp.* and *Rhizomucor sp* are the common etiological agent of mucormycosis (zygomycosis). Rapid identification of mucormycetes is important due to its dreadful nature as it increases the morbidity and mortality rate. High-Resolution Melting Analysis (HRM) is a post-PCR method performed using the region of interest within the DNA sequence that is first amplified using the polymerase chain reaction. This study focuses on rapidly detecting and identifying Mucorales from culture isolates using High-Resolution Melting Analysis (HRM) which easily aids in differentiating the various genera among the order Mucorales, thus helping in the proper management of patients.

AIM:

- To detect and identify clinically important Mucorales from culture using HRM analysis.
- To compare the results of HRM analysis with the phenotypic identification of Mucorales.

MATERIALS & METHODS:

A total of 45 isolates belonging to the order Mucorales received in the Mycology division, Sri Ramachandra Institute of higher education and Research, Porur, Chennai were considered

for the study. Phenotypic confirmation of the isolates was done by performing Lactophenol cotton blue (LPCB) wet mount and also by studying the colony morphology on Sabouraud's dextrose agar. Extraction of DNA for all the cultures was performed by the Phenol-chloroform method. Real-time PCR followed by the HRM analysis using KAPA HRM Fast qPCR (dye-based) kit using pan-Mucorales-specific primers designed manually by MegaX software was carried out for the extracted DNA products. ATCC strains of *Rhizopus, Rhizomucor, Mucor, Apophysomyces, Lichtheimia, Cunninghamella*, and *Syncephalastrum* were used as the positive controls, and *Aspergillus flavus* ATCC 9643 as a negative control.

RESULTS & CONCLUSION:

Among the 45 isolates received, the following were the distribution of various genera of the order Mucorales identified phenotypically: *Rhizopus sp* (31), *Rhizomucor sp* (3), *Mucor sp* (1), *Apophysomyces sp* (6), *Lichtheimia sp* (2), *Cunninghamella sp* (1) and *Syncephalastrum sp* (1). Real-time PCR followed by HRM analysis was performed and the HRM curves of various genera under the Order Mucorales differed based on the GC content of the organisms. The results of the phenotypic identification and the PCR-HRM analysis were in concordance. Studying the melting temperatures and the HRM curves produced by the various genera helps in the rapid detection and identification as well as in the right choice of treatment as the resistance pattern differs among them.

P166

Laboratory capacities to diagnose and treat invasive fungal infections in Italy: local results from an ECMM survey

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Laboratory capacities to diagnose and treat invasive fungal infections in Italy: local results from an ECMM survey

Background

Different patients are at risk for invasive fungal infections (IFI). In Italy, most of them are immunosuppressed. Adequate access to diagnostic and treatment is of utmost relevance. Thus, we aimed to depict the current status of such tools.

Methods

Collected variables included a) institution profile, b) perceptions on IFI in the respective institution, c) microscopy, d) culture and fungal identification, e) serology, f) antigen detection, g) molecular tests and h) therapeutic drug monitoring.

Results

47 centers replied to the survey. IFI incidence of IFI was considered as moderate in 53% of the sites, while high/very high in 15%. Candida spp. (94%), Aspergillus spp. (90%) and Cryptococcus spp. (26%) were considered the most relevant pathogens. All laboratories had access to cultures (100%), two thirds of which could also implement susceptibility tests in molds and yeasts and almost 30% only in yeasts. In addition, 92% could perform microscopical analyses, 85% molecular tests (mainly PCR), and 81% serological ones. In every analyzed site there was access to at least one antigen detection test, and in all but one to an Aspergillus test. More than 90% of the clinics could administer at least one triazole, mainly fluconazole and voriconazole (92%, each). Echinocandin access was guaranteed in 92% of the sites, always at least caspofungin (92%). Liposomal amphotericin B was available in 89% of the clinics, while terbinafine and flucytosine in 17% and 53%, respectively. Only the access to TDM was significantly higher in Northern-Central regions as compared to Southern-Islands (82% versus 29%, $p<0.001$).

Conclusions

Italy is well-prepared to overcome the diagnostic and treatment of IFI. No statistically significant differences were observed between institutions based on their geographical location, except for TDM. Efforts from all stakeholders are necessary to guarantee an even access to diagnostic tools regardless of geographical the setting.

P167

Effect of different aliquot volumes on bronchoalveolar lavage galactomannan as a biomarker for diagnosis of pulmonary aspergillosis: a proof-of-concept study

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Objectives: Bronchoalveolar lavage (BAL) galactomannan is now part of newer criteria for diagnosis of both invasive and chronic pulmonary aspergillosis. However, unlike serum galactomannan, BAL-GM could possibly be affected by the volume of aliquots used during broncholaveolar lavage, which is not standardised. Varying amounts of aliquots used during bronchoalveolar lavage can potentially result in variable dilutions of the samples obtained for measuring BAL-GM (Image 1), the impact of which has not been well explored in previous studies.

Materials & Methods:

We included 250 samples obtained during BAL from 50 patients with suspected pulmonary aspergillosis, undergoing bronchoscopy for evaluation of chronic respiratory symptoms. We performed GM assay in BAL samples obtained as shown in Image 2 to study if the GM values vary with a difference in aliquot volume used during BAL. From each patient, 5 samples were obtained at varying 'dilution' after instilling 40 ml of normal saline for four times (i.e cumulative instillation of 40 ml, 80ml, 120 ml, 160 ml). The fifth sample was obtained by mixing 1 ml of each of the previous samples. We compared the galactomannan values obtained from different aliquot volumes as well as the discordance/agreement in galactomannan positivity between the various samples using a BAL-GM cut-off of 1 using McNemar test and Kappa statistic respectively.

Results: This study included 50 patients (Male:Female=1:1) with chronic respiratory symptoms with a mean age of 42 (±15) years, 40% with history of previous tuberculosis. Symptoms in these patients included cough (72%), hemoptysis (37%),fever (38%), loss of weight (30%), loss of appetite (28%) and shortness of breath (33%). 42% had likely chronic pulmonary aspergillosis while 20% had active pulmonary tuberculosis. The median [IQR] BAL GM value for 40 ml,80ml,120ml,160ml and mix of samples were 0.48 (0.16-0.81), 0.49 (0.15-1.34), 0.38 (0.20-0.57) , 0.47 (0.14-1.58) and 0.71 (0.25-1.38) respectively. Using a BAL-GM cut off of 1, 13.3% of 40ml samples, 30 % of 80ml samples, 23.3% of 120 ml samples, 26.7% of 160 ml samples and 30% of the mixture of samples were positive. The discordance of BAL-GM between 40 ml sample and other samples were 16.7% for 80ml (p=0.02), 10% for 120 ml (p=0.08), 13.3 % for 160ml (p=0.04) and 16.7% for mix of all samples (p=0.02). Similarly the level of agreement based on Kappa statistic between 40 ml sample and subsequent samples were 0.53, 0.67, 0.59 and 0.53 respectively, showing unsatisfactory agreement in BAL-GM positivity using samples from different aliquots used for BAL. The best correlation of serum galactomannan was with the 160 ml sample (r= 0.84) while the best correlation of 40ml sample (which was the least diluted sample) was with the mix of all samples (r= 0.91).

Conclusions: This study highlights the variation in measured BAL galactomannan values with different cumulative volumes of aliquots used during bronchoscopy. Different volumes of instillation of lavage fluid can result in variable BAL galactomannan indices and interpreted

positivity rate . Future studies should further evaluate BAL galactomannan cut-offs using standardized aliquot volume in patients with suspected pulmonary aspergillosis.

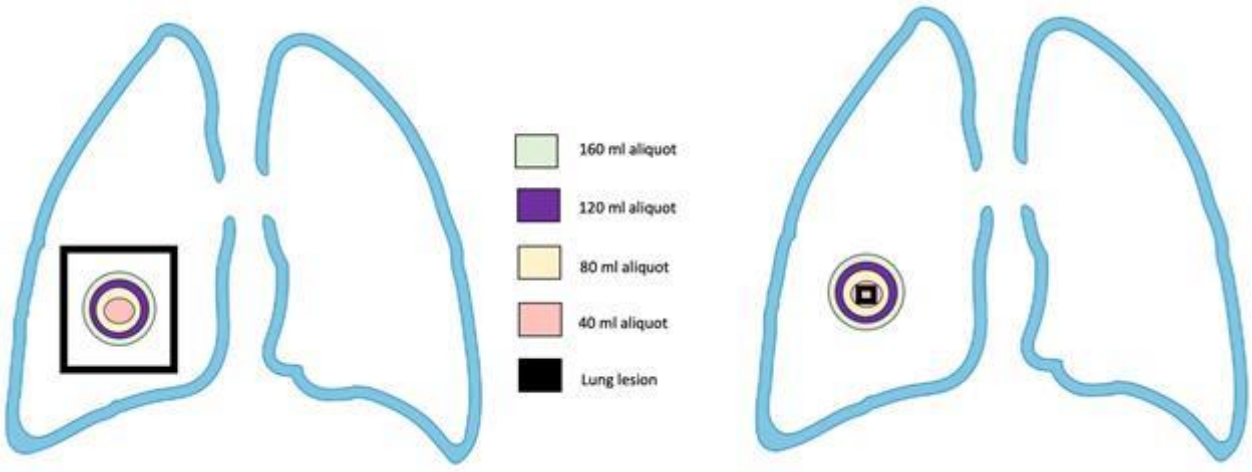
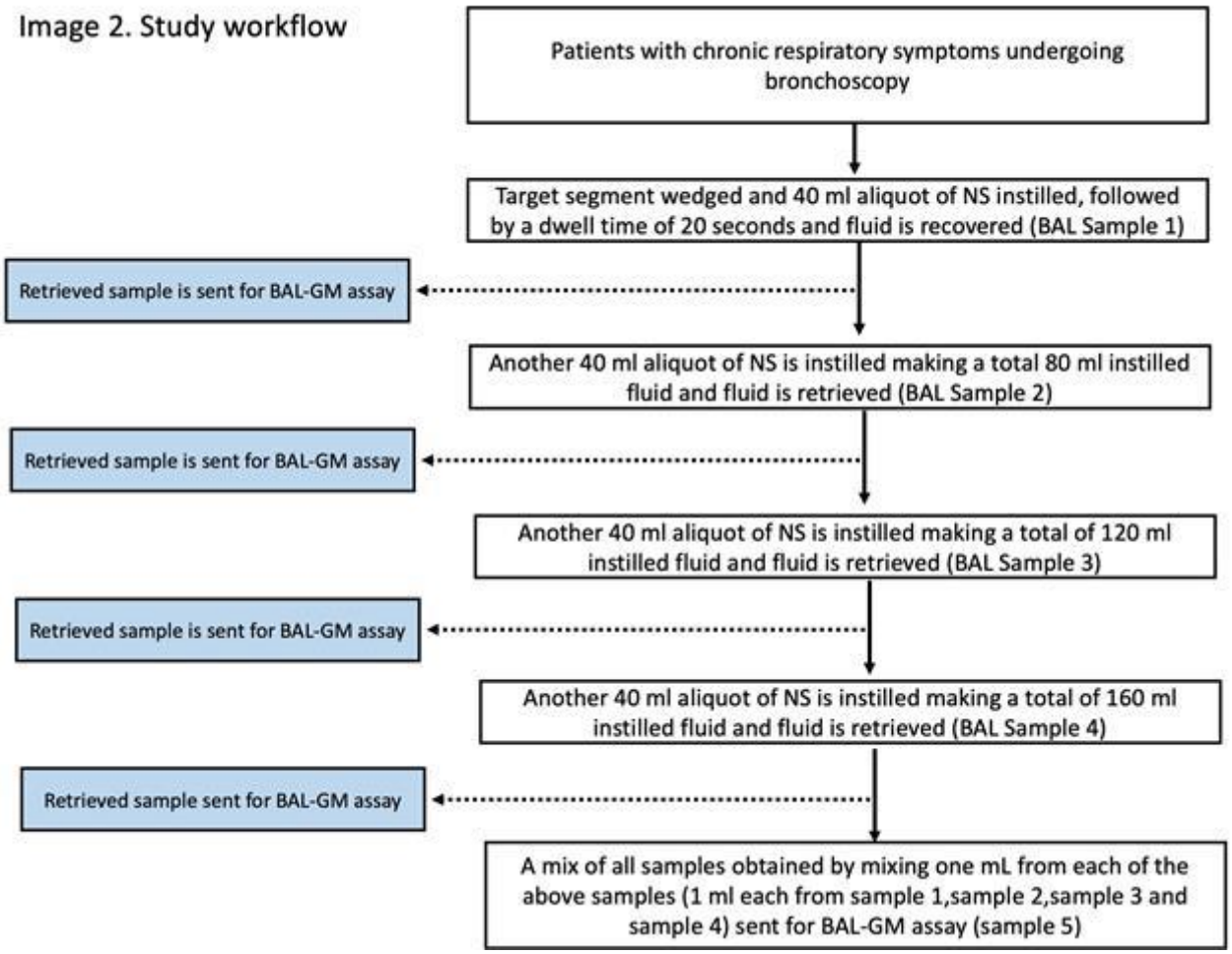


Image 1. Probable effect of varying volumes on BAL-GM
Panels above show the difference in fluid extension based on aliquot volumes and distribution with respect to lesion size. With subsequent aliquots, a larger volume of the lung tissue is percolated. The circles represent the extent of lung tissue percolated by serial aliquots of fluid instilled while the square represents lung lesion. With a larger lesion (left panel), larger volumes possibly extent into a wider region of the lesion and possibly resulting in retrieval of a larger amount of fungal antigen with higher BAL GM values and positivity. With a smaller lung lesion (right panel) or heterogenous involvement, larger volumes are likely to extend into uninvolved lung tissue much more than smaller volumes resulting in lower BAL-GM values

Image 2. Study workflow



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EVALUATION OF NEW TOOLS FOR THE DIAGNOSIS OF HISTOPLASMOSIS

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Objectives: Histoplasmosis is difficult to diagnose, probably due to a lack of epidemiological information, insufficient training and awareness among front-line healthcare workers, and clinical features very similar to those of tuberculosis, which can be misleading. In particular, this could explain the low number of diagnoses in Sub-Saharan and West Africa, compared with Latin America. This fungal infection mainly affects immunocompromised patients and particularly advanced HIV-patients, with a high case-fatality rate in the absence of treatment (from <10% to >40%). Implementing non-invasive diagnostic tools will allow to improve histoplasmosis diagnosis for the most exposed patients but also to evaluate the prevalence of this fungal infection in countries where data are still lacking. Recently, two Rapid Diagnostic Tests (RDTs) have been made commercially available for the diagnosis of histoplasmosis, based on urinary monoclonal antigen detection: (I) *Histoplasma capsulatum* Urinary Antigen Rapid Test from Optimum Imaging Diagnostics (OIDx) and (II) *Histoplasma* Urine Antigen Lateral Flow Assay from MiraVista Diagnostics (MV). Our objective was to evaluate two histoplasmosis Rapid Diagnostic Tests (OIDx and MV) feasibility and diagnostic performances for histoplasmosis diagnosis in French Guiana and Suriname.

Materials & Methods: We performed OIDx and MV tests on frozen urine samples from EDIRAPHIS biobank, conducted on hospitalized PLHIV from French Guiana and Suriname and on healthy control subjects from French Guiana. Proven histoplasmosis was defined according to EORTC criteria. As the fungal culture and the direct exam may not be feasible in future prospective studies in some settings, we decided to compare the probable histoplasmosis samples, defined by *Histoplasma* Galactomannan EIA Immy. This ELISA is widely used in endemic areas with a good performances Se=90,5% and a Sp=96,3% in urines.

Results: Our evaluation was done on urine samples from 35 patients with proven histoplasmosis, 96 with probable histoplasmosis, 87 without biological criteria for probable or proven histoplasmosis, and on a group of healthy controls n=30. In patients with proven histoplasmosis, OIDx tests was positive in 62.7% and MV in 65.7%. For patients with probable histoplasmosis, we calculated a sensibility (Se) =70.5% and a specificity (Sp) =92.8% for OIDx and a Se=84.1% and a Sp=94.9% for MV. OIDx were negative in 76.7% of the group of healthy controls while MV reached 100%. In this whole evaluation (n= 248) there was an excellent inter-observer correlation with a Kappa coefficient of 97% for both tests.

Conclusions: Mira Vista test showed higher performances than OIDx for samples from proven histoplasmosis or probable histoplasmosis and for healthy patients. These first results will be completed with further analysis to have the whole picture of these two RDTs. The next step will be to

implement these new tools at bedside or in laboratories together with other tests in different settings across Sub-Saharan Africa and on fresh urine samples. Diffusion of RDTs together with appropriate training of clinical and laboratory teams and increase accessibility to treatment may help reduce the burden of histoplasmosis in endemic areas of SSA and West Africa where the prevalence of advanced-HIV disease is high.

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Cross-reactivity of Aspergillus galactomannan antigen test among emerging non-fumigatus Aspergillus species

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Objectives: Invasive aspergillosis (IA) is a serious and sometimes lethal mold infection, which is most frequently encountered in immunosuppressed patients. *Aspergillus fumigatus* is the most common etiologic agent to cause IA worldwide. However, several other members of genera *Aspergillus* are reported to cause infections in humans and animals, including: *A. nidulans, A. udagawae, A. pseudoviridinutans, A. felis, A. flavus, A. tanneri, A. felis, A terreus, A lentulus, A. subramanianii,* and *A. fumisynnematus.*

Galactomannan (GM) is a polysaccharide antigen component of cell walls, characterized in *Aspergillus fumigatus* and some other ascomycetous molds. Measurement of GM antigen may aid in early diagnosis of IA. In the current study, we investigated the diagnostic value of GM testing to detect emerging non-fumigatus Aspergillus species.

Materials & Methods: A collection of 18 clinical *Aspergillus* isolates (belonging to 11 different sections of genera *Aspergillus*) obtained from NIH hospitalized patients were tested. The identity of each isolate was confirmed by colony morphology, microscopic characteristics, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper® Bruker Daltonics Inc. Billerica, MA) and PCR-sequencing of the internal transcribed spacer (ITS) region of ribosomal RNA and β-tubulin genes.

The *Aspergillus* isolates were cultured on Sabouraud dextrose agar for 5-7 days at 35-37℃, to obtain conidial suspensions. The conidia were inoculated in Sabouraud broth at a final concentration of 1 × 10^6 conidia/50 mL and incubated on a rotary shaker at 150-200 rpm for 48 hours at 30-37℃. The supernatants were collected by filtering through a sterile BD Falcon 40-µm-pore-size cell strainer (Coming Incorporated, Coming, NY) and centrifuged at 5,000g for 5 minutes at room temperature. The Bio-Rad Laboratories Platelia Aspergillus enzyme immunoassay was used to detect GM in centrifugated culture filtrates according to the manufacturer's instructions, and the results are reported as the GM index.

Results: Overall, all non-*fumigatus Aspergillus* isolates produced positive reactions in concentrated culture filtrates (10^3 dilution). The mean GM index for *Aspergillus fumigatus* supernatant (5.30) being comparable to those of non-*fumigatus Aspergillus* species.

Conclusions: In conclusion, our data provide supporting evidence that *Aspergillus* GM testing may have a role in the evaluation of patients with non-fumigatus *Aspergillus* infections. Additional testing in clinical samples and experimental animal model of disseminated aspergillosis are warranted to confirm the diagnostic value of GM testing in non-fumigatus *Aspergillus* infections.

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Mycoflora of a University Hospital; fungal contamination in air, water, and surface samples

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Mycoflora of a University Hospital; fungal contamination in air, water, and surface samples

Objectives:
Hospital indoor environments such as air, tap water and inanimate surfaces contains wide variety of microorganisms. The composition and concentration of airborne microorganisms in hospital indoor air has been reported to contain airborne bacteria and fungi concentrations ranged 10^1–10^3 CFU/m3 in inpatients facilities which mostly exceed recommendations from the World Health Organization (WHO). The role of the environment in transmission of bacteria is clearly defined, however studies on fungi are limited in the literature. Fungi are important component of the microorganisms of the air and are widely distributed in soil and water. The aim of this study is to evaluate distribution of fungal species in hospital indoor air, water and surfaces collected from different hospital wards and intensive care units.
Materials & Methods:
This study was conducted in Gazi University Hospital, Ankara, Turkey, during 2022-2023. Ethical approvel was obtained from Gazi University Ethics Committee (E-77082166-604.01.02-622780). Water samples from tubs, taps and toilets is monitored through the evaluation of microbiological parameters. The samples were taken after letting the water run for five minutes to clear stagnant water from the pipe. Microbiological surface sampling of high hand-touch surfaces with sterile disposable cotton swabs is performed by sampling with the top of a swab on surface area (conventionally 100 cm^2). Air collections were taken from hospital interiors, outdoor areas adjacent to the hospital at the approximate same time. Air samples were collected using the Air Sampler®, bioMerieux, which was a final air volume of 500 L per sample. Sabouraud dextrose agar (SDA) plates were incubated at 25 and 30°C for 7 days and checked daily. The resulting colonies were counted and reported as colony-forming units per cubic meter of air (CFU per m3). Surface and water samples were evaluated by counting growing colonies on the agar plates and documented as well.
Results:
A total of 22 hospital wards were surveyed; 110 air samples, 20 water samples, 45 surface samples were collected. The fungal genera in the indoor air and outdoor air were more or less homogenous. Key taxa specific to the hospital were *Penicillium*, *Cladosporium*, *Aspergillus*. Air samples revealed *Penicillium*, *Cladosporium*, *Aspergillus, Rhizopus, Paecilomyces, Curvularia, Alternaria* spp. contamination, whereas water samples were resulted in *Penicillium*, *Aspergillus* growth and surfaces were found to be contaminated with *Penicillium*, *Cladosporium* spp, and *Cryptococcus albidus*.
Conclusions:
The results showed controlled contamination levels on high touch surfaces in the patient environment and a high level of contamination of the indoor air suggesting deficiencies in the decontamination process.

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ISOLATION AND IDENTIFICATION OF FUNGI ON MOBILES PHONES AND HANDS OF HEALTHCARE WORKERS AND MEDICAL STUDENTS IN TERTIARY CARE CENTRE

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ABSTRACT

Background:

The presence of microorganisms on mobile phones is a well proven fact supported by many literatures. These microbes can be transmitted between individuals from mobile phones. The use of mobile phones remains mandatory for effective communication between the Health Care Workers (HCWs) and its usage in highrisk areas like Intensive Care Unit, burn wards and operative rooms has to be overlooked closely as these group of patients are more prone for developing infections. But only few studies are there in this category with respect to fungi.

Objective:

This study was conducted to investigate the presence of fungi colonising the hands and mobile phones of HCWs, in order to take necessary remedial measures thereby reducing the morbidity and mortality in immunocompromised individual and medical care costs.

Materials & Methods:

This case control study was conducted in a tertiary care centre, India**.** A total of 150 samples were collected from both HCWs and non HCWs by Glove juice technique for hands and sterile swabs were used for mobile phones. The collected samples were inoculated in Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) and incubated at 25˚C and 37˚C for 5 days and the growth was checked at regular intervals. The plates without any growth after 5 days were considered sterile. The fungal isolates grown were further identified by standard mycological tests.

Results:

In HCWs, about 46.7% (35/75) of the samples were positive for fungal isolates. Out of 35 positive samples 14(18.7%) were positive for mobile phones, 12(16%) were positive for hands and 9 were positive for both mobile phones and hands. In HCWs, Dual fungal organisms were isolated from 5 mobile phones.

In Non HCWs, about 49.3% (37/75) of the samples were positive for fungal isolates. Out of 37 positive samples 19(25.3%) were positive for mobile phones, 5(6.7%) were positive for

hands and 13 were positive for both mobile phones and hands. Dual fungal organisms were isolated from 2 mobile phones and 1 hand. Triple fungal organisms were isolated from a single mobile phones and hand.

In our study, mobile phones (55/150) were more contaminated compared to their hands (39/150) of both HCWs and Non HCWs. Among the HCWs and non HCWs, same organisms has been isolated from both mobile phones and hands in 8 and 11 individuals respectively which shows cross contamination of fungal microflora between hands and their mobile phones. *Candida*, *Aspergillus*, *Penicillium*, Phoma, Alternaria and Cladosporium were the fungi isolated from the contaminted mobile phones and hands. *Candida albicans* and *Aspergillus fumigatus* were found to be the predominant isolate obtained from both mobile phones and hands of HCWs and Non HCWs.

Conclusion:

Mobile phones were more contaminated than hands of both HCWs and Non HCWs. Cross contamination of hands from these mobile phones are potential source of risk for critically ill patients. Therefore it is necessary to regularly decontaminate the mobile phones and always wash hands before and after contact with mobile phone. Thereby, the spread of nosocomial infections can be prevented.

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Of yeasts and birds: identification and antifungal susceptibility of Candida spp. from the digestive tract of wild birds in France

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Objectives: Previous investigations indicated that *Candida* yeasts could be found in healthy birds, as part of the digestive microbiota of several avian species such as poultry, pigeons, turkeys, raptors and migratory birds. The present study aimed to identify *Candida* yeasts from the gastrointestinal tract of different wild bird species from a wildlife rehabilitation center in western France.

Methods: For the isolation of yeasts, samples were collected from birds admitted to the Wildlife Health Centre of Oniris in Nantes, France. For each animal, samples were collected from the oesophagus/crop and from the cloaca, using sterile cotton swabs. Samples were further seeded in Petri dishes containing Sabouraud dextrose agar supplemented with chloramphenicol and incubated at 37°C, for 7 days. When several colonies of yeasts developed on Sabouraud agar, samples were also inoculated onto CHROMID® *Candida* (Biomérieux, Marcy-l'Etoile, France), a chromogenic medium for the selective isolation of yeasts. Mycological identification of the isolates was performed by MALDI-ToF mass spectrometry (Vitek MS and Bruker Biotyper). Antifungal susceptibility to azoles and amphotericin B was assessed by a gradient strip method.

Results: During the study period, 100 wild birds were examined. Most of them were columbids (34 wood pigeons, *Columba palumbus*, and 24 Eurasian collared doves*, Streptopelia decaocto*) and raptors (39 animals amongst different species of owls and falcons). Few corvids were also included. Mycological cultures revealed the presence of yeasts in digestive tract of 23 birds. From the oesophagus, a positive culture was obtained in 15, 24 and 33% of raptors, columbids and corvids, respectively. From the cloaca, a positive culture was obtained in columbids only (10.3%). Four wood pigeons and one Eurasian collared dove were colonized by yeasts in both crop and cloaca. Eleven yeast species were identified: *Candida albicans, C. glabrata (Nakaseomyces glabrata), C. krusei (Pichia kudriavzevii), C. guilliermondii (Meyerozyma guilliermondii), C. tropicalis, C. parapsilosis, C. boidinii, Clavispora lusitaniae, Rhodotorula mucilaginosa, Kazachstania bovina* and *K. telluris.* The most frequent ones were *C. albicans* (60% of the positive cultures), *C. glabrata* (10%), *C. guilliermondii* (10%) and *C. krusei* (7%). *Candida albicans* was the only species detected in raptors whereas columbids and corvids could be colonized by different yeasts, sometimes in the same animal. In four cases of co-occurrence, *C. albicans* was present in association with one or two other yeast species. In one case, *C. glabrata* was associated to *C. krusei*. *In vitro* antifungal susceptibility is currently ongoing.

Conclusions: Wild birds may be colonized by various yeasts species, including many described as opportunistic pathogens in humans, therefore potentially contributing to their dissemination in the environment. We evidenced the presence of *C. albicans* and other human pathogenic yeasts in several types of wild birds, especially in synanthropic ones. Further genetic studies should be performed to investigate the gene flow between yeast populations carried by wild birds and humans.

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Culturomics analysis of gut mycobiota in patients with ulcerative colitis and characterization of Candia albicans isolates

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Objective: The human gut is colonized by diverse microorganisms, including bacteria, viruses, protozoa, and fungi. Several studies have suggested that the gut fungal microbiota (mycobiota) impacts the host immunity and the development and progression of human diseases. However, most gut microbiota studies have focused exclusively on bacteria, and the mycobiota in the organ have largely been unexplored.

Methods and Materials: We developed a culturomics platform and isolated the fungal strains from fecal samples of a cohort of Korean patients with ulcerative colitis (UC) and compared them with those of healthy subjects (HT).

Results: Our culturomics analysis showed that most identified fungal colonies belonged to the phylum Ascomycota followed by Basidiomycota both in the HT subjects and UC patients. The total number of colonies from the fecal samples of the UC patients was significantly higher than that of the HT subjects, suggesting that more fungal strains may persist in the intestines of the UC patients compared to that of the HT subjects. Moreover, we collected some *Candida albicans* isolates, which was one of the most dominant fungal species in the fecal samples. The phenotypic and genotypic characteristics of the *C. albicans* isolates from fecal samples were analyzed and compared with that of the same fungal species isolated from the different niches, such as the gut mucosal layer and blood. The results of the comparisons between the different *C. albicans* isolates are presented.

Conclusion: Our study emphasizes the importance of the gut mycobiota and provides useful information on *C. albicans* residing in the human gut.

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Clinically Relevant Yeasts in Sand: A Multiannual Evaluation of the Romanian Black Sea Coast

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Clinically relevant yeasts in sand: a multiannual evaluation of Romanian Black Sea Coast

Objectives: Sandy beaches represent a possible source of contamination with pathogenic microorganisms of people using those areas for recreational activities. The present study investigated the yeast contamination of sand in twenty beaches from the Romanian Black Sea coast.
Methods & Materials: The sites for sampling were chosen by the Mycosands team and included 20 coastal beaches from both urban and non-urban areas. The samples were collected quarterly in 2018 and 2021 (i. e. March, June, September, and December), and monthly during summer in 2022 (i. e. June, July, August). The total number of yeasts per gram of sand (cultivable at 37°C) has been registered for each sample. Fungal identification to species level was done by MALDI-TOF MS and Internal Transcriber Spacer Region (ITS) sequencing. *In vitro* antifungal susceptibility testing of the isolates (n=84) was performed using EUCAST E. Def. 7.3.2 for fluconazole only.
Results: A seasonal increase in yeast burden has been detected in some beaches, probably related to human activity and specific pollution sources. The total number of yeasts per gram of sand varied between 0 and 160 cfu (2018), between 0 and 1150 cfu (2021), and between 0 and 825 cfu (2022), respectively. Among clinically important species, the following taxa were identified: *Candida albicans*, *Candida blankii*, *Candida glabrata*, *Candida inconspicua*, *Candida lambica*, *Candida parapsilosis*, *Candida tropicalis*, *Clavispora lusitaniae*, *Geotrichum candidum*, *Hyphopichia burtonii*, *Meyerozyma guilliermondii*, *Naganishia albida*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, *Trichosporon asahii*, *Wickerhamomyces onychis*, *Yarrowia lipolytica*. The MICs to fluconazole varied between 0.125 mg/L and more than 64 mg/L.
Conclusions: The results showed that the beaches on the Romanian Black Sea coast can be contaminated with clinically important yeasts – some of them exhibiting reduced susceptibility to azoles. They should be monitored for this fungal parameter for a better management of sand and water safety.

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Surveillance for airborne and linen-associated fungi within hospitals in a U.S. city

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Objectives. Nosocomial outbreaks of fungal infections are increasingly reported. There are scant data on fungal burdens in hospital environments. Standardized surveillance methods are lacking. Our objectives were to develop methods for surveillance of fungi, and to assess airborne and healthcare linen burdens longitudinally in hospital environments.

Methods. We developed culture and culture-independent (real-time PCR, reference to standard curve) methods for measuring airborne fungal burdens using SAS Super 100 and SASS 3100 Dry Air samplers. We used our previously published methods for culturing healthcare linens. We performed serial airborne surveillance in 7 units with high-risk, immunosuppressed patients in 2 hospitals in different areas of Pittsburgh, as well as outside of the hospitals. Linens were tested upon hospital delivery from an offsite laundry.

Results. Air sampling methods were optimized for multiple variables, including volume, duration, and flow of air; culturing techniques (direct impact plates, cultures at different stages of filter processing); filter DNA recovery (extraction protocols, stage of filter processing); PCR (targets, primers, PCR parameters). Using optimized methods, we have surveyed each unit ≥6 times (over 3 months to date). Pathogenic moulds were cultured in >50% of outdoor and indoor air samples [Figures 1A-B]. Fungal burdens were significantly greater outside than inside hospitals, by both positive culture % and DNA burden (1- to 3-$\log_{10}$ genome equivalents (GEs) lower immediately inside door than outside). There was no correlation between outside and inside GEs on given days; $R^2<0.06$), nor were there significant differences in GEs within a given unit (nursing stations, patient rooms, positive pressure rooms; all $p>0.1$). GEs were significantly greater in culture-positive than culture-negative samples (Aspergillus, 2.4 vs. 1.5 $\log_{10}$; pan-fungal, 2.8 vs. 1.5 $\log_{10}$GE; $p<0.0001$; best results with sonicated filters). Fungal burdens within individual units varied by 0-3 $\log_{10}$ GE over time. Differences between outside and inside burdens were greater than differences between units within hospitals. Outside fungal GEs did not significantly differ between a hospital with extensive ongoing construction and a hospital 3 km away without construction. Overall, direct impact cultures were less sensitive than air filter cultures. On each date, <10% of linens were culture-positive for fungi.

Conclusions. We developed standardized methods for sampling airborne fungi in hospitals that yield reproducible results. Fungal burdens were significantly higher outside hospitals than those immediately inside the door. In this urban setting, outside burdens did not differ based on presence or absence of on-campus construction. Outside fungal burdens could not be used to estimate relative burdens within hospitals. Although burdens within hospitals were relatively low, fungal DNA and viable Aspergillus and other moulds were commonly recovered from units housing high-risk patients, including from positive pressure rooms. Linen burdens were significantly lower than previously reported at our center. Direct impact cultures, commonly used in surveillance, lacked sensitivity for detecting viable fungi and did not correlate with DNA burdens. We are optimizing direct metagenomic sequencing from airborne samples. We will assess correlations between environmental surveillance data and nosocomial fungal infections.

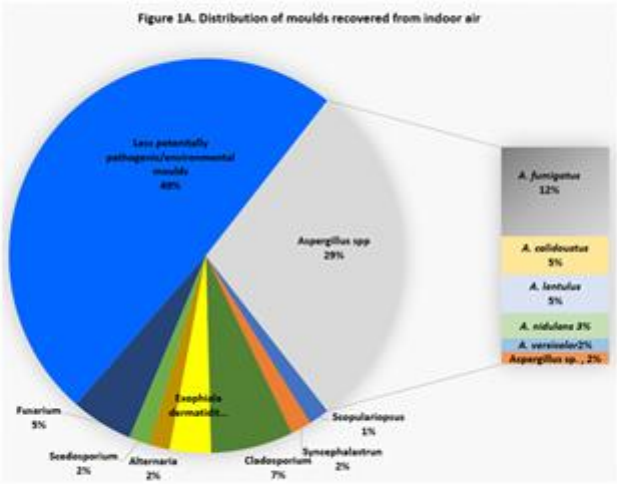
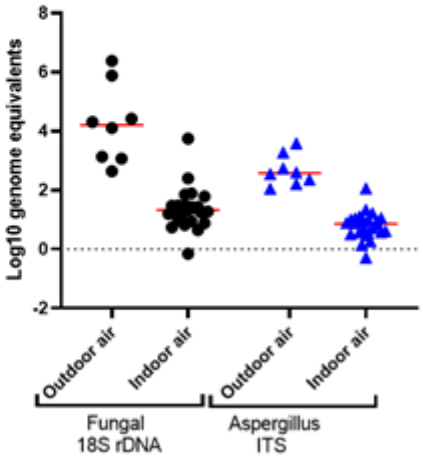


Figure 2. Overall fungal (black circles) and Aspergillus (blue triangles) burden recovered from outdoor and indoor air. Burden is presented as log10 genome equivalents. Fungal and Aspergillus burden was determined using 18S rDNA and Aspergillus ITS primers, respectively. Note that fungal burdens were significantly greater outside than inside hospitals, by both positive culture % and DNA burden (Aspergillus ITS: median 3.6 vs. 0.9 $\log_{10}$ genome equivalents (GE); pan-fungal 18S: 4.2 vs. $\log_{10}$1.3; p<0.001).



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Pan-Malassezia qPCR: a tool to quantify Malassezia burden in human mycobiota

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Background:
Malassezia spp. organisms are an integral part of the cutaneous, gut, oral, ears, nose, and throat (ENT) mycobiota. Moreover, they have been involved in various pathologies. Since *Malassezia* are difficult to grow in the laboratory, unexhaustive molecular biology methods have been developed to detect them.

Objectives:
The aim of the study was to evaluate an in-house pan-*Malassezia* quantitative PCR (panM-qPCR) on various clinical human samples and determine *Malassezia* burden in various human mycobiota.

Materials & Methods:
Efficiency was evaluated using 10-fold dilution of a *Malassezia pachydermatis* (CNRMA15.353). The panM-qPCR was designed to target the repeated 28S rDNA gene from all *Malassezia* species (17 species targeted *in silico* and 8 species tested *in vitro*). Cross reactivity with other fungi (n=82) was also tested.
We used the assay to quantify the *Malassezia* burden on 361 samples from 161 patients. A total of 80 skin swab samples with a dedicated protocol were collected from 10 healthy volunteers, 13 samples from 2 seborrheic dermatitis patients (SD) and 90 samples from 19 burned patients. Moreover, 119 stool samples from 89 immunocompromised patients were analysed and 59 ENT samples from 41 patients were collected. Each amplification that was run contained positive control (*Malassezia pachydermatis* (CNRMA 15.353) at 10^{-4} ng/µL) and negative control.

Results:
The qPCR assay yielded a PCR efficiency of 84.5% (CI 95%, 84.1 – 85.7]) and a R^2 value of 0.99. The intra assay variation was 0.99%, and the limit of detection was 10^{-6} ng/µL. The analytical specificity was 100% for *Malassezia* species with no cross reactivity with other pathogenic fungi tested.
For HV, the amount of *Malassezia* was different according to the swabbed areas (Ficher's test, p=0.0013), with lower Cq on the face (31.8 [IQR = 30.3-33.6]) compared with the other sites (32.8 [31.7-35.8], p=0.016). In total, Cq in SD are lower than in HV (26.2 [24.2-28.5] vs. 32.1 [30.9-34.6], p<0.001). In details, Cq in SD are lower on the face (25.6 [22.68-27.83] vs. 31.4 [29.9-32.3], p<0.001) and on other sites (27.6 [24.2-32.5] vs. 32.8 [31.7-35.8] p<0.01) compared to HV. In burned patients, Cq was significantly lower (33.9 [32.1-35.9]) compared to HV (35.8 [35.0-36.7], p<0.001).
Next, to detect the presence of *Malassezia* in stool, 119 stool samples were tested and 6.7% (n=8) were positive for *Malassezia* spp., with a median Cq of 37.28 [36.77-37.62]. For the ENT

area, a higher proportion of positive specimens were detected in ears samples (75%, n=15/20) than in nose samples (41%, n=13/32) (p=0.0016).

Conclusion:
Our findings emphasized the importance of qPCR, confirming elevated *Malassezia* spp. levels on individuals' faces, increased burden in seborrheic dermatitis patients and a higher fungal load in hospitalized burn patients than in healthy volunteers. Screening stool samples of immunocompromised individuals showed rare or absent *Malassezia* spp., while confirming the ear as a preferred colonization site. A comparison with a standardization of samples is currently underway and will enable us to refine these results. Therefore, we developed a pan-*Malassezia* PCR assay and showed that it could a useful tool for human mycobiota studies.

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Effect of pH on lipase production by clinical Candida isolates

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Objectives: In recent years there has been an increase in the incidence of diseases caused by *Candida*, such as candidemia and invasive candidiasis, with high mortality rates, together with an increase in the number of strains that show resistance to antifungal drugs, which hinders the treatment of *Candida*-associated infections and highlights the need for new therapeutic alternatives. Virulence factors including hydrolytic enzymes are among the promising targets to design inhibitory molecules potentially able to prevent infection and disease development. *Candida* virulence factors include various hydrolytic enzymes including lipases. Since candidiasis occurs at different sites of the human body with different pH, the main objective of this study was to detect the secretion of lipases by clinical *Candida* species isolated from different sites of infection and to analyse the effect of pH and strain origin on lipase production.

Materials & Methods: Fifty-nine clinical strains of *Candida* spp. from six different species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. auris*) and four different origins (blood, skin, mouth and vagina) were analysed. *C. albicans* NCPF 3153, *C. auris* 17-257 and *C. parapsilosis* ATCC 22019 were used as positive controls (lipase producers) and *C. tropicalis* NCPF 3111 as a negative one. To detect lipolytic activity, the strains were grown on malt agar plates supplemented with Tween 80 and calcium chloride. The assays were performed at different pH (5, 6.5 and 7.5) of the medium and the halos formed around the colonies were further examined to calculate the corresponding E_z (enzymatic zone) indexes.

Results: The secretion of lipases by *Candida* was higher at acidic pH values (pH 5 and pH 6.5): 18 of the 59 isolates secreted lipases at these pH values, and only 11 isolates secreted lipases at basic pH (pH 7.5). Among the species tested, *C. auris* showed the highest frequency of lipolytic activity (100% of isolates) observed at all pHs, which is consistent with the high pathogenicity of this species. *C. albicans*, *C. parapsilosis* and *C. glabrata* also showed lipolytic activity, which was observed in 7 out of 13 isolates, 6 out of 10 isolates and 3 out of 12 isolates, respectively. No lipase secretion was detected by testing *C. tropicalis* and *C. krusei* species. In terms of origin, the clinical isolates with the highest lipolytic activity were among those isolated from skin and blood thus indicating that the strains isolated from lipid-rich environments (e.g., skin) are better lipase-producers.

Conclusions: Production of lipase-like enzymes depends on the species, pH and clinical origin. *C. auris* was the species more efficient producing lipases, followed by *C. albicans* and *C. parapsilosis*, among which the isolates obtained from skin and blood showed lipase production at acidic pH.

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P178

A citizen science project to investigate environmental yeasts in urban soils (FungiSol)

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Objectives: Although recent studies showed several yeasts usually considered to be human commensals can also evolve in the environment, its role as as a reservoir of pathogenic yeasts is still largely unexplored. To gain further insights into this topic, we aimed to set up a citizen science project related to fungal biodiversity of yeasts, in urban soils.
Materials & Methods: Between february and april 2024, 126 kids (aged 10 to 15), from four schools of Nantes (France) participated in this project. Each kid collected two distinct soil samples in its school (playgrounds, vegetable garden, potted plants, flowerbeds), leading to a total of 256 samples (n = 51 to 76 per school). Once collected, samples were transferred to our research lab for mycological cultures. For each sample, 2 g of soil were added to YPD broth medium with antibiotics and incubated at 30°C under constant agitation. After three days, 10^{-4} and 10^{-5} dilutions were inoculated onto YPD agar plates for yeasts isolation. For each sample growing yeasts, up to 24 colonies were subcultured on both Sabouraud and chromogenic media plates (Chromagar Candida, BioRad) to screen for distinct morphotypes (based on variation in colony texture, color, surface, elevation, and margin). Each individual morphotype was then identified by Maldi-TOF mass spectrometry (Bruker biotyper), or ITS rDNA barcoding when necessary.
Results: Environmental yeasts could be isolated in more than half of the samples (n = 159, 62%), under our conditions. Overall, among the 2042 yeasts colonies screened, a total of 216 morphotypes were distinguished. Of these, 28 distinct yeast species, belonging to 17 different genera, have already been identified by Maldi-TOF mass spectrometry. Species distribution and diversity differed between schools although *Torulaspora delbrueckii* was the main isolated species (20-38%, n = 81) followed by *Hanseniaspora uvarum* (3-17%, n = 25). Beyond *C. tropicalis* which appeared to be the more prevalent *Candida* species (n = 13), several other human opportunistic yeasts were identified, including *Pichia kudriavzevii* (*C. krusei*), *Pichia norvengensis* (*C. norvegensis*), *Kluyveromyces marxianus* (*C. kefyr*), *Nakaseomyces glabrata* (*C. glabrata*), and *Clavispora lusitaniae* (*C. lusitaniae*). Some isolates remain to be identified by ITS rDNA barcoding.
Conclusions: Bringing together citizens and researchers on this topic is both an opportunity to raise public awareness of fungi and medical mycology and a mean of speeding up scientific research in this field. Alltogether, our preliminary findings underline the role of urban soils as potentiel reservoir of opportunistic yeasts, under the One Health umbrella. Further experiments are underway to assess the in vitro susceptibility of these environmental yeasts to medical antifungals.

P180

Environmental surveys for the risk assessment of invasive fungal infections in Spanish hospitals

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Objectives: The hospital environment requires special attention to protect patients against hospital-acquired infections. There, opportunistic pathogens cause life-threatening invasive fungal infections (IFI) associated with immunocompromising conditions. In addition, the current emergence of antifungal drug resistance points out the need for further environmental studies to find out the origin of this problem. Considering these challenges, in the *Mycospitalomics* project, we studied three tertiary care hospitals: (VR) Virgen del Rocío in Seville, (LF) La Fe in Valencia, and (SO) Severo Ochoa in Leganés, Madrid; addressing three objectives: (i) to evaluate the methods for monitoring indoor fungi in hospitals, (ii) to characterise the fungal communities in different building zones and substrates (air and surfaces), and (iii) to generate a representative fungal collection from hospital environments.

Materials & Methods: A first pilot survey in VR was conducted to evaluate the methodology for sampling and culture-dependent analyses. Afterwards, the major sampling campaigns were carried out in winter 2023. We studied three indoor zones with different levels of exposure: (IA) admission halls and waiting rooms near the building entrances, (IB) regular patient rooms, and (IC) intensive care units protected with HEPA filters. For comparison, some areas outside buildings (OUT) were also sampled. Twelve sampling points (3 per zone) were studied in each hospital. Particulate matter, relative humidity and air temperature were recorded *in situ* using a Trotec PC200 particle counter. Air samples were directly collected on SDC and DRBC agar plates using a VWR SAS Super Duo 360 sampler, while surface samples from ventilation grilles and high-contact areas were taken with 3M sponge-sticks. We assessed viable counts and isolated representative colonies of the observed morphological types. Fungal identification was performed by PCR and Sanger sequencing of the ITS regions.

Results: The pilot study was useful to validate our methods and to decide some key points such as the recommended volumes of air samples depending on the zones: 100L in OUT and IA, 500L in IB, and 1000L in IC. In general, VR and SO hospitals showed higher concentration of airborne indoor fungi compared to LF. As expected, inside the three hospitals we observed a clear gradient in both particle counts and airborne cultivable fungi from the most protected zone (IC) to the zone with the highest numbers of occupants near the entrances (IA), which showed similar concentrations to outdoors (OUT). Relatively low numbers of colonies grew from surface sponges (for both vents and high-contact areas), except for several vents at IA zones that reached high CFU counts. A collection of 418 isolates from air (69%) and surfaces (31%) have been kept in our laboratories and molecularly identified, its taxonomical composition and relevance for the risk of IFI will be discussed.

Conclusions: These prelimnary results and the valuable fungal collection from the hospital environment will be the basis of future planned researches: (i) comparison of other approaches, e.g. more selective cultivation or direct eDNA analyses, to monitor indoor fungi, (ii) testing the antifungal susceptibility of relevant isolates, and (iii) comparative genomics into pathogenic and resistant species.

P183

Ochratoxins and deoxynivalenol contamination of cereals in kibra

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Background; Mycotoxins are secondary metabolites of fungi affecting agricultural products in the food value chain. Among mycotoxins,Ochratoxin (OTA) and Deoxynivalenol (DON) are widely distributed in cereals and grains products. They are of public health importance due to their nephrotoxic, hepatotoxic, and genotoxic effects in humans.Poor pre-harvest
and post-harvest handling practices leads to the formation of OTA and DON in various
food commodities due to fungal infestations.

Objectives; To characterize the OTA and DON producing fungi and determine the contamination levels in cereals stores in Kibra.

Methodology;Thecross- sectional lab based study was conducted in kibra targeting cereal vendors. A total of 20 cereal samples consisting of; muthokoi 40.0%, njahi 15.0 %, beans 15.0 % maize 25 .0% and millet 5.0%)were collected and subjected to fungal culture and ELISA test for OTA and DON using standard laboratory procedures.

Results; The Ochratoxin contamination was 52.0% and fungal contamination with;*Aspergillus niger, Aspergillus flavus,*Rhizopus*spp* and Penicillium *spp*. Detection of Deoxynivalenol was 48.0% with Fusarium spp. infestations.

Conclusion; Mould and mycotoxin (OTA and DON) contamination of cereals is a significant public health concern with potential health consequences. Mycotoxins have been associated with stunting, malnutrition, acute mycotoxicoses, cancer, immune modulatory and infectious disease severity. We recommend more studies and interventions to reduce the effects of mycotoxin exposure.

P184

Invasive fungal infections caused by rare yeast-like fungi. Results of a prospective study

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Objective: to study the clinical and laboratory features of severe fungal infections caused by rare yeast-like pathogens to optimize their diagnosis and treatment.

Materials and methods. A prospective study was conducted in the period 2000-2020. We used EORTC/MSG (European Organization for Research and Treatment of Cancer Mycoses study group), 2020 to diagnose mycoses.

Results. We examined 30 adult patients aged 18 to 60 years (median – 30 years), men – 61%, women - 39%.

The main background conditions were oncohematological diseases - 50%. injuries/burns – 17%, AIDS – 13%, surgical interventions – 10%, cancer of solid organs - 7%, cirrhosis of the liver – 3%.

The main risk factors were ICU stay (93%), CVC for more than 14 days (90%) and agranulocytosis (47%). Mycosis proceeded mainly as a fungemia (77%), as well as damage to the central nervous system (20%), lungs (10%) and skin (10%). 2 or more organs were affected in 30% of patients.

The causative agents of mycoses were: *Trichosporon* spp. (40%), *Rodotorula* spp. (33%), *Geotrichum* spp. (17%), *Saccharamyses* spp. (7%), *Malassesia* sp. (3%).

13% of patients were diagnosed postmortem, the remaining patients received antimycotics: fluconazole (53%), voriconazole (20%), amphotericin B deoxycholate (50%) and echinocandins (33%). The overall survival rate of patients within 30 days was 63%.

Conclusions:

1. Invasive mycoses caused by rare yeast–like micromycetes develop mainly in patients staying in the ICU for a long time - 93% of patients.
2. The main risk factors: the use of CVC for more than 14 days (90%) and agranulocytosis (47%).
3. The main pathogens: Trichosporon spr. (40%) and Rodotorula spr. (33%).
4. The main clinical variant is fungemia (77%).
5. The overall 30-day survival rate was 63%.

P185

Chaetomium spp: a rare case of severe fungal keratitis and endophthalmitis

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Objectives: To present a case of rare fungal keratitis and endophthalmitis caused by *Chaetomium*. *Chaetomium* species belong to the genus of saprobic ascomycetes and are melanized ascosporulating fungi, found in cellulose-containing materials in indoor environments that have suffered water damage and also in decaying plant material, soil and dunk. Species of the genus have been, scarcely, implicated in opportunistic human infections and have been associated with immunodeficiency of the host. Up until now, only a few *Chaetomium* keratomycosis cases have been reported in the literature.

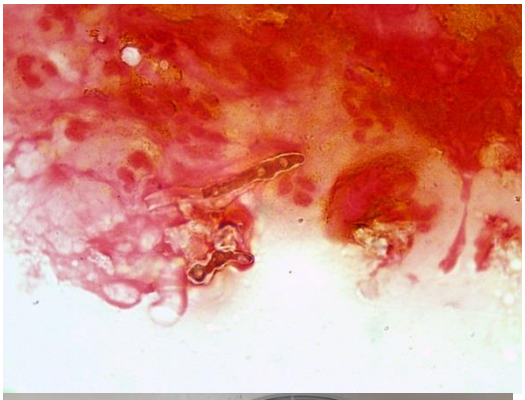
Case description

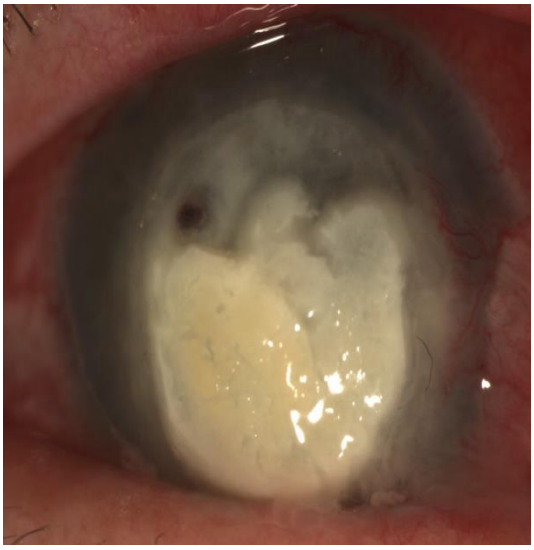
A 78-year-old male patient, from the province, was referred to our hospital, due to corneal melting and suspected left eye endophthalmitis. The patient's ophthalmological history revealed ocular trauma, caused by metal burr, three weeks before referral. During this time, he visited the local hospital, where corneal ulcer was diagnosed and after unsuccessful topical treatment, conjunctival flap graft was applied on the cornea. His medical history revealed that he suffered from high blood pressure and coronary artery disease. Upon examination the patient's left eye exhibited: edema and redness of the upper and lower eye lid, conjunctival hyperemia, old corneal melt with conjunctival graft placement, hypopyon under the central part of the conjunctival graft and vitreous humor slightly blurry. Corneal and vitreous samples were taken for culture.

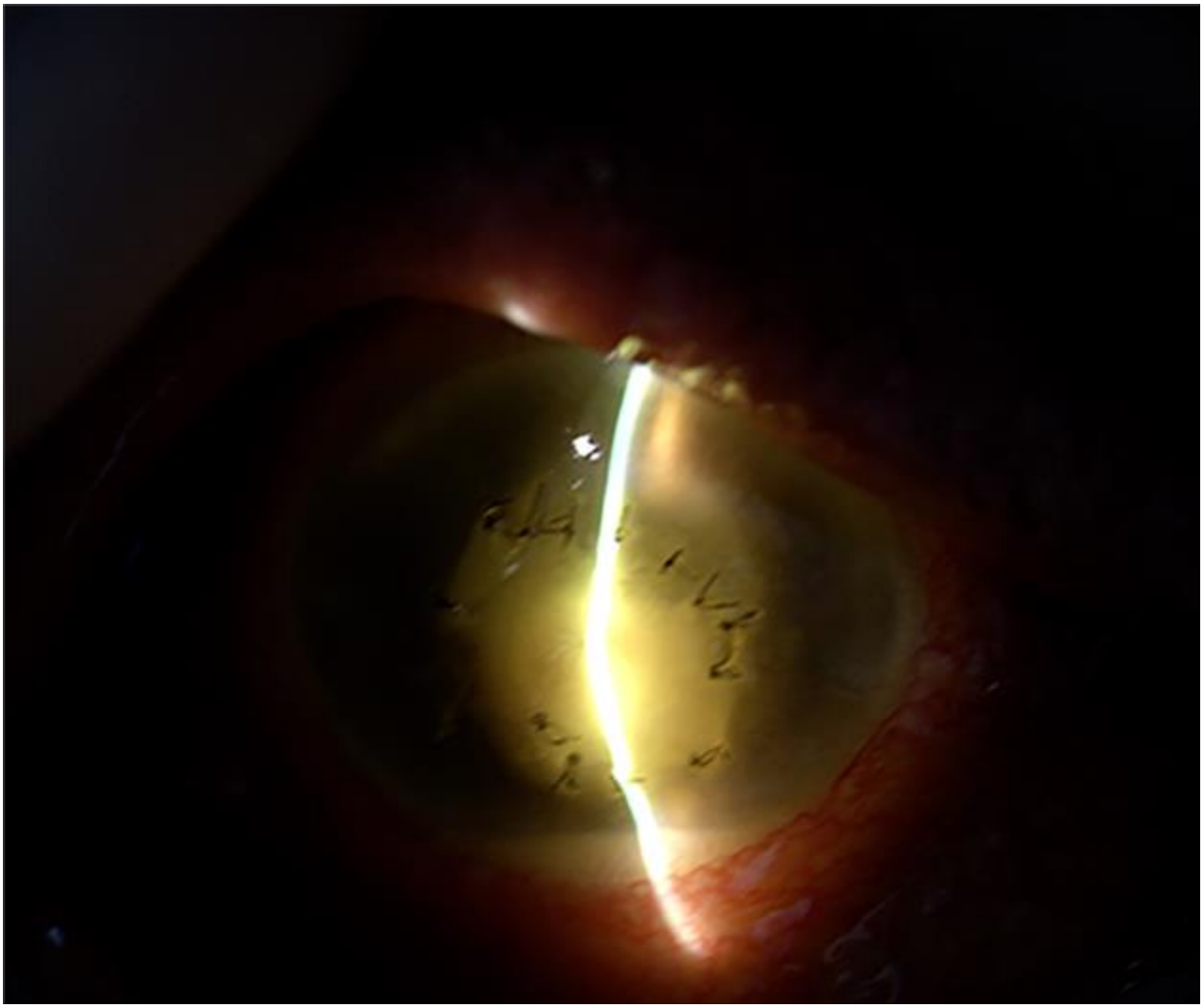
Due to the suspicion of fungal keratitis, voriconazole 0.02% and amikacin eye drops were immediately applied and iv administration of voriconazole and moxifloxacin started. Simultaneously, considering the suspicion of fungal endophthalmitis, tap and intravitreal injection of voriconazole and amikacin was performed. Four days later, *Chaetomium* spp was isolated from both corneal and vitreous humor cultures, confirming the clinical suspicion of fungal infection. The strain was identified in genus level by MALDI Biotyper MSP (BRUKER). Susceptibility test was performed and it appeared susceptible to voriconazole, posaconazole and amphotericin-B, and resistant to fluconazole. Accordingly, iv treatment was modified to amphotericin and isavuconazole, while topical instillation of voriconazole and amikacin continued, and vancomycin eye drops were added. Despite the efforts, patient did not respond to antifungal treatment, instead, the infection of both corneal and vitreous humor aggravated rapidly. Within a week of his admission, the patient underwent evisceration of the eye.

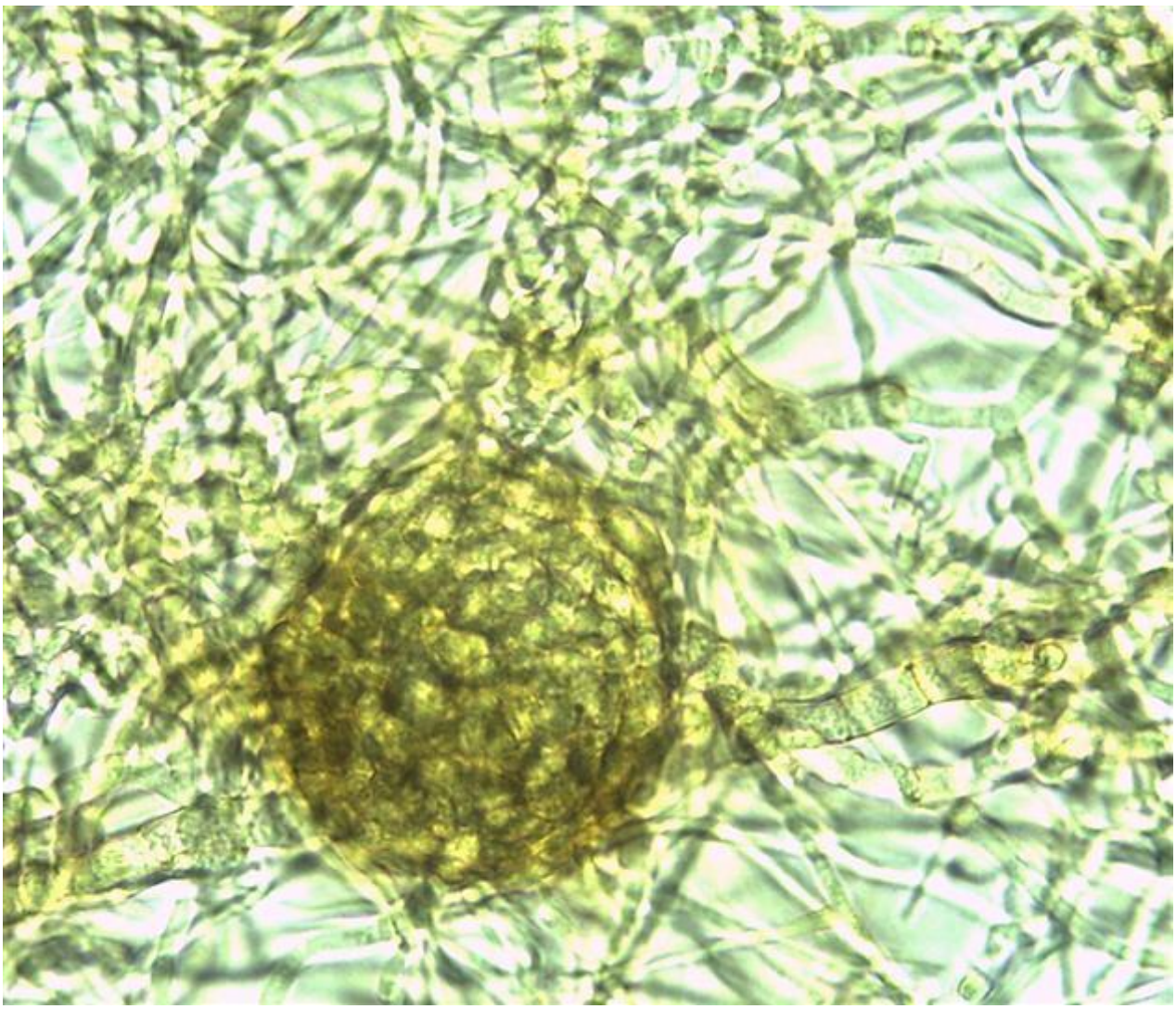
Discussion

To our knowledge this is the first case of *Chaetomium* ocular infection in Greece. Reviewing the literature, only a handful of *Chaetomium* ocular infections have been reported worldwide, most of which had a positive outcome. Although the genus *Chaetomium* is a rare cause of human disease, such as systemic mycoses, endocarditis and subcutaneous lesions, it should not be overlooked in cases of ocular trauma.









P186

Necrotizing pneumonia caused by Curvularia hawaiiensis and Mycobacterium tuberculosis coinfection in a patient with ascariasis: a case report

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Objectives:
Describe the clinical presentation and management of the first report of co-infection of *Curvularia hawaiiensis* (syn. *Bipolaris hawaiiensis*) and *Mycobacterium tuberculosis*.

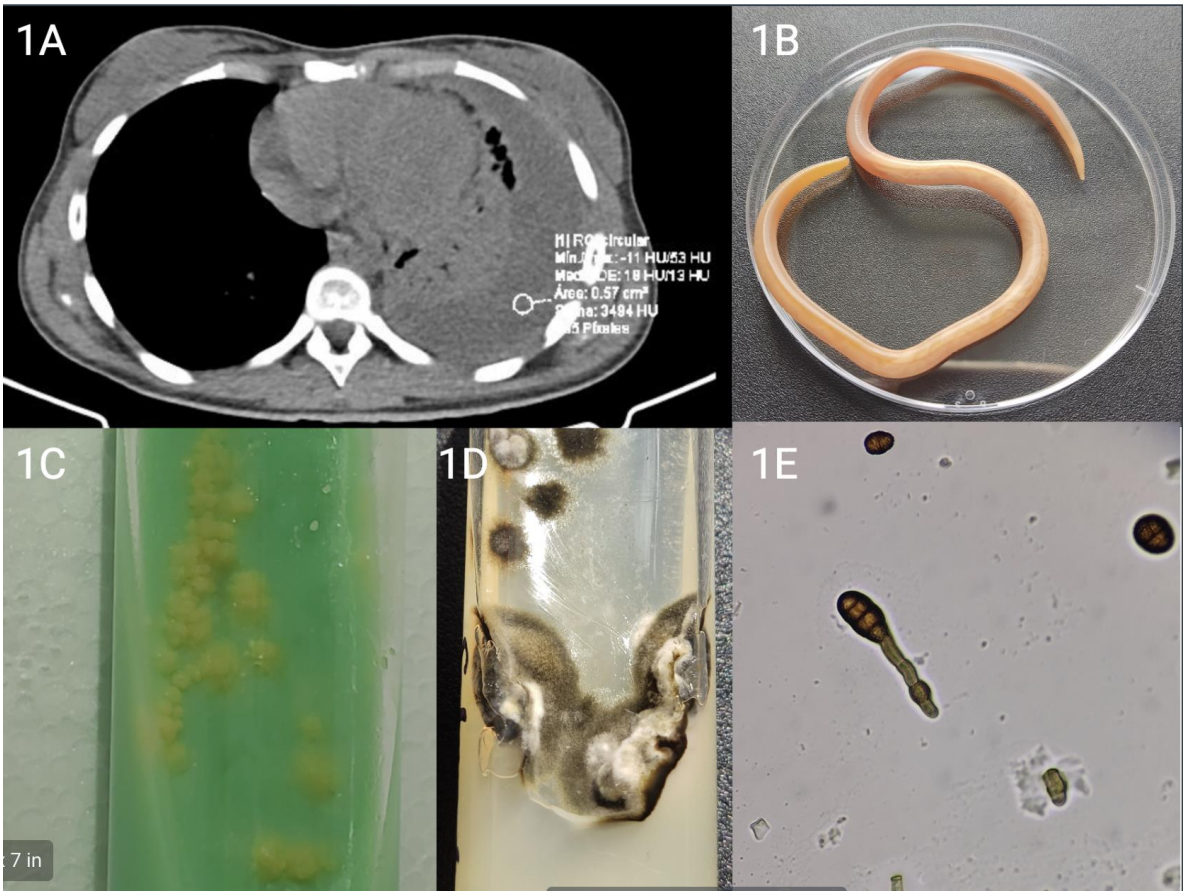
Materials & Methods:
Based on care guideline recommendations for case reports. We describe A 16-year-old female patient with no comorbidities was admitted to the emergency department for fever and chest pain. We describe the first case of co-infection with *Curvularia hawaiiensis* and necrotizing pneumonia due to *Mycobacterium tuberculosis*.

Results:
6-year-old female patient who presented to the emergency department with moderate oppressive headache, mild pressing retrosternal pain, and dyspnea at rest. She had a family history of high blood pressure, diabetes, breast cancer, and pleural effusion. Physical examination revealed absent breath sounds in the left lung base and diminished air entry in the right lung base with fine crackles. Chest tomography revealed pleural effusion in the left hemithorax. Thoracentesis was performed, and examination of the pleural fluid revealed the presence of leukocytes with 97% monocytes, erythrocytes, and LDH 735. GeneXpert MTB/RIF detected *Mycobacterium tuberculosis* in the pleural fluid (Figure 1A). The patient also vomited a female *Ascaris lumbricoides* during her hospitalization (Figure 1B).
The patient was started on antituberculosis first line therapy for six months and albendazole 400 mg orally one dose for the parasitic infection. However, she continued to have fever and chest pain, and a lung biopsy of necrotic tissue obtained by thoracoscopy revealed the presence of *Curvularia hawaiiensis* in her lung tissue. Identification by traditional microbiology and sequencing confirmed *C. hawaiiensis* (figure 1 C,D,E). The patient underwent lung surgery and was treated itraconazole 200 mg twice daily for 16 weeks. Follow-up showed no relapses or new hospitalizations after one year.

Conclusions:
Active pneumonia TB may be associated with other pathogens. Among fungal infections in patients with TB, the most critical microorganisms reported are *Candida* sp., *Aspergillus* sp., *Histoplasma capsulatum*, and *Cryptococcus neoformans*. In addition, it is essential to mention the sum of risk factors in fungal infections. For example, patients with *Aspergillus fumigatus* and cystic fibrosis colonization are associated with high mortality and an unfavorable prognosis. There was no underlying disease in the patient studied in this case report. However, contact with TB was likely prolonged based on the calcifications noted on tomography (Figure 1b). And according to some reports, active tuberculosis becomes a risk factor for fungal

infections in certain previously healthy patients. This case highlights the importance of considering multiple possible diagnoses when treating patients with complex medical histories and symptoms. It also emphasizes the need for thorough diagnostic testing to identify and treat all underlying infections.

Figure 1. Relevant radiological and microbiological findings of the case.



1A.- Thoracic tomography shows pleural effusion in the left hemithorax, approximate volume 485 ml, maximum density 38 hu, parietal and visceral pleura thickening, and mediastinal calcifications. Left anterior apical calcified granuloma, subpleural laminar and segmental atelectasis left basal consolidation with aerial bronchogram. Pre-aortic and subcarinal reactive nodules. Small right pericardial effusion. 1B.- Female helminth of *A. lumbricoides* obtained from the patient's vomit. 1C.- *Mycobacterium tuberculosis* isolated from lung biopsy on Lowenstein Jensen agar. 1D.- Culture of a lung biopsy on Sabouraud agar with chloramphenicol showing filamentous growth of *Curvularia hawaiiensis*. 1E.- Microscopic view of *C. hawaiiensis* ha colony with ellipsoid conidia, rounded at the ends, pale brown, medium reddish brown to dark brown, three septa.

P187

Candida auris fungus spreading in a Greek hospital

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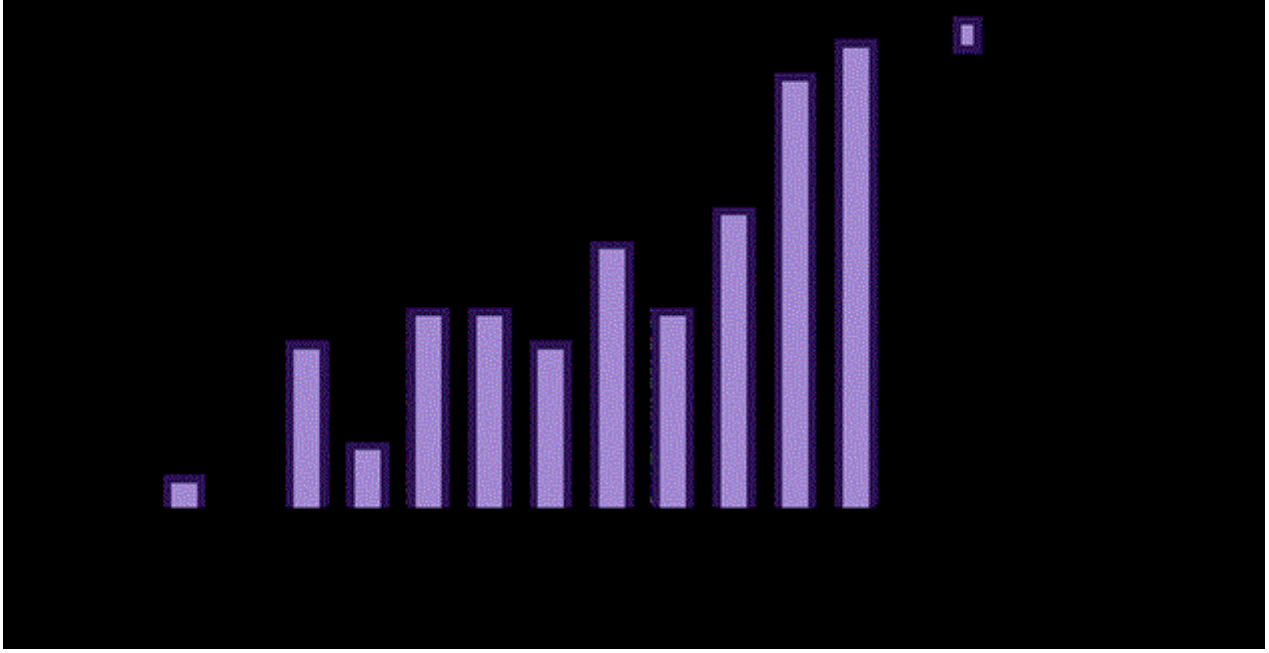
Objectives: *Candida auris* is an emerging, multidrug-resistant yeast causing hospital outbreaks around the world. In January 2022, *C. auris* was first isolated at a university hospital in Greece and since then the implementation of early and strict surveillance and control measures have been taken.The purpose of the study is to report the course of the spread of the fungus (infection or colonization) in the 1st and 2nd semesters of 2022 in the hospital , to highlight the characteristics of the patients in whom it was detected and the resistance of the strains to antifungal drugs
Materials & Methods: *Candida auris* was isolated from 75 patients hospitalized at our hospital in 2022. Isolation of patients in a single-bed room or co-horting, obtaining colonization cultures, observing contact precautions, and disinfecting surfaces and equipment were the measures applied to prevent transmission inside the hospital and to prevent the development of infections in patients who are already colonized. Data analysis was done with SPSS software.
Results: An increasing frequency of *C. auris* isolation was found (20 patients in the 1st and 55 in the 2nd half of 2022) with a maximum value of 14 new cases in the month of December. 55% of patients were male.The majority of patients (64%) were hospitalized in the intensive care unit, 28% of cases was related to Covid-19 infection , while 38.6% of the patients had recent hospitalization in a health facility. *C. auris* was isolated in blood, bronchial secretions, central catheter tip, urine and skin in 30.7%, 14.7%, 13.3%, 5.3% and 36% of the cases respectively. The isolates showed 100% resistance to Fluconazole, 25.3% to Liposomal Amphotericin B, 9.3% to Echinocandins. Twenty-eight patients (37.3%) developed candidemia, 90% of patients received an echinocandin as treatment.Thirteen patients (13) with candidemia died (46%)but a*s candida auris* infection typically occurs when a person is already in severe condition, it may be difficult to disentangle the attributable mortality Comparing the two semesters of the year, a 2.5-fold increase in strain isolation was observed in the 2nd semester, with an increase in the *C. auris* carriage of hospitalized patients (10% vs 41.8%; p=0.005) ,no difference in resistance was noted. The increase in incidents in the second semester could be attributed to the inability to faithfully implement the disinfection-isolation measures due to the lack of natural and human resources.
Conclusions:The rapid spread of candida auris infection in our hospital highlights the complexity of handling and containing a *C. auris* outbreak. Persistent colonization of patients and medical surfaces by the fungus have become a challenge for treatment and infection control.

Candida auris Cases	
2022	
N	
Age (IQR)	
Gender	
Female/Male(%)	
Ward	
ICU / Non-ICU (%)	
Admission Type	
Covid / Non-Covid (%)	
Recent hospitalization (%)	
First Positive Culture	
Blood (%)	
Bronchial Secretions (%)	
Central Catheter Tip (%)	
Urine (%)	
Skin carriage (%)	
Antifungal resistance	
Fluconazole (%)	
Amphotericin B (%)	
Echinocandins (%)	
Patients with Fungemia (%)	
Treatment	
Echinocandins (%)	
Amphotericin B (%)	



P188

An imported case of histoplasmosis in an immunocompetent patient

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Background :

Histoplasmosis, a disease caused by the fungus *Histoplasma capsulatum var. duboisii,* is an uncommon yet likely underestimated infection found in the intertropical regions of Africa. Although this infection is closely linked to HIV infection, cases occurring i immunocompetent patients have been described.

We report the case of a Tunisian patient having lived in Congo, admitted to the infectious diseases department of La Rabta hospital for a histoplasmosis due to *Histoplasma capsulatum var. duboisii.*

Case report :

Our patient is a 49-year-old man, originally from Tunis, having stayed in Congo Brazzaville from 2008 to 2022, transferred from the internal medicine department for the management of polyadenopathy with altered general condition.

The patient had type 2 diabetes on oral anti-diabetics.

The history of the disease goes back to one year before admission, marked by the appearance of bilateral axillary adenopathies that had progressively increased in size with no fever. The patient was then hospitalized. The clinical examination showed a good general condition , no fever, Labstix: no proteinuria, no hematuria, right submandibular adenopathy, painless, without local inflammatory signs, two firm, slightly painful, mobile jugulocarotid nodes, a magma of centimetric left axillary adenopathies with a productive fistula leaking serohematic fluid.

The biological work-up was normal

CT scan showed multiple ganglion formations with necrotic centers above and below the diaphragm, associated with an excavated lung nodule in the right upper lobe. Micronodules of low density, centro-lobular and scattered, predominantly in the two lower lobes.

Tests for HIV, brucellosis, tuberculosis and other viruses were negative. A microbiopsy of a left axillary adenomegaly was performed, and pathological examination revealed fibrofatty tissue with a foreign-body macrophagic granuloma. Muller's cells include rounded yeasts with reinforced contours. Histological appearance suggestive of *Histoplasma duboisii* infection. The case discussed with parasito-mycologists.

Our course of action was to put the patient on amphotericin B deoxycholate with a cumulative dose of 700 mg, but the patient developed renal failure. We had to switch to Itraconazole 200 mg per day orally with good clinical and biological evolution.

Conclusion :

The exact mode of acquiring histoplasmosis, a severe fungal infection, has yet to be determined. There is a requirement for affordable and more precise diagnostic tools. Itraconazole and amphotericin B are the most effective treatment options and should be accessible in all low-income countries.

P189

Isolation of resistant yeast species including Candida auris from soil samples-emergence of resistant yeast strains from soil to clinical settings.

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Objectives: *Candida auris* is an emerging public health threat and is resistant to most antifungal agents. After its first reporting in Japan in 2009, *C. auris* has spread rapidly worldwide and caused outbreaks in hospitals. World Health Organisation declared *C. auris* as one of the four 'critical priority' fungal pathogens. Outdoor of the hospital settings, *C. auris* has been isolated from surface of stored apples, tidal marshes, hypersaline environments and recently from wastewater suggesting that this yeast can survive in harsh conditions. Soil is main niche for all kinds of microorganisms including clinically important fungal species.

Materials & Methods: In our present work, we investigated a total of 50 soil samples from different sites including river banks of Ganga and Yamuna, different ponds of Haryana, India, Okhla Bird Sanctuary, Kamla Nehru Ridge area (a public park) and agricultural lands to explore the medically important fungal species and to check their resistance patterns. Samples were processed within the 24 hours of collections. Different dilutions (1/10,1/50,1/100) of soil were prepared in normal saline along with adding chloramphenicol, gentamicin and streptomycin to inhibit bacterial growth. A volume of 100µl was transferred on Sabouraud Dextrose Agar and CHROMagar plates and plates were incubated at 37 ℃ for 24 and 48h respectively. Identification of yeast colonies was done by MALDI-TOF MS and isolated yeast species were subjected to antifungal susceptibility testing, determined using CLSI broth microdilution method.

Results: Nine different medically important yeast species (n=35) including, *Candida tropicalis(n=19), Candida krusei*(n=10), *Trichosporan asahii* (n=3), *C. rugosa* (n=2), *C. guillirmondii* (n=2), *C. auris (*n=1), *Candida palmioleophila* (n=1), *Candida lusitaniae*(n=1),

Candida rugosa (n=1), were recovered. *Candida auris* isolation was done from soil collected from agricultural land having usage of azole related fungicides.

AFST testing revealed that one *C. auris* isolate was resistant to FLU (MIC >128 mg/L) and 21.4% *C. tropicalis* were resistant (MIC ≥8 mg/L) to fluconazole. Out of three recovered *T. asahii*, one showed high resistance to echinocandins (MICs ≥8 mg/L) but was susceptible to all other antifungal drugs. All other yeast species were found to be susceptible to the tested antifungal drugs.

Conclusions: Isolation of fluconazole resistant *C. auris* from agricultural land where use of agricultural fungicides is already taking place and FLU resistant *C. tropicalis* strains from ponds soil samples which were near to agricultural lands confers underlining possibility of acquired cross-resistance due to enhanced use of de-methyl inhibitors in agricultural fields that share similar structure to clinical azoles. Soil environment is also the most common route for the emergence of azole resistant isolates in hospitals hence exploration of other types of soils is still warranted.

P190

Antifungal resistance profile and azole resistance dynamics of Candida auris in a 13 year collection of clinical isolates across India

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Objectives:
To study the drug resistance profile and to explore the azole resistance associated mechanisms in *C. auris* clinical isolates, in a large collection across India over a period of 13 years (2009-2021).

Methods:
596 *C. auris* clinical isolates, which were deposited to our National Collection Center of Pathogenic fungi (NCCPF) from 37 healthcare centers across India over a 13 year duration (2009 through 2021) through the AMR network, were included. Isolates were screened for resistance against 8 antifungal drugs using the CLSI Broth Microdilution (BMD) method (M27-A3). ERG11 gene mutations were analyzed in 45 total isolates (30 fluconazole-resistant and 15 fluconazole-susceptible) by Sangar sequencing. The expression of ERG11 gene and efflux pumps (MDR1 & CDR1) were assessed in 20 *C. auris* isolates (10 fluconazole-resistant and 10 fluconazole-susceptible) by SYBR Green qRT-PCR. The estimation of cell wall ergosterol content and rhodamine 6G efflux assay for ABC-type drug transporter activity were also determined. Genotyping using Fluorescent amplified fragment length polymorphism (FAFLP) was done in a 64 total isolates (28 fluconazole-resistant, 26 fluconazole-susceptible and 10 Pan-resistant), along with reference isolates from all 5 clades.

Results:
Of the 596 *C. auris* clinical isolates, 92.7% were from fungemia [blood (81.5%) and urine (11.2%)] while 7.3% were from other invasive infections. North India contributed the highest number of isolates (n=255, 50.1%) followed by East India (n=165, 27.6%) and South India (n=81, 13.6%). A striking rise in *C. auris* isolation was observed in both 2019 and 2021, with a sudden drop in 2020 during the COVID-19 pandemic. 80% of isolates showed resistance to fluconazole, while the 27.9% observed voriconazole resistance was much higher than in previous reports. A significant increase in amphotericin B (52.85%) and caspofungin (12.08%) was observed since 2017 . We found that 19 total (3.18%) isolates were Pan-resistant. Out of 15 fluconazole-susceptible isolates, three were wild genotype isolates. Y132F (n=24) and K143R (n=18) mutations were present in both fluconazole susceptible and resistant isolates, as reported earlier. The expression of all three genes were highly elevated [7.4±8.19 vs 1.47±1.18, P=0.0007 for ERG11; 2.19±0.39 vs 1.02±0.36, P<0.0001 for CDR1 and 3.21±0.69 vs 0.65±0.53, P<0.0001 for MDR1]) in fluconazole-resistant isolates, where 3 out of 10 fluconazole-susceptible isolates also showed elevated ERG11 expression (>2 to 4 folds) upon fluconazole treatment. Those three fluconazole-susceptible isolates also had elevated ergosterol content after drug exposure. Basal level cell wall ergosterol were comparatively higher (32.1±1.34 vs 21.5±0.62, P=0.036) in fluconazole-susceptible isolates. Rhodamine-6G efflux activity were correlated with the CDR1 gene expression level. All isolates belonged to Clade-I with percent similarity of >85% where three distinct clusters were observed with inter-cluster difference of <8%.

Conclusion:

This is the first report of the antifungal susceptibility profile and its distribution of the largest collection of Indian *C. auris* clinical isolates till date. The paradigm shift in the resistance profile including amphotericin-B and caspofungin is concerning. Besides mutations and gene expressions, other pathways governing drug resistance, like stress response, must be studied to better understand the evolution of this multidrug resistant pathogen.

*__Presenting author__

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Trends in Medical Mycology

P191

A disseminated Arhtrocladium infection associated with tuberculosis in a pregnant woman

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Objectives:
Arthrocladium is a black-yeast fungus rarely responsible of human infection. We report the first case of a disseminated *Arthrocladium* infection associated with tuberculosis in a Tunisian pregnant woman.

Materials & Methods:
We report the case of a patient hospitalized in the infectious diseases ward, Rabta Hospital, Tunis, Tunisia, in 2019.

Results:
She was a pregnant 24-year-old woman with no medical history. She developed a spinal adenopathy at 28 weeks of amenorrhea. One week later, she had a focal seizure. Brain MRI revealed multiple focal lesions with a left parietal lobe lesion and perilesional edema.
Cytopuncture of the spinal adenopathy revealed multinucleate epithelial cells. As tuberculosis is endemic in Tunisia, cerebral and nodal location was suspected, and the patient was treated with first-line tuberculosis regimen, corticosteroids and anti-seizure medications.
Two months later, during labor and on tuberculosis medication, she had another seizure. Brain MRI showed an extension of the left lesion and the perilesional edema.
The parietal brain lesion was entirely removed surgically. It had a white thick shell and caseous-like material. Its histological examination found tuberculous granuloma with caseous necrosis, as well as PAS+, Grocott+, extra and intra-cellular branched and septated mycelial fragments. It was not addressed to mycology.
A full body CT-scan revealed a spinal adenomegaly, a left sus-clavicular adenomegaly and pulmonary nodules. Pus culture from lymph node puncture was positive to *Arthrocladium*.
We started Amphotericin B deoxycholate (1mg/Kg/day) and Itraconazole (300mg b.i.d. because of interaction with Rifampicin and anti-seizure medications).
We looked for immunodepression. Blood cell count was normal. Human immunodeficiency virus serology was negative. Protein electrophoresis was normal.
Blood culture was negative for bacteria and fungi. Histological exam of the spinal adenectomy concluded to a fungal granulomatous adenitis. There was no proof of tuberculosis in the lymph nodes.
The patient had side-effects to Amphotericin B, including fever, shaking chills, vomiting and cytopenia.
Seizures continued to occur as brain lesions and edema initially extended. A spondylitis contiguous to the spinal lymphoadenopathy appeared. Drug interactions were suspected, and

we changed tuberculosis and anti-seizure medications. Corticosteroids were prescribed once again at high doses.
The lesions shrunk slowly, as the frequency of seizures decreased.
The patient had received 12 months of TB medication, six weeks of high doses of corticosteroids, one month of Amphotericin B and 20 months of Itraconazole to which she developed blue lips, itching, headache and vomiting.
The lymphadenopathies and the spondylitis completely regressed. In the last brain CT-scan performed six months after the end of the antifungal treatment, only the now-calcified parietal lesion persisted.
Our final diagnosis was a disseminated *Arthrocladium* infection involving the brain, lymph nodes, lungs and spine, associated with a brain tuberculosis.
Presently, the patient is 27-year-old. She hasn't have any symptoms for the last three years.
Conclusions:
Human infections due to *Arthrocladium* are scarce. We know of only three observations involving this fungus, the third one being our patient.

P192

In vitro whole leukocyte infection model and detection of hydrophobic surface-binding protein A (HsbA) in L. corymbifera

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In vitro* whole leukocyte infection model and detection of hydrophobic surface-binding protein A (HsbA) in *L. corymbifera

Objectives:
Explore the possibility of detecting the HsbA protein produced by *L. corymbifera* in a model of co-infection with human leukocytes.

Materials & Methods:
In this study, we present the interaction between *L. corymbifera* with professional phagocytes which represents the first line of defense after infection. Human leukocytes were obtained from human donors with ethics committee approval. After extraction, the erythrocytes were removed with an erythrocyte lysis buffer[1]. Leukocytes were collected in PBS with EDTA and subsequently co-incubated with *L. corymbifera* spores at an MOI of 1:2 (leukocyte:spores). The supernatant of the *in vitro* whole leukocyte infection model detection of hydrophobic surface-binding protein A (HsbA)[2] were performed by liquid chromatography-mass spectrometry analysis (LC-MS /MS) (Figure 1).

Results:
Interestingly, it was possible to detect the secretion of HsbA by *L. corymbifera* spores. Subsequently, coinfection (MOI 1:2) of leukocytes2 with *L. corymbifera* spores3 allowed detection of HsbA in the supernatant after 72 hours by LC-MS/MS. Finally, HsbA was shown to bind predominantly to monocytes and macrophages. Altogether, our results suggested that HsbA plays a key role in interaction with the host immune system and helps to unravel the pathogenicity mechanism during mucormycosis.

Conclusions:
Proteomic analysis of the *L. corymbifera* co-infection model with human leukocytes showed the detection of HsbA in the culture supernatant at 72 hours.

References

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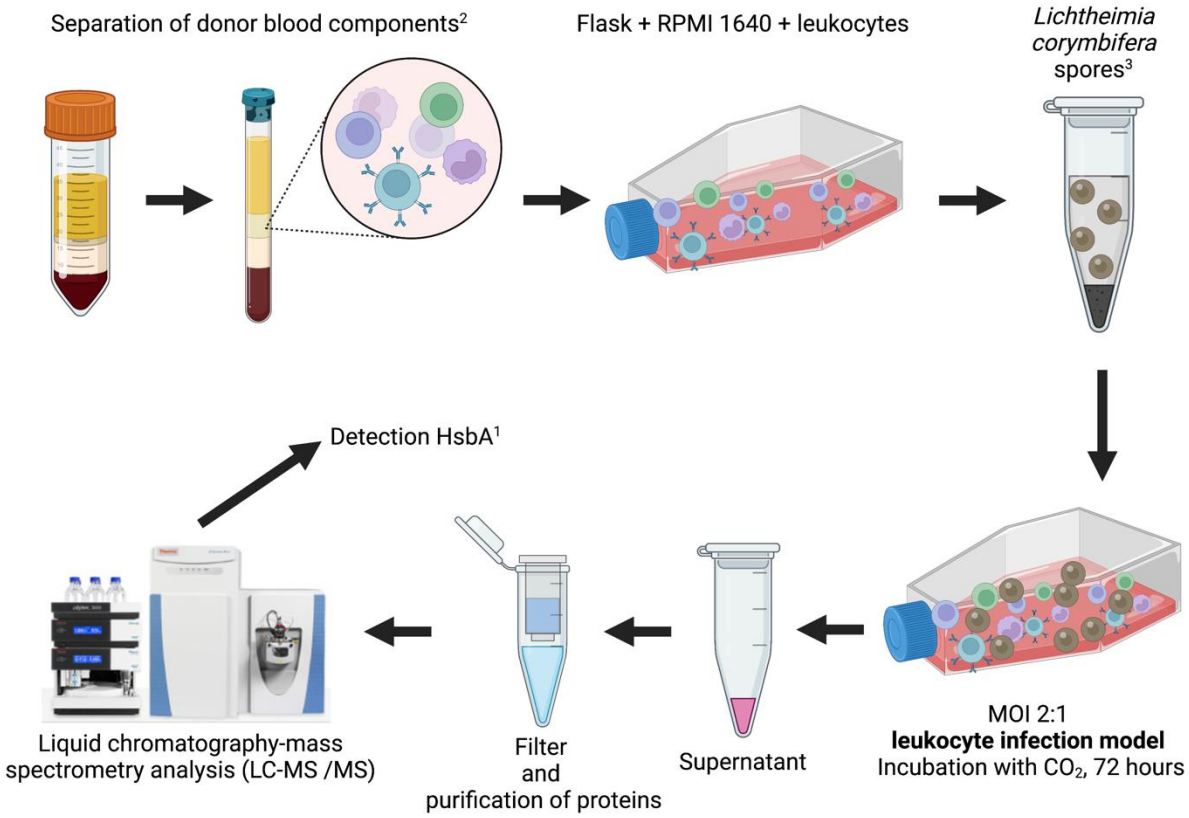
2. Hassan, M. A. *et al.* P343 Novel hydrophobic binding surface proteins are instrumental for phagocytosis of *Lichtheimia corymbifera* by macrophages. *Med Mycol* **60**, (2022).

Figure 1. Method


Separation of donor blood components[2]
Flask + RPMI 1640 + leukocytes
Lichtheimia corymbifera spores[3]
Detection HsbA[1]
Liquid chromatography-mass spectrometry analysis (LC-MS /MS)
Filter and purification of proteins
Supernatant
MOI 2:1
leukocyte infection model
Incubation with CO_2, 72 hours

P193

An emerging Aspergillus granulosus disseminated infection following a long-term course of azole antifungal therapy: a case report

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Aspergillus granulosus is an uncommon pathogenic A*spergillus* within the *Aspergillus* of section *Usti*. It is a ubiquitous mold found in outdoor and indoor environments. It is tolerant to high temperatures that can cause invasive aspergillosis (IA) in humans, particularly immunocompromised individuals. Herein, we demonstrate the emergence of *A. granulosus* disseminated infection in a patient who received prolonged azole therapy due to prior invasive *A. fumigatus* infection.

Case report
A 78-year-old Thai woman was diagnosed with primary myelofibrosis receiving intermittent blood transfusions, weekly erythropoietin injections, and oral ruxolitinib 300 mg/day since February 2018. Seven months later, she had a new onset of fever, non-productive cough, and left shoulder pain for 3 weeks. A physical exam discovered a large ill-defined mass at her left shoulder, including a few painful subcutaneous nodules at both thighs. Magnetic resonance imaging (MRI) of the left shoulder revealed heterogeneous enhancing soft tissue masses and suspected abscesses. Aspiration of the shoulder abscess and skin biopsy of the left thigh nodule were sent for microbiological diagnoses. Gram stain and KOH fresh smear of both samples demonstrated many septate hyphae. Serum galactomannan (SGM) was 2.63. In addition, computed tomography (CT) of chest showed a heterogeneous enhancing soft tissue mass, sized 3x4 cm. at left lower lung, and sputum Gram stain was positive for septate hyphae. Disseminated aspergillosis was suspected, and oral voriconazole was commenced, whereas ruxolitinib was discontinued after the diagnosis. Later, *A. fumigatus* was isolated from all those samples sent. The fever and the subcutaneous lesions were resolved well after the voriconazole therapy. After 1 month of therapy, the antifungal was switched to an oral posaconazole solution since the patient developed cutaneous vasculitis on both legs, likely caused by voriconazole. After that, the patient continued posaconazole therapy with a dosing of 800-1200 mg/day, and the follow-up levels were 0.5-1.7 mg/L. The chest CT at 3 months post-treatment showed a reduced size of the left lung mass, and all subcutaneous lesions disappeared. However, she had a new onset of fever and frequent cough for 3 weeks again and had a large ill-defined painful mass at the right side of the buttock during posaconazole therapy for 8 months. CT of chest and lower abdomen demonstrated a lung abscess at right lung and a large abscess, sized 10x6x13 cm. at the right buttock. Aspiration of the buttock abscess obtained 85 mL of bloody fluids. Aspirated fluid and sputum sent for a microscopic exam were positive for septate hyphae, and SGM testing was 4.69. A combination of posaconazole, liposomal amphotericin B 300 mg/day, and caspofungin 70 mg/day were administered to treat a breakthrough IA. All samples sent for fungal cultures grew velvety brown cinnamon colonies, subsequently identified as *A. granulosus* by 18S rRNA sequencing. However, the patient rapidly deteriorated and died 10 days after receiving the combined antifungal therapy.

Conclusion
Following long-term azole therapy, *A. granulosus* can cause a breakthrough IA and disseminated infection with a fatal outcome. Therefore, early diagnosis and combined antifungal therapy are required to prevent adverse outcomes.

P194

In-silico chromosome-level assembly of Sporothrix pathogenic genomes using a hybrid long- and short-read sequencing approach

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Objectives:

Sporotrichosis is an (extra)cutaneous infection usually caused by only 4 (*Sporothrix schenckii*, *S. brasiliensis*, *S. globosa* and *S. luriei*) of the 62 species currently listed in the genus *Sporothrix*. Although this disease is an emerging global health problem, very little progress has been made to date in exploring the genetic changes implicated in genome evolution of pathogenic lineages. This gap in knowledge reflects the lack of fully sequenced and well-annotated genomes for most of pathogenic *Sporothrix* strains, thus limiting our understanding of their biology and pathogenicity.

In this study, we expand the current knowledge on the genome organization, gene content and genetic diversity of *S. schenckii*, *S. brasiliensis* and *S. globosa* by providing well-annotated whole-genome assemblies of multiple strains obtained by using a hybrid sequencing strategy which combines PacBio SMRT long-read data with complementary datasets generated by Illumina short-read-based technology.

Materials & Methods:

Using PacBio and Illumina sequencing platforms, we sequenced the genomes of a total 30 *Sprotorhix* strains (11 *S. schenckii*, 12 *S. brasiliensis* and 7 *S. globosa*) from different geographical areas and sources (Fig. 1a). *De novo* hybrid assembly of high-quality long- and short-reads data was performed following the steps illustrated in Fig. 2 which also provides an overview of the entire bioinformatics workflow adopted in this study.

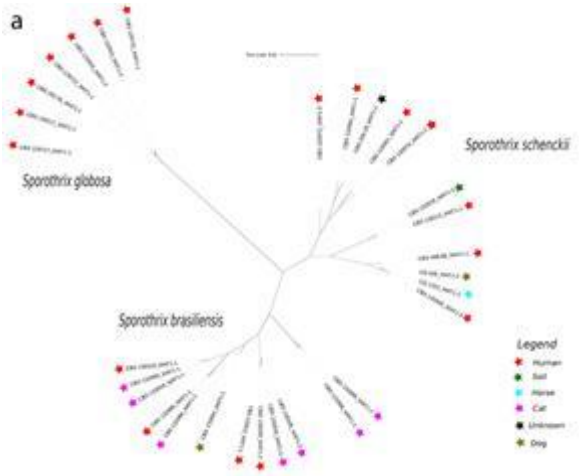
Results:

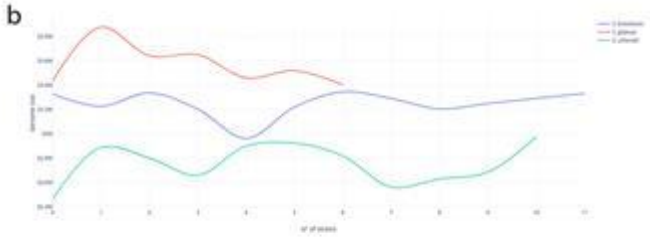
Hybrid sequencing of 30 *Sporothrix* strains produced a total of 8,785,014 long-reads and 315,163,649 short-reads that, after quality analysis, were assembled into chromosome-level assemblies containing each 7 different nuclear C-scaffolds, or chromosome-length scaffolds, and one mitogenome. Variation in nuclear genome size among strains is shown in Figure 1b. Phylogenetic analysis, using protein orthogroups, separated the strains into 3 distinct clades according to their currently known phylogeny (Fig. 1a). Interestingly, most of *S. brasiliensis* strains were further divided into 2 major sub-clades based on their mating-type alleles and regardless of their origin (animal or human) (Fig. 1a). One additional subclade, containing two MAT1-1 isolates from cats, was also observed. Genome-wide core SNPs analysis also supports the existence of uneven distribution of a single mating-type idiomorph in *S. brasiliensis*, a phenomenon not observed in *S. schenckii* and *S. globosa* populations.

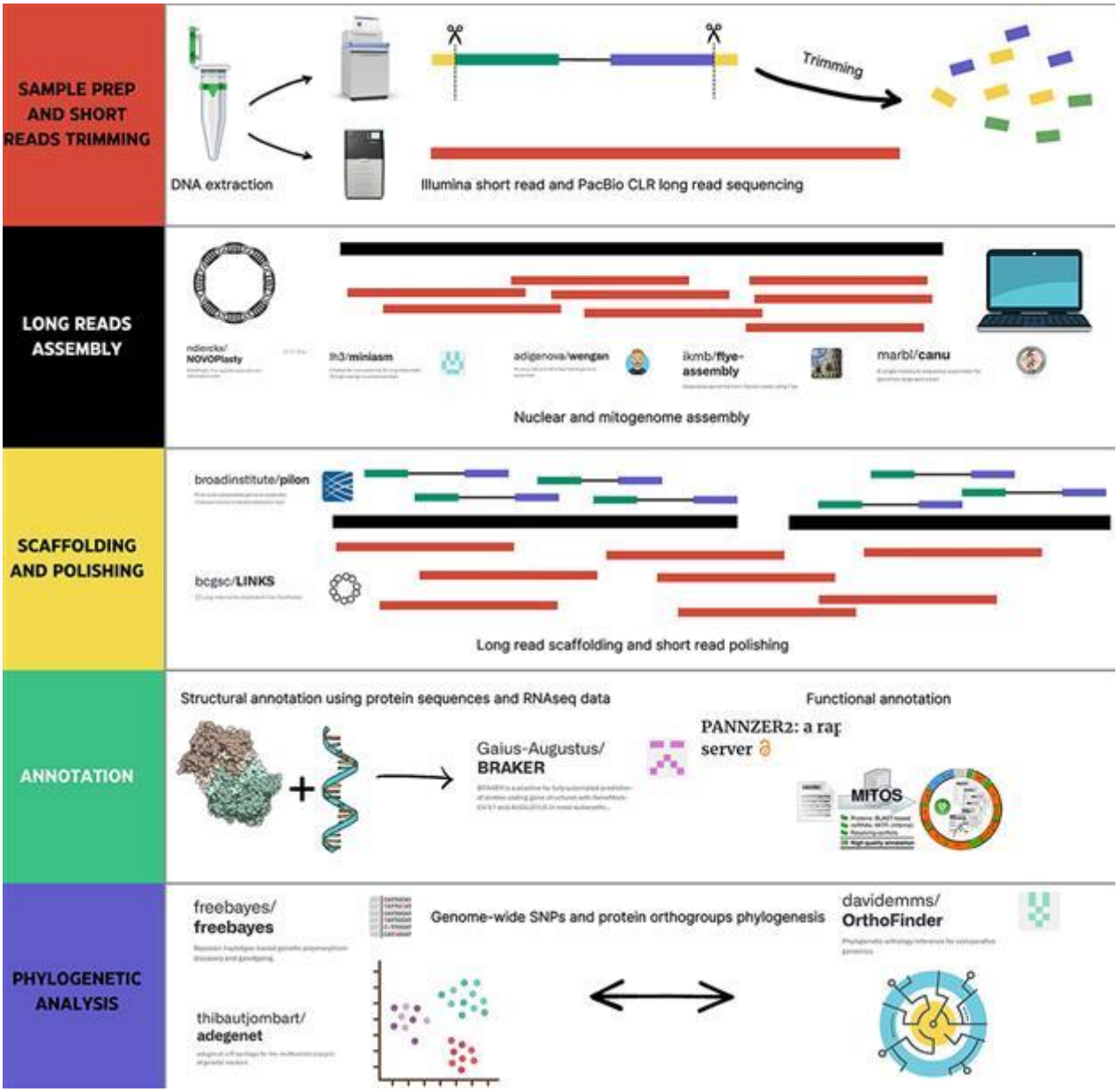
Funcional annotation of mitogenomes reveals, for the first time, the presence of genes encoding homing endonucleases (HEs) in two *S. globosa* strains (CBS129722 and CBS132924). These HEs belonged to GIY-YIG and LAGLIDADG families and are encoded by free-standing genes inserted in intergenic regions.

All genomic data, obtained in this study, can be freely accessed via the Sporothrix Genome DataBase (http://sporothrixgenomedatabase.unime.it)

Conclusions:
Our study expands the current understanding of genome organization and genetic diversity in members of the *Sporothrix* pathogenic clade by providing a rich source of genomic data greatly useful for comparative genome analysis addressing strain diversification, gene evolution and population dynamics.






SAMPLE PREP AND SHORT READS TRIMMING
DNA extraction
Trimming
Illumina short read and PacBio CLR long read sequencing
LONG READS ASSEMBLY
ndierckx/NOVOPlasty
lh3/miniasm
adigenova/wengan
ikmb/flye-assembly
marbl/canu
Nuclear and mitogenome assembly
SCAFFOLDING AND POLISHING
broadinstitute/pilon
bcgsc/LINKS
Long read scaffolding and short read polishing
ANNOTATION
Structural annotation using protein sequences and RNAseq data
Functional annotation
Gaius-Augustus/BRAKER
PANNZER2: a rap server
MITOS
PHYLOGENETIC ANALYSIS
freebayes/freebayes
Genome-wide SNPs and protein orthogroups phylogenesis
davidemms/OrthoFinder
thibautjombart/adegenet

P195

CLINICOMYCOLOGICAL PROFILE OF DEMATIACEOUS FUNGI AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN- A STUDY FROM SOUTH INDIA

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BACKGROUND
Melanised fungi though rare few years back, are being increasingly recognized as emerging pathogens causing infection both among immuno-compromised patients and healthy individuals. Prompt diagnosis and early initiation of treatment may facilitate reduction of morbidity and mortality. Subcutaneous mycoses caused by these fungi run a chronic course and hence need to be treated for a long duration of time. Antifungal susceptibility studies will help to choose the appropriate antifungal drug which will in turn help to improve clinical outcome in these patients.
OBJECTIVES
To identify the dematiaceous fungi isolated from patients using phenotypic and genotypic methods and to study their antifungal susceptibility pattern.
METHODOLOGY
A total of 8 samples suspected of dematiaceous fungi were collected over a period of one year and identified by phenotypic and genotypic methods. Phenotypic identification was done by LPCB from slide culture and banana peel culture method. Genotypic identification was done by DNA extraction using phenol-chloroform method using certain modifications. Following PCR amplification, the products were sequenced. Antifungal susceptibility testing was performed according to CLSI M38-A guidelines. Six antifungal drugs Amphotericin B (Sigma A4888), Fluconazole (Sigma F8929), Itraconazole (Sigma PHR1834), Voriconazole (Sigma PZ0005), Posaconazole (Sigma SML2287) and Terbinafine (Sigma T8826) were tested against these dematiaceous fungi.
RESULTS
Out of the 8 isolates, 4 isolates identified by phenotypic methods were as *Phoma sp*. (1), *Bipolaris sp*. (2), and *Fonsaceae sp*. (1) and 4 isolates did not sporulate. All the isolates were sent for gene sequencing and identified as *Macrophomina phaseolina, Pleosporales sp., Curvularia spicifera, Medicopsis romeroi, Curvularia rouhanii, Hypoxylon lignicola, Rhytidhysteron rufulum* and *Pseudochaetosphaeronema sp.* The *in vitro* activity against these dematiaceous fungi tested and MIC values were found to be high for Amphotericin B and Fluconazole and remain susceptible to other triazoles and Terbinafine.
CONCLUSION
The melanised fungi generally thrive on plant and soil environment and rarely cause infection in humans, especially the rare ones as isolated and identified from the various clinical

specimens. Even rare is the data available from India regarding the susceptibility pattern of these fungi. An attempt has been made to collect the melanized fungi from various centers for a period of one year and study their morphology and resistance pattern.

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A case of Millerozyma farinosa (Pichia Farinosa) fungemia: Recognition and management of a rare fungal infection

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Objectives:
Invasive fungal infections are an area of concern in the scarcity of new antifungals. *Millerozyma farinosa* (*Pichia farinose*) is a homothallic diploid and osmotolerant yeast. It rarely causes infection in humans and only few cases have been reported previously. To raise awareness and emphasize diagnostic challenges, we are reporting first case of fungemia caused by ***Millerozyma*** (***Pichia) farinosa*** in an immunocompetent patient with no prior risks or morbidity to provide valuable insight into the diagnosis, treatment, and prognosis.

Materials & Methods:
A 39-year-old woman with no prior known diseases underwent elective total abdominal hysterectomy for menorrhagia due to multiple fibroids. Surgery was uneventful and post-procedure she was discharged in stable condition. A week later, she was readmitted with fever, surgical site infection and abdominal discomfort. She had a WBC count of 15,000 cells/mm^3, CRP of 35.0 mg/dL and procalcitonin level was 0.82 ng/ml. She had a pelvic collection on ultrasound. Blood and wound cultures were sent and empirical treatment with intravenous meropenem and vancomycin was started but patient continued to spike fever and experience abdominal discomfort.
On the 4th day, wound culture was negative but blood cultures flagged positive for yeast identified as *Millerozyma farinosa* on Vitek2 Yeast ID card (BioMerieux, France). Antifungal susceptibility tested on YeastOne Sensititre™ revealed MICs of 4 mcg/mL against fluconazole, 0.15 mcg/mL against voriconazole, ≤0.12 mcg/mL against amphotericin and ≤0.008 mcg/mL against caspofungin. She was started on IV amphotericin B deoxycholate at dose of 0.5mg/kg/day with close monitoring for response to therapy. She became afebrile after 3 days and blood culture after 48 hours of antifungal therapy remained negative. On discharge, she was shifted to oral fluconazole and a week later, there was complete resolution of pelvic collection. Total duration of antifungals were 14 days including 3 days of amphotericin and 11 days of fluconazole.

Results:
The molecular identity of the isolate was confirmed by amplification of internal transcribed region 1 and 2 (ITS1 and ITS 2) that surround the 5.8S rRNA gene and sequenced.. The organism exhibited a 97-99% identity with *Millerozyma farinosa* strains originating from Brazil, Norway, and Iraq. Phylogenetic analysis using MEGA11 software revealed closest match with a skin isolate from Iraq. *M. farinosa* is a thermotolerant and osmotolerant environmental yeast found most commonly in food sources. It is usually thought to be non-pathogenic, but there are a few case reports of central line related fungemia in immunocompromised individuals. Ours appears to be first case of *M. farinosa* in a post-surgical immunocompetent host. Translocation from gut seems to be a possible route of infection, and global warming may be leading to an emergence of thermotolerant opportunistic fungal pathogens.

Conclusions:
In the absence of any epidemiological cut-off values, clinical breakpoints or recommendations, diagnosis and treatment of rare fungal infections is a challenge faced by clinicians, microbiologist and patients alike.

TIMM
Trends in Medical Mycology

P197

Profile of genes encoding efflux pumps in Candida auris clade V; no relationship with resistance to fluconazole

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Objectives:
Candida auris is an emerging opportunistic multidrug-resistant fungal pathogen with a high mortality rate and seriously threat to public health. Based on genetic analysis obtained from whole genome sequencing (WGS) data, *Candida auris* isolates are grouped into five geographically clades: clade I (South Asia), clade II (East Asia), clade III (South Africa), clade IV (South America) and clade V (Iran). In general, the majority of *C. auris* isolates demonstrate resistance to fluconazole, but variable susceptibility to echinocandins, amphotericin B, and other azoles. The second Iranian isolate of *C. auris* clade V (IFRC 4050) has been evaluated for probable mutations related to fluconazole resistance in the previous study, while the expression of genes encoding efflux pumps was not investigated. Therefore, the expression profile of different genes encoding efflux pumps including *CDR1*, *CDR2*, *MDR1*, and *MDR2* was tested for probable relation with fluconazole resistance in this unique strain.
Materials and Methods:
In this study, 2 isolates of fluconazole-resistant and one fluconazole-susceptible *C. auris* clade V were cultured on Sabouraud dextrose agar. These isolates were collected from the ear of Iranian patients with otitis. Then, total RNA was extracted from fresh yeasts exposed to sub-MICs concentrations using phenol-chloroform protocol. Real-time PCR was applied to evaluate the possible alteration in the expression of *CDR-1*, *MDR-1*, *CDR-2*, *MDR-2* and *ACT1* (as reference gene) genes, using specific primers. REST® software was used to analyze the results.
Results:
According to the REST output, there were no significant changes in the expression of *CDR-1*, *CDR-2* *MDR-1*, and *MDR-2* genes both in fluconazole-resistant and fluconazole-sensitive *C. auris* isolates.
Conclusion:
According to the obtained results, this resistance to fluconazole can be caused by biofilm or mutation in *ERG11* and *TAC1B* genes, which needs further study and investigation. Undoubtedly, the results of this study will help in understanding molecular mechanisms involved in fluconazole resistance in this mysterious emerging yeast.
Key Words: *C. auris* clade V, *CDR-1*, *CDR-2*, *MDR-1*, *MDR-2*, RT-PCR

P198

Characterisation of key amino acid residues in CYP51 that confert intrinsic short-tail azole resistance in Mucor circinelloides

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Objectives:
Mucormycosis infections are on the rise and treatment options are limited due to intrinsic drug resistance against short-tailed azoles and echinocandins. Based on in silico analysis in a previous study, the amino acid substitutions (AA) Y129F and V293A within the substrate-binding pocket of the sterol-14-α-demethylase (CYP51) paralog F5 were postulated to cause short-tailed azole resistance. Today we present the experimental proof.

Materials & Methods:
McCYP51 paralogues (CYP51A and CYP51B) and modified version of CYP51A carrying AA reversions (F129Y, A293V, F129Y & A293V) were overexpressed with and without their cognate cytochrome-P450-reductase (CPR) in our heterologous host S. cerevisiae. MIC valus and growth kinetics were determined. Protein expression was verified using protein chemistry. Sterol composition profiles were generated with and without azole exposure.

Results:
Strains harbouring functional CYP51 versions and CPR showed expression levels between 38% -69%. Our results suggest that short-tailed azole resistance is based on the CYP51A homolog. The growth rate and ergosterol composition of CYP51A-CPR remained unchanged when challenged with voriconazole. Voriconazole MIC values were 4.0 mg/L for. CYP51B reacts in all assays susceptible to high dose (1.0 µM) voriconazole and exhibits a MIC of 0.17 mg/mL.

Conclusions:
In conclusion, we were able to confirm that AA substitutions Y129F and V293A in combiantion are responsible for intrinsic short-tailed azole in M. circinelloides.

P199

Invasive and subcutaneous infections due to rare species of filamentous fungi, Parengyodontium album and Microascus cirrosus

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Objectives:

Invasive infections due to filamentous fungi have emerged as prominent causes of morbidity and mortality worldwide. Although *Aspergillus fumigatus* heads the list of these opportunistic moulds, infections due to less common but antifungal-resistant species of filamentous fungi are being reported with greater frequency. Importantly, opportunistic moulds pose a growing threat to human health, as several of these fungal pathogens show resistance to some or all current classes of antifungals. The aim of this study was to molecularly characterize the rare mold species originating from five patients of cutaneous, subcutaneous and invasive fungal infections and to analyse their antifungal susceptibility patterns.

Materials & Methods:

Preliminary macroscopic phenotypic identification was done on potato dextrose agar (PDA) incubated at 28°C for 14 days. Microscopic phenotypic identification was done by preparing slide cultures on potato dextrose agar (PDA) and examining under the scanning electron microscope. All isolates were identified by sequencing of the internal transcribed spacer (ITS) region. Antifungal susceptibility testing (AFST) was carried out according to CLSI-broth dilution method (M38-A2 guidelines).

Results:

All the 5 isolates were identified as *Parengyodontium album* (n=2), *Microascus cirrosus, Medicopsis romeroi* aand *Sporothrix globosa*, using ITS sequencing. The AFST results revealed that multiple antifungal agents were inactive against the *Microascus cirrosus,* with MIC value of >16mg/L for itraconazole, 2mg/L for posaconazole, 1mg/L for isavuconazole and voriconazole and 4mg/L for amphotericin B. This isolate of *M. cirrosus* was isolated from skin biopsy of deep subcutaneous infections of the right ankle with osteomyelitis in a 7-year-old girl. Fluconazole exhibited MIC values of 4mg/L and 16mg/L against both the isolates of *Parengyodontium album*. Other tested azoles and amphotericin B exhibited lower MIC values

for *P. album* (MIC ≤0.5mg/L). *Parengyodontium album* isolates were recovered, one from diffuse lesions of cutaneous infection in a patient suffering from systemic sclerosis and other from splenic abscess of a patient suffering from acute lymphoblastic leukemia. *Sporothrix globosa* exhibited high MICs values against all tested azoles: fluconazole, 256mg/L; itraconazole, 1mg/L; voriconazole, 16mg/L, isavuconazole, 8mg/L; posaconazole, 1mg/L and amphotericin B, 8mg/L. This *S. globosa* isolate was recovered from skin biopsy of a patient with subcutaneous involvement, with linear papulonodular lesions with history of unknown plant injury. AMB and azole showed good activity against *Medicopsis romeri*. Echinocandins showed no activity against any of the five moulds species tested.

Conclusions:

The present study reports a series of rare opportunistic mould infections in patients of cutaneous, subcutaneous and invasive mycoses. *Microascus cirrosus* and *Sporothrix globose* exhibited high MIC values against azoles, AMB and echinocandins. Sseveral of these less common opportunist rare mould species already exist in our environment, are intrinsic resistance to even the newest antifungal agents and may emerge as opportunistic pathogens. Both clinicians and microbiologists must become familiar with the various fungi, their epidemiologic and pathogenic features, and the optimal approaches to diagnosis and therapy.

P200

The first domestic isolation of terbinafine- and itraconazole-resistant Trichophyton indotineae in Chinese mainland

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ABSTRACT

Background: *Trichophyton indotineae*, a new species of dermatophytes, has become a significant concern in treating dermatophytosis due to the high level of terbinafine resistance reported in India and even worldwide.

Objectives: *This study aimed to report the terbinafine- and itraconazole-resistant T. indotineae in Chinese mainland, by identifying the phylogenetic classification of the isolate strain, and detecting the drug resistance, gene mutation and expression.*

Patients/Methods: *The skin scales of the patient were cultured on SDA and the isolate was authenticated by DNA sequencing and MALDI-TOF MS. Antifungal susceptibility testing was performed following the M38-A2 CLSI protocol to examine the MICs values of terbinafine, itraconazole, fluconazole, etc. The strain was screened for mutations in the squalene epoxidase (SQLE) gene by Sanger sequencing and detected the expression of CYP51A and CYP51B by qRT-PCR.*

Results: *A multi-resistant ITS genotype VIII sibling of the T. mentagrophytes complex (T. indotineae) was isolated in Chinese mainland. The strain harbored high terbinafine MIC of >32 μg/mL and itraconazole MIC of 1.0 μg/mL, which was identified a mutation in the squalene epoxidase gene with amino acid substitution (Phe$_{397}$Leu, mutation 1191C>A). In addition, overexpression of CYP51A and CYP51B was observed. With multiple relapses, the patient finally achieved clinical cure by itraconazole pulse therapy and topical clotrimazole cream for 5 weeks.*

Conclusions: *The first domestic strain of terbinafine- and itraconazole-resistant T. indotineae from a patient in Chinese mainland was isolated*. Itraconazole pulse therapy can be an effective method for the treatment of *T. indotineae*.

P201

Genetic characterization of emerging multidrug resistant Candida auris isolates in Malaysia

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Title: Genetic characterization of emerging multidrug resistant *Candida auris* isolates in Malaysia

Abstract

Background

Candida auris, a newly emerging healthcare associated yeast pathogen from the Metschnikowiaceae family, was first described in the ear canal of an elderly Japanese patient in 2009. The yeast is one of the causative agents of candidemia, which has been linked with nosocomial outbreaks and high mortality rates in healthcare facilities worldwide. This present study is the first comparative analysis by whole genome sequencing (WGS) of Malaysian clinical *C. auris* genomes that highlights clonal expansion of *C. auris* strains in Malaysia.

Methods

A total of 4 *C. auris* clinical isolates, from University Malaya Medical Centre, were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and tested for antifungal resistance using Sensititre™ YeastOne YO10 plate method. The complete genomes were determined by WGS using Illumina sequencing to reveal the clade distribution, and resistance mutation analysis.

Results

C. auris exhibited resistance against fluconazole (256 µg/mL), amphotericin B (4 µg/mL), and caspofungin (8 µg/mL) and showed susceptible towards micafungin (range 0.12-0.5 µg/mL) and anidulafungin (0.5 µg/mL). Phylogenetically, *C. auris* genomes belonged to South Asian clade I and showed limited genetic diversity, suggesting clonal transmission. Among key antifungal resistance genes studied, a single mutation (Y132F) was detected in *ERG11* gene of the fluconazole resistant strains.

Conclusion

The genetic characterization and antifungal susceptibility testing of *C. auris* isolates from Malaysia in the present study fills a gap in the literature by providing molecular epidemiologic information as well as antifungal susceptibility profile of Malaysian *C. auris* isolates. Rapid identification and appropriate infection prevention measures are required to control the transmission of the multi drug resistant fungal pathogen in healthcare facilities in Malaysia.

P202

Invasive mycoses caused by rare mold fungi. Results of a prospective study

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Objective: to study the clinical and laboratory features of severe fungal infections caused by rare mycelial pathogens.

Materials and methods. A prospective study in the period 2000-2022 used the criteria EORTC/MSG, 2020 for the diagnosis of invasive mycoses.

Results. 70 patients from 14 hospitals in St. Petersburg were examined, men - 60%, women – 40%, median age – 42.5 [26;55].

The main background conditions were oncohematological diseases of 74% (n=52): acute myeloid leukemia – 34%, acute lymphoblastic leukemia – 24%, non–Hodgkin's lymphoma – 12%, Hodgkin's lymphoma – 8%, chronic lymphocytic leukemia – 8%, chronic myeloid leukemia – 4%, myelodysplastic syndrome - 4%, undifferentiated acute leukemia – 4%, multiple myeloma - 2%. "Other" background diseases (n=18) included: COPD (39%), AIDS (17%), type 2 diabetes mellitus (17%), chronic sinusitis (11%), a condition after surgical treatment of a solid tumor (11%), polytrauma and burn disease (11%), medicinal agranulocytosis (6%), the condition after organ transplantation (6%).

We analyzed risk factors with control group of patients. The main risk factors were: agranulocytosis (53%), lymphocytopenia (60%), the use of GCS (70%), TCSC and GVHD (23%), stay in the ICU (33%), the use of ventilators (21%).

The main localizations of the development of the infectious process were the lungs (73%), less often in the paranasal sinuses (17%), the central nervous system (7%), skin and soft tissues (7%), disseminated mycosis (16%). The lesion of two or more organs was observed in 20% of patients.

The causative agents of mycoses were: Fusarium spp. (27%), Paecilomyces spp. (20%), Acremonium spp. (14%), Trichoderma spp. (9%), Exophiala spp. (6%), Scopulariopsis spp. (7%), Scedosporium spp. (6%), Alternaria spp. (4%), Aureobasidium pullulans (4%), Cladosporium spp. (3%).

Antimycotic therapy was performed: voriconazole (67%), amphotericin B deoxycholate (19%), itraconazole (23%), echinocandins (16%), amphotericin B lipid forms (14%) and posaconazole (11%). Combined antimycotic therapy was used in 23% of patients. Surgical treatment was applied in 20% of patients.

The overall survival rate of patients within 12 weeks was 63%.

Conclusions:
for the successful treatment of invasive fungal infections caused by rare mycelial pathogens, early diagnosis with obtaining a culture of the pathogen and determining sensitivity to antimycotics, early targeted antimycotic therapy in combination with surgical treatment and control of risk factors are necessary.

P203

Romania on the Candida auris Roadmap – First Outbreak in a Tertiary Hospital

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Objectives: We present an outbreak of invasive *Candida auris* infection occurring in the intensive care unit of a tertiary hospital from Romania.

Materials & Methods: The isolates of *C. auris* (n=9) have been obtained between August - December 2022 from the blood cultures of nine patients hospitalized at the intensive care unit of a tertiary hospital in South Romania, during a candidemia surveillance multicentric study. All patients showed predisposing factors for fungal infection and the average length of ICU stay exceeded 7 days. The isolates were identified by VITEK2 MS and later molecularly confirmed using a tetraplex PCR and ITS-rDNA sequencing, followed by long-read genome sequencing for outbreak analyses. *In vitro* antifungal susceptibility testing was performed by the colorimetric broth microdilution panel Sensititre YeastOne Y010 (SYO) according to the manufacturer's instructions.

Results: NCBI Blast search of the ITS sequences had a 100% match with *C. auris* (NCBI GenBank accession numbers OQ581776-OQ581784). Genome data was deposited in the NCBI Genome databases, accessible via BioProject PRJNA932422, BioSample SAMN33191480-SAMN33191488, and Sequence Read Archive SRR23363415-SRR23363423. The isolates included in this study cluster with the southern Asian isolates (Clade I) that have been previously described in European and US hospitals, and related to outbreaks of invasive infections. However, phylogenetic analysis based on SNP-number comparison showed that the new Romanian isolates are slightly different from other Clade I isolates. All nine isolates showed similar antifungal susceptibility pattern independent of the patient. According to CLSI tentative breakpoints defined for *C. auris*, all our isolates were resistant to fluconazole (MICs >256 µg/mL) and amphotericin B (MICs of 4 µg/mL). Alternatively, the use of SYO wild type upper limit value proposed by Siopi *et al* [1] resulted in 100% susceptibility to amphotericin B. The crude mortality rate in these patients was 55.6% (n=5/9).

Conclusions: To the best of our knowledge, this is the first report of a healthcare-associated *C. auris* infection in Romania. The emergence of this highly pathogenic yeast in Romanian hospitals is alarming

due to its rapid transmission within intensive care unit environment and the high mortality rate among *C. auris*-infected patients.

References: [1] Siopi M, Peroukidou I, Beredaki MI, Spruijtenburg B, de Groot T, Meis JF, Vrioni G, Tsakris A, Pournaras S, Meletiadis J. Overestimation of Amphotericin B Resistance in *Candida auris* with Sensititre YeastOne Antifungal Susceptibility Testing: a Need for Adjustment for Correct Interpretation. Microbiology Spectrum. 2023 Apr 10:e04431-22.

P204

Candida haemulonii complex, an emerging threat from tropical regions?

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Objectives
Within the *Metschnikowiaceae* clade, *Candida haemulonii* complex-related species are pathogenic yeasts closely related to *Candida auris* and share several pathogenicity-related traits, like adhesion on prosthetic materials, phenotypic switching, and multidrug resistance. While the origins of the worldwide *C. auris* outbreak are still poorly understood, only limited data are available on the epidemiological situation of these potentially emerging yeasts.

Materials & Methods
We analyzed clinical and demographic characteristics of patients with fungemia due to *C. haemulonii* complex and related species (*C. pseudohaemulonii*, *C. vulturna*) reported in France during 2002-2021, and compared them to data of *C. parapsilosis* fungemia rather than *C. albicans* because preliminary information revealed the presence of these study species on human skin. We also conducted a study on adult inpatients and outpatients colonized by *C. haemulonii* complex, managed at the University Hospital of Martinique during 2014-2020. Finally, we performed a literature review of fungemia due to *C. haemulonii* complex and related species reported in Medline (1962-2022).

Results
In total, we identified 28 fungemia due to *C. haemulonii* complex in France. These episodes were frequently associated with bacterial infection (38%) and high mortality rate (44%), and differed from *C. parapsilosis* fungemia by their tropical origin, mainly from Caribbean and Latin America. All isolates showed decreased *in vitro* susceptibility to amphotericin B and fluconazole. In Martinique, we found that skin colonization was frequent in the community population, while colonization was strongly associated with the presence of foreign materials in ICU patients. The literature review identified 274 fungemia episodes, of which 56 were individually described. As in our national series, published cases originated mainly from tropical regions and occurred between 2006 and 2022. These infections occurred in adult patients with risk factors for fungemia but also, but also neonates and pediatric patients, all with a high crude mortality. Strains also presented high MIC values for amphotericin B and fluconazole, but also for echinocandins for some strains. Intra-hospital outbreaks involving *C. haemulonii* complex suggest the capacity for horizontal transmission.

Conclusion

Multidrug-resistant *C. haemulonii* complex-related species are responsible for fungemia and colonization in community and hospital settings, especially in tropical regions, warranting closer epidemiological surveillance to prevent a potential *C. auris*-like threat.

a.

Variable		N	Odds ratio	
Region	Mainland France	877		Reference
	Overseas territories	88		70.77 (23.67, 280.01)
Solid cancer	No	676		Reference
	Yes	289		1.91 (0.65, 5.44)
Chronic renal failure	No	861		Reference
	Yes	104		1.83 (0.25, 8.79)
Liver cirrhosis	No	920		Reference
	Yes	45		6.62 (0.83, 37.97)
Intensive Care Unit	No	641		Reference
	Yes	324		1.14 (0.40, 3.18)
Context of bacterial infection	No	774		Reference
	Yes	191		2.91 (1.10, 7.64)

b.

Variable		N	Odds ratio	
Candida species	*C. parapsilosis*	757		Reference
	C. haemulonii complex and related species	22		2.39 (0.95, 5.86)
Solid cancer	No	548		Reference
	Yes	231		1.97 (1.34, 2.90)
Hemopathy	No	671		Reference
	Yes	108		1.92 (1.18, 3.09)
Intensive Care Unit	No	522		Reference
	Yes	257		3.17 (2.23, 4.54)
Liver cirrhosis	No	742		Reference
	Yes	37		0.72 (0.31, 1.56)
Context of bacterial infection	No	628		Reference
	Yes	151		1.05 (0.68, 1.59)

Figure 1. a. Explanatory model for the occurrence of *C. haemulonii* complex, *C. pseudohaemulonii* or *C. vulturna* fungemia rather than *C. parapsilosis (adjusted odds ratio from logistic regression coefficients with 95% confidence interval)*
b. Explanatory model for the death within 3 months of *C. haemulonii* complex, *C. pseudohaemulonii, C. vulturna* or *C. parapsilosis* fungemia. (adjusted odds ratio from logistic regression coefficients with 95% confidence interval)

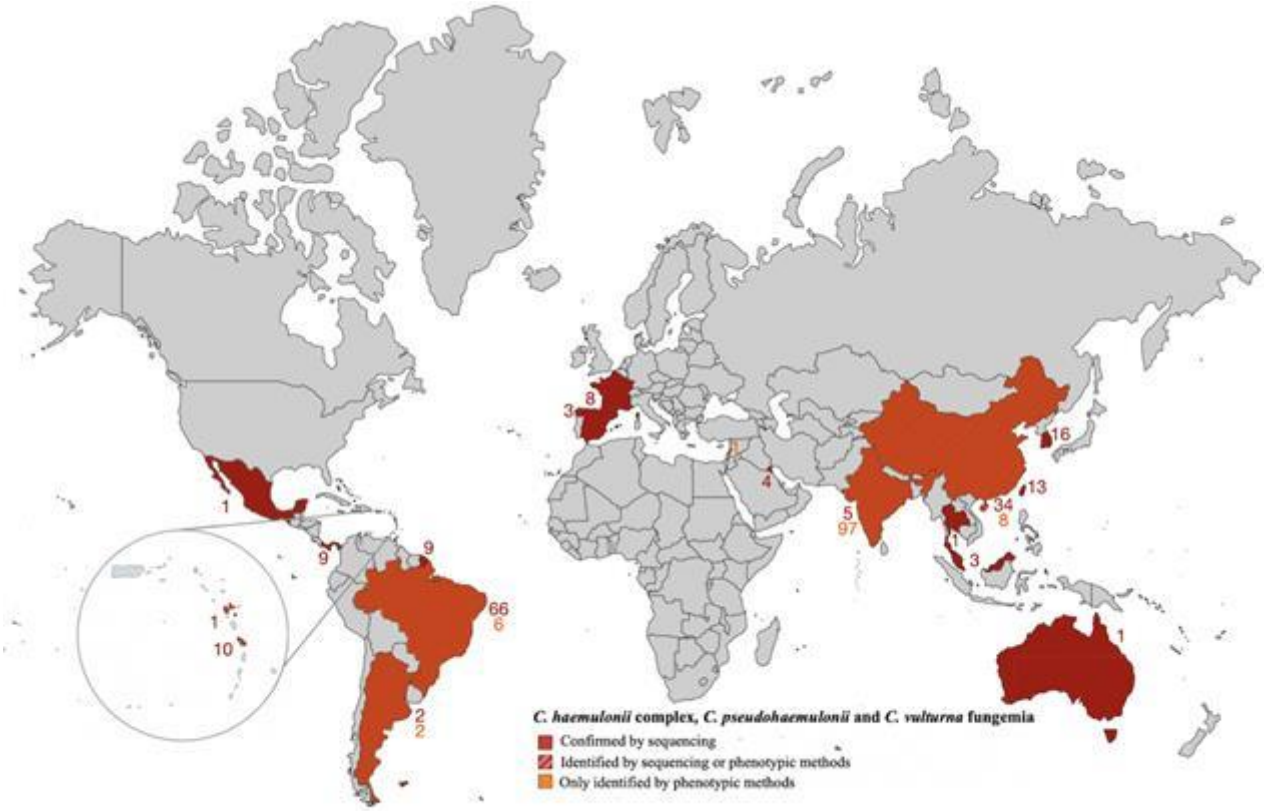

Figure 2. World mapping of cases of fungemia due to *C. haemulonii* complex, *C. pseudohaemulonii* or *C. vulturna*, according to our case series (obtained from the YEASTS program 2002-2021 and the RESSIF Network 2012-2021) and the literature review (Medline, 1962-2022).

P205

Genotyping analysis of a Candidemia Outbreak Caused by Candida parapsilosis isolates in a Northern Italy hospital.

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Objectives:
Candida parapsilosis (CPA), the most common species of *Candida* non *albicans* isolated from bloodstream infections, although usually susceptible to azoles, has been recently reported as an emerging multi-resistant yeast. Azoles play an important role in the therapeutic management of invasive candidiasis. In recent years, *Candida* isolates with acquired resistance to azoles have been reported frequently. Therefore, antifungal susceptibility testing and the detection of genes' mutations are becoming increasingly important to detect antifungal resistance mechanisms. The aims of this study were:1) to detect mutations in resistance genes of azole-resistant strains by sequencing, focusing on mutations of the ERG11 gene.
2) to perform a genotyping analysis using microsatellite markers in order to evaluate their genomic distribution.

Methods & Methods:
This study was conducted at the AUSL Piacenza Hospital (Piacenza, Northern Italy). Antifungal susceptibility to fluconazole was investigated in 65 CPA isolates from blood cultures during the period 2021-2023.
Yeast identification was performed by using MALDI-TOF MS (Vitek MS, bioMérieux, France). Antifungal susceptibility was evaluated with the Vitek2 system (bioMériueux, France) and confirmed with Sensititre Yeast One YO10 (Thermofisher Scientific,USA) microdilution broth. Internal transcribed spacer 1 and 4 (ITS2 and ITS4) regions and ERG11 gene of R-CPA isolates were analyzed by sequencing according to Sanger's method, with the purpose of confirming respectively the species identification and the mutation involved in fluconazole resistance of the isolates.
Microsatellite genotyping was performed on all the 65 susceptible and resistant strains, as described by Sabino et al.. In order to evaluate genetic diversity between isolates, data from microsatellite analysis were used to perform unweighted pair group method with arithmetic mean (UPGMA) dendrogram.

Results:
Within 65 CPA isolates, 20 (30.8%) were respectively susceptible (S-CPA) and 45 (69.2%) were resistant (R-CPA) to fluconazole.
Sequencing of ITS regions confirmed the MALDI-TOF MS identification: the whole of R-CPA tested were identified as *Candida parapsilosis sensu stricto*.
ERG11 gene analysis has shown the presence of different mutations in R-CPA isolates: among fluconazole resistance-related mutations, Y132F was the most prevalent (42/45; 93.4%), followed by R398I (2/45;4.4%), and A114V + R398I (1/45; 2.2%).

The figure 1 shows high genetic diversity between S-CPA strains, with single isolate representing a single cluster, while R-CPA were divided into 5 clusters (labeled in the figure 1): 4 (A to D) considered as single strain and 1 (E) that include all the remaining 41 isolates.

Conclusions:

Genotyping studies with microsatellites, similarly to NGS, offer the opportunity to have data on local epidemiology and information on the distribution of emerging microorganisms.
Of note, isolates belonging to the same cluster may exhibit different mutations in the ERG11 gene, suggesting that strains could collect new mutations over time.
In addition, strains with different mutations show similar phenotypes in terms of susceptibility and resistance to azoles.
The finding of isolates belonging to the most populous cluster in different departments, even geographically distant, shows that the distribution of these microorganisms is now ubiquitous.

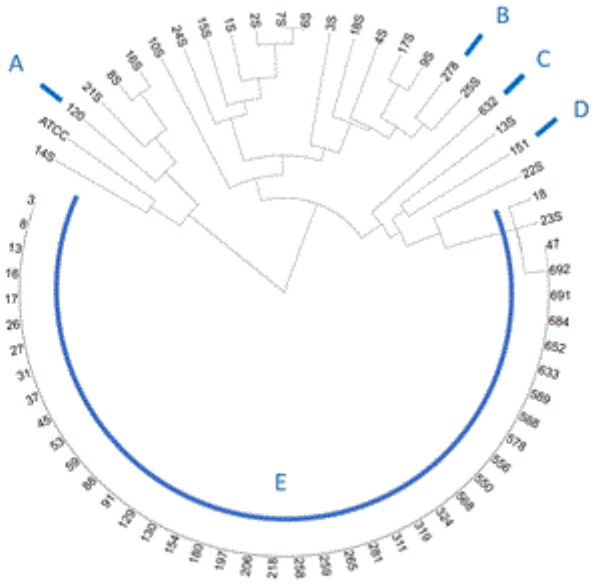

Figure 1: UPGMA dendrogram of 65 CPA isolates typed by microsatellite approach. Fluconazole-susceptible strains are marked with "S". The 5 R-CPA clusters are signed with letters from A to E.

P206

Development and evaluation of a rapid molecular diagnostic strategy for the detection of T. indotineae from patient samples

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Objectives: Terbinafine-resistant dermatophytosis caused by *Trichophyton indotineae* are emerging across the globe, characterized by extensive tinea of the glabrous skin. The routine practice of direct examination and culture in the mycology laboratory is unable to distinguish *T. indotineae (TI)* from *T. mentagrophytes* and *T. interdigitale* (i.e. *T. mentagrophytes* complex, *TMC*). Precise species identification relies on ITS region sequencing of the isolate, resulting in delayed diagnosis in most settings. The aim is to propose a rapid diagnostic strategy based on quantitative PCR (qPCR), designed to detect, and differentiate *TI* from *TMC* directly from patient's skin scrapings.

Materials & Methods: Primers were designed by aligning ITS sequences from 20 *TI* and 33 *TMC* from all genotypes and qPCR was optimized to highest efficiency. The specificity of our *T. indotineae*-qPCR (*TI*-qPCR) was evaluated against 96 other fungal pathogens DNA (including seven dermatophytes). A total of 86 and 19 skin scraping specimens positive for *TI* and *TMC* respectively previously diagnosed by a positive culture and ITS sequencing of the isolate were included between 2020 and 2022. In addition, 48 specimens positive for *T. rubrum* and 24 negative specimens were included as controls.

Results: qPCR design: By aligning the ITS sequences of *TI* and *TMC*, only three sparse single nucleotide polymorphisms were found to be different. Out of 96 fungal pathogen we only amplified *TMC*. At identical amount of *TI* or *TMC* DNA per reaction (0.1 ng), mean Cq for *TI* and *TMC* was respectively 27,9 (±0,1) and 38,9 (±0,3). Therefore, our strategy was based on Cq values difference between *TI* and *TMC*. We normalized our *TI*-qPCR Cq values with a pan-dermatophyte qPCR[1] Cq values to obtain ΔCq = (TI-qPCR) – (pan-dermatophyte-qPCR).

qPCR evaluation on samples: All control specimens (n=72) were not amplified and all samples with a positive culture of *TI* (n=86) were amplified (sensitivity = 100%). For the samples positive for *TI (n=86)* or *TMC (n=19)*, the mean±SD ΔCq for *TMC* was 9.6±2.7 and ΔCq for *TI* was -1.46±2.1 ($p<0.001$). By defining a ΔCq=4.5 as a cut off-value, the specificity to detect *TI* was 100% (figure 1).

Conclusions: We developed and validated a qPCR to quickly identify *TI* directly from skin scrapings from infected patients with a sensitivity of 100%. Using this technique, we can deliver results in 2-3 days at best, instead of waiting for culture growth and molecular identification (1-2 weeks). We propose a molecular diagnostic strategy (Figure 2) that enables the differentiation between *TI* and *TMC*, leading to early diagnosis and improved therapeutic adequacy for *TI* dermatophytosis.

[1]Paugam *et al.* (2013) J Microbiol Meth

Figure 1. Mean ΔCq values of skin scraping specimens from patients infected with *Trichophyton indotineae* (TI) or other genotypes of *Trichophyton mentagrophytes* complex (TMC). Proposed cut-off of 4.5 to distinguish TI from TMC (red dotted line). ΔCq=(*TI*-qPCR) – (pan-dermatophyte-qPCR)

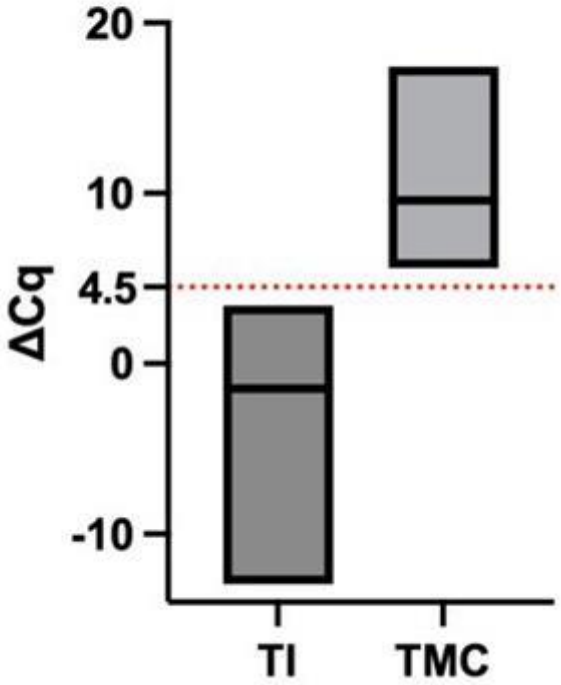
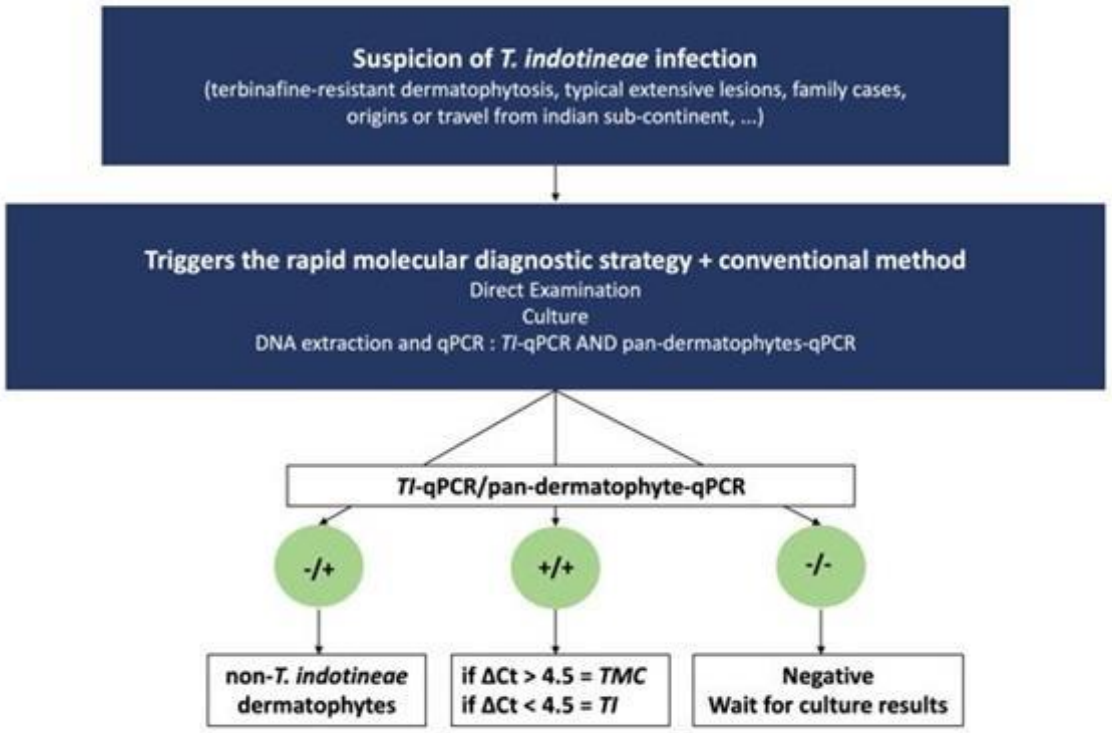


Figure 2. Rapid molecular strategy for the diagnosis of *Trichophyton indotineae* infection. TI: *Trichophyton indotineae*; TMC: *Trichophyton mentagrophytes* complex



P208

First report of a Candida auris outbreak in a tertiary hospital in Northern Greece

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Objectives
Candida auris is an emerging drug-resistant fungus that presents a serious global health threat. Isolation from clinical samples has been reported globally. According to the 2020/21 ECDC report, 15/30 European countries have been affected. Greece reported its first isolate in 2019 with a logarithmic increase of its documented cases up to date. The region of Northern Greece, which hosts a population of approximately 4 million inhabitants, was free of *C. auris* until late 2022. The aim of this report is to describe the first *C. auris* outbreak in the area of Northern Greece.
Methods
Since October 2022, when *C. auris* was isolated for the first time in Northern Greece from the central venous catheter tip of an ICU patient with prolonged hospitalisation, all patients testing positive were recorded. Positive patients were either colonised when axillary and groin surveillance swabs grew the pathogen, or infected if the fungus was cultured from clinical samples (blood, urine, bronchoalveolar lavage). Screening was performed on chromogenic media. The isolates were verified by MALDI-TOF MS and molecular methods in the reference laboratory. The departments which hosted the affected patients upon recognition were recorded. Clinical outcome of the affected patients was monitored.
Results
Patients with *C. auris* were isolated, and extensive screening of close contacts was performed. In departments with outbreaks, or high-risk departments, screening of the inpatients was repeated regularly. A total of 49 (24♂) patients (age: 68 [32-92] years) with *C. auris* were recorded from October 2022–May 2023. All of them were fluconazole-resistant but echinocandin-sensitive. Two positive case clusters were documented (1st cluster when the blood culture from a septic patient grew the fungus, who died shortly afterwards, and the 2nd when an inpatient was found colonised by PCR prior to transfer to the ICU). Twenty-two patients were hospitalised in the ICU upon diagnosis. Twenty-four patients grew the fungus from clinical samples. During extensive surveillance, the pathogen was not detected on any screen from medical equipment or the patients' environment. Twenty-one patients died [21/49 (47.7%)], due to multiple complications, reflecting the severity of the clinical course. Death was directly attributable to the fungus in one patient with fungaemia.
Conclusions
The emergence of *C. auris* presents new challenges for clinicians, due to its multi-drug resistance. The ability to colonise skin and the mucosa and spread rapidly horizontally calls for prudent management within the healthcare facilities. Containment of the transmission demands adherence to a series of measures and strict administrative policies. Additional contributing factors responsible for in-hospital spread include the extended length of stay of the affected population, and the impeded transfer to rehabilitation facilities.

P209

Scedosporium and Lomentospora Emerging Ocular Infections in India: Case series

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Objectives: To report clinical presentation, predisposing factors and final outcome of ocular infections caused by *Scedosporium*/*Lomentospora.* Also to identify the predominant species and antifungal susceptibility patterns cases of *Scedosporium*/*Lomentospora* isolates causing ocular infections, in North Indian population.

Materials and Methods: Over a period of five months between December 2023 to May 2023, seven cases of culture positive *Scedosporium*/*Lomentospora* ocular infections diagnosed were included in the study. Definitive diagnosis was based on isolation, visualization and detection of the fungus from clinical specimens by microscopy techniques, culture.

Results: Of the seven cases, *Lomentospora prolificans* (4 cases) was the predominant isolate followed by *Scedosporium apiospermum* (1 case) and *S. boydii* (2 cases). In one patient synanamorphic forms of *Pseudallescheria boydii* i.e. *Graphium* was isolated. Co-infection with other fungal pathogens namely *Alternaria sp* and *Phaeoacremonium sp* was noted in 2 patients. Six patients presented with keratitis while one case of *Lomentospora prolificans* causing endophthalmitis was also noted. Exposure to unspecified foreign body injury or organic matter injury was noted in majority of the cases. Most *Scedosporium* infections of the cornea alone responded well to commercially available antifungals with natamycin as the primary drug. Combination of medical therapy along with therapeutic keratoplasty was performed for progressing cases not responding to medical treatment.

Conclusion: Being a rare filamentary fungus to affect the eye, reports of *Scedosporium*/*Lomentospora* ocular infections in literature are very few. Our report highlights *Scedosporium*/*Lomentospora* as an

emerging cause ocular infections in India**.** As these fungi are resistant to many antifungal agents, early diagnosis is essential for initiating targeted drug therapy. Majority of our cases responded to medical therapy with natamycin as the primary drug, though cases with progressed disease required therapeutic keratoplasty.

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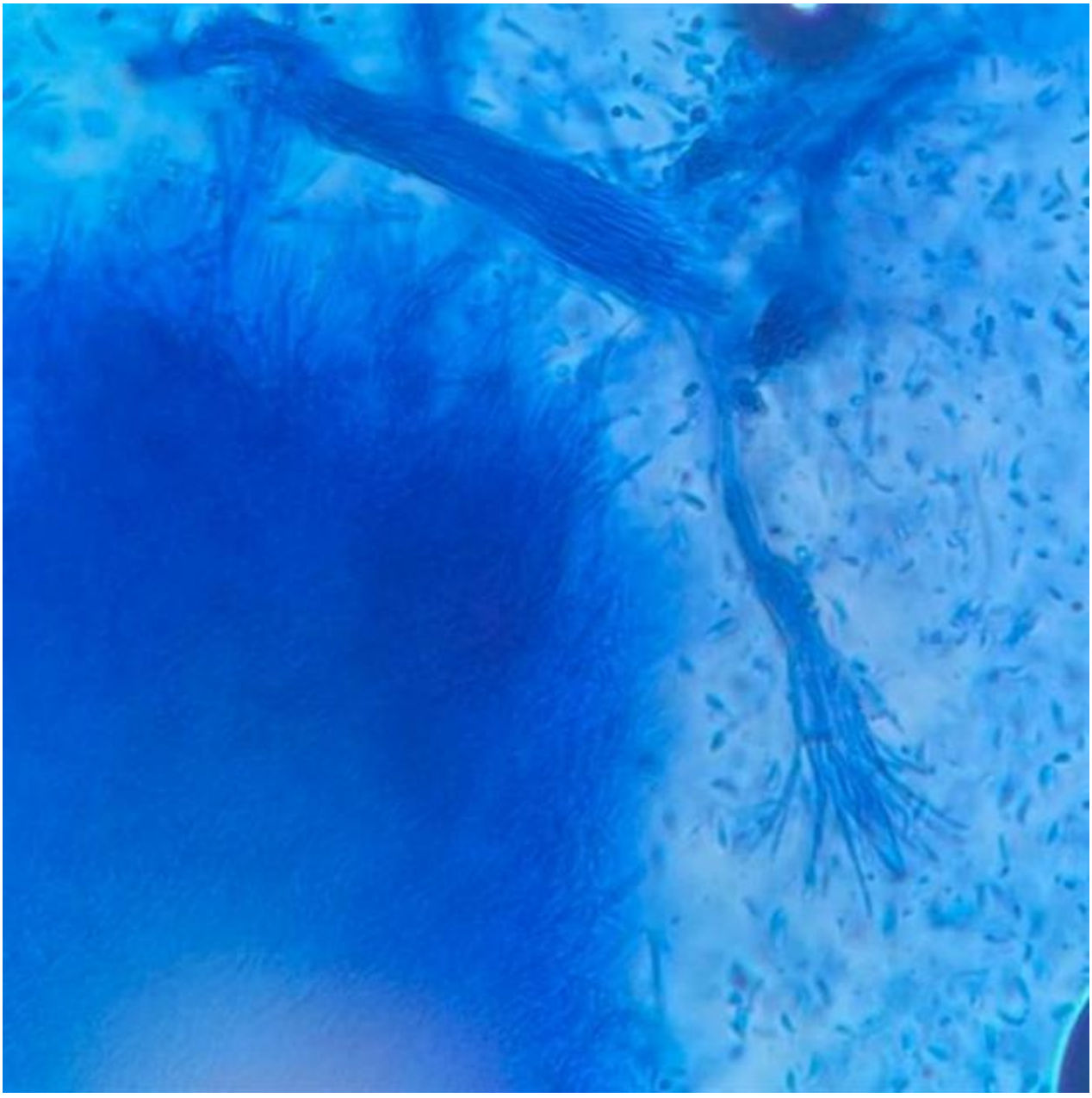

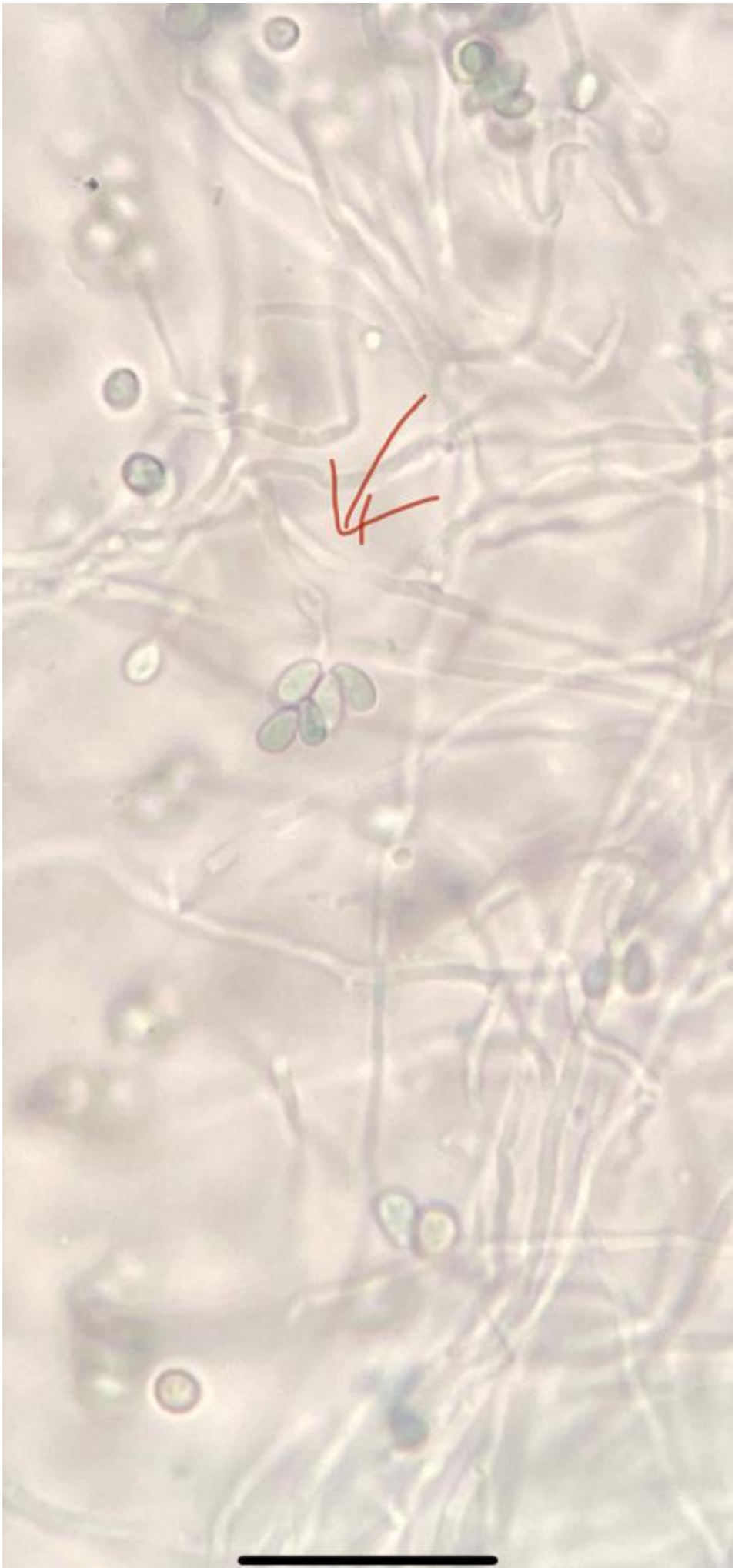

P210

Development of a microsatellite typing panel for the pathogenic yeast Trichosporon asahii

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Objectives: *Trichosporon asahii* is a basidiomycetous yeast able to cause life-threatening infections in susceptible hospitalized patients. We developed a novel microsatellite panel to molecularly characterize clinical *Trichosporon asahii* isolates.
Materials & Methods: The genome of the *T. asahii* type-strain CBS 2479 was de novo assembled from long-read nanopore sequence data to cover complex genetic regions like microsatellites. The newly generated CBS 2479 genome was used as input for the Tandem Repeat Finder software using the standard parameters, 500bp flanking regions were included for each locus to enable primer design. An initial list of 26 loci were selected to developed primers using Primer3 v0.4.0. During primary tests set to check if designed primer sets yielded amplicons for 16 *T. asahii* strains, a total of six microsatellite markers were selected. Monoplex PCRs amplified three di-, tri-, or tetranucleotide repeats. The six microsatellite markers were used to analyze 95 presumably unrelated *T. asahii* strains, including clinical and environmental sources, obtained from South America and Europe. The strains were obtained from different anatomical sites, including deep-seated and superficial infections, representing the five prevalent IGS1-genotypes already described. Raw data and relatedness between strains were analyzed using Bionumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium) via the unweighted pair group method with arithmetic averages (UPGMA) as previously described. The discriminatory power of the microsatellite panel was determined using the Simpson index of diversity (*D*). A *D* value of 1.0 indicates that the typing method was able to discriminate between all isolates, while a D value of 0 indicates that all isolates were identical (clonal).
Results: For each marker, between 11 and 37 alleles were found in our *T. asahii* population. Thirty two different fingerprinting profiles were obtained. The Simpson's diversity index for the individual markers ranged from 0.461 to 0.773 and of 0.947 for the combined microsatellites, indicating the high discriminatory power of this developed panel.
Conclusions: In summary, this panel combines high reproducibility and specificity suitable to be used in epidemiological studies of *T. asahii* and outbreak investigations potentially caused by this pathogen.

P211

Clinico-epidemiological features and Mycological Profile of Keratitis from a tertiary care teaching hospital in central India

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Objectives:

1. To analyze the clinical and epidemiological characteristics of fungal keratitis cases.
2. To study the mycological profile of keratitis cases from central India

Methods:

This study was carried out in a tertiary care teaching hospital of central India for a period of three years (January 2020 to December 2022). This was a retrospective analysis of clinical and demographic records and mycology reports of clinically suspected fungal keratitis cases. After taking informed consent, corneal scraping was collected from the patients by an experienced Ophthalmologist. Specimen was inoculated, under the supervision of Microbiologist, on Sabouraud dextrose agar in “C” shaped manner (Figure 1 A) and also smear was made on glass slide for KOH mount. Standard mycological procedures were followed for the identification of fungal pathogen. A diagnosis of fungal keratitis was made on the basis of KOH positivity and/or culture positivity or both. Antifungal susceptibility testing for Voriconazole was performed for Aspergillus & Fusarium species using E-test and MIC values were determined and interpreted using EUCAST ECOFFs breakpoints.

Results:

A total of 59 clinically suspected keratitis cases were studied, out of which 30 (50.84 %) were diagnosed of having fungal keratitis. We observed male to female ratio of 1:1. Maximum cases were from 40 – 60 years (53.34%) followed by 21 – 40 years (30%), with a mean age of 44 years (range 15 – 80 years).

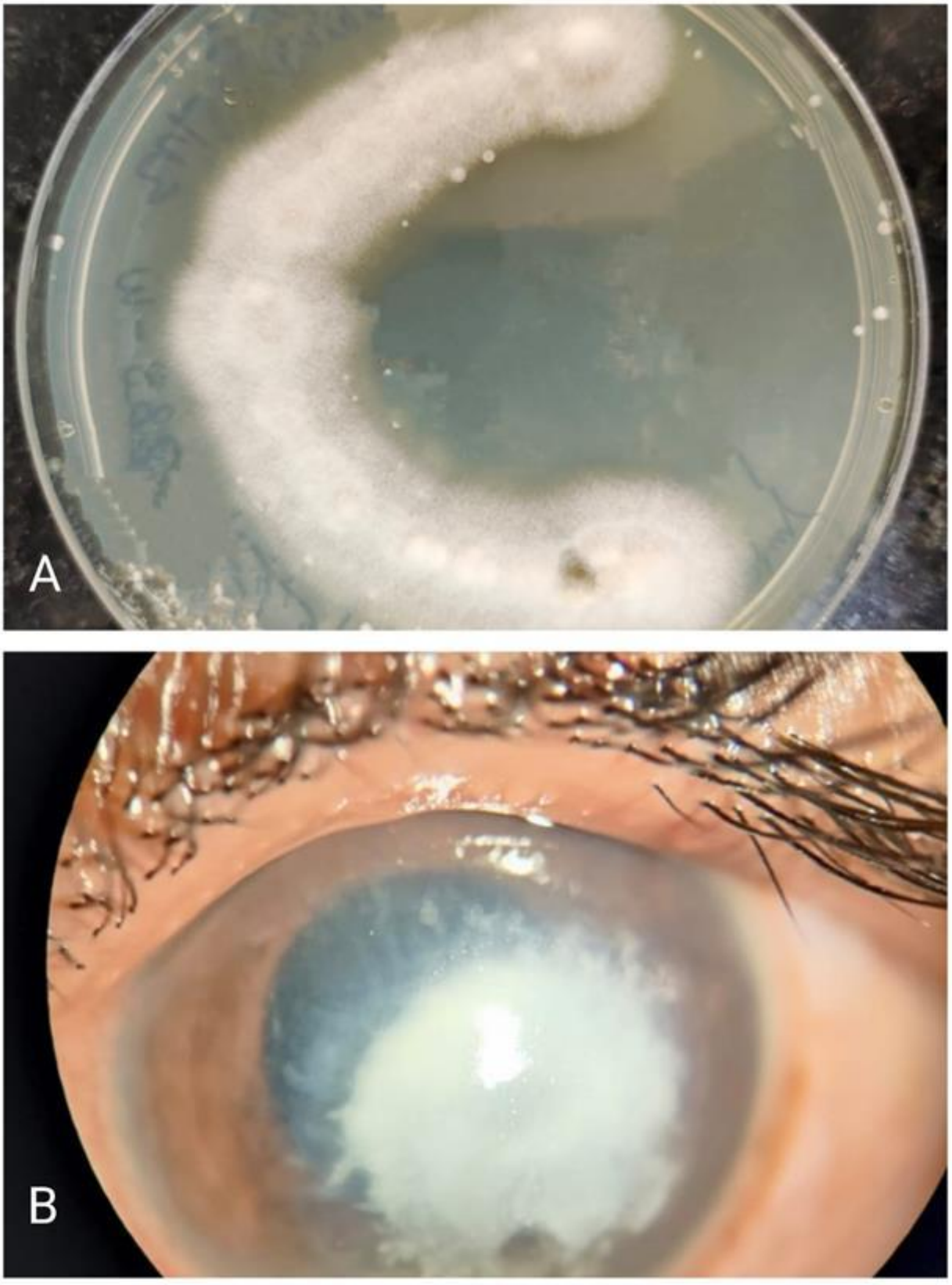
Maximum patients were from rural areas (53.34%) involved in agricultural activities (36.67%). Trauma with the vegetative matter was the most common risk factor. There was a seasonal variation with increased case in monsoon months (June to August) (50%) which coincides with paddy cultivation, which is the staple food of people in central India. Also, increased cases were observed in cooler and dry months (November to February) (36.67%), which coincides with harvest season.

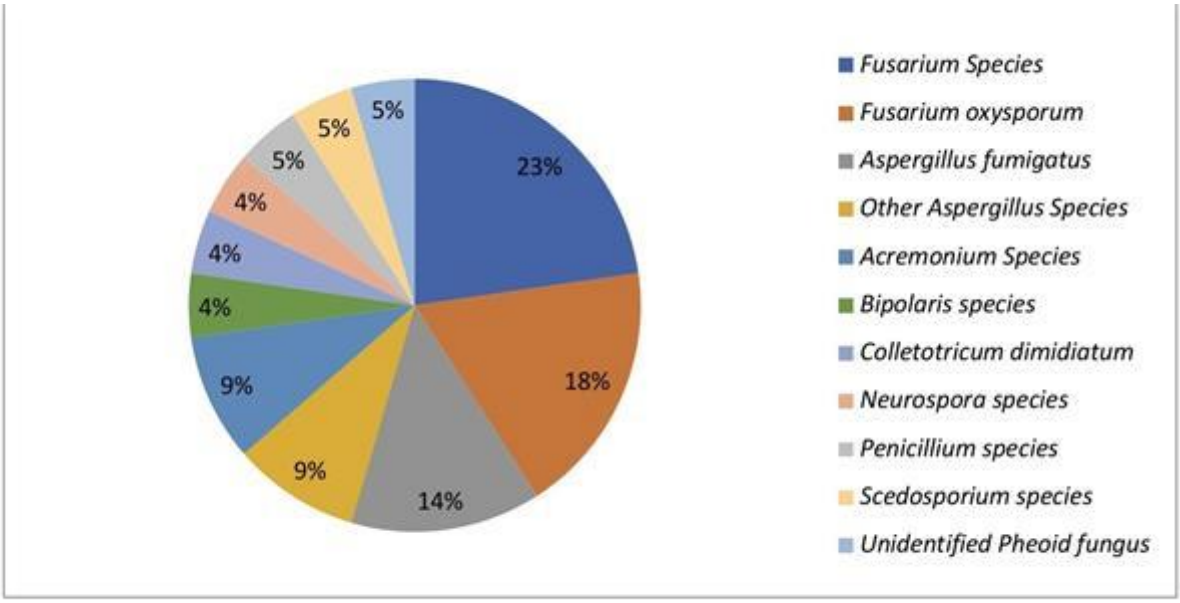
Among ulcer characteristics of suspected keratitis cases, typical fungal corneal ulcer with feathery margins (Figure 1 B) was present in only 30% cases, followed by hypopyon in 13% cases and corneal opacity with satellite lesions in 10% cases.

In our study, we observed relatively more KOH positivity (44%) than culture positivity (37.28%). This was due to prior usage of topical antifungal eyedrops (e.g. Natamycin, and Voriconazole) before specimen collection and also due to insufficient sample collection. Most common fungal pathogen isolated was *Fusarium oxysporum* (23%) and other *Fusarium* species (18%), followed by *Aspergillus fumigatus* (14%) and other *Aspergillus* species (9%) and *Acremonium* species (9%). We isolated some rare fungal pathogens of keratitis like *Colletotrichum dimidiatum*, *Scedosporium* species and *Neurospora* species. (Figure 2) *Aspergillus* & *Fusarium* species are 100% susceptible to Voriconazole by E-test.

Conclusion:

In our region, fungal keratitis is predominant in adults from rural areas involved in agricultural activities. Since typical corneal ulcer suggestive of fungal aetiology is not always present, KOH mount and fungal culture is always necessary for identification and isolation of fungal pathogen. The regional information of common etiological agents and predisposing factors from our study can be helpful in starting an empirical therapy based on high degree of clinical suspicion and formulating preventive measures in a population at risk of fungal keratitis.


A
B


23%
18%
14%
9%
9%
4%
4%
4%
5%
5%
5%
Fusarium Species
Fusarium oxysporum
Aspergillus fumigatus
Other Aspergillus Species
Acremonium Species
Bipolaris species
Colletotricum dimidiatum
Neurospora species
Penicillium species
Scedosporium species
Unidentified Pheoid fungus

P212

Kodamaea ohmeri: an emerging pathogen of fungemia in Pakistan

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Objective:

Kodamaea ohmeri is an environmental yeast that may cause serious infections, particularly in immunocompromised patients leading to high mortality. *K. ohmeri* has been linked to outbreaks in neonatal intensive care units. Our laboratory noticed increased isolation of *K. ohmeri* fungemia, from September 2022-March 2023.

Materials and Methods:

This study was performed at the Aga Khan University laboratory, Karachi, Pakistan that receives specimens across Pakistan. Yeast isolated from blood cultures was Identified using VITEK 2 YST ID card system and susceptibility testing was performed with YeastOne Sensititre YO10. Multiplex sequencing library preparation was performed on reconstituted specimens using PlexWell 24 kits (seqWell, MA USA) and sequencing performed on the NovaSeq platform (Illumina, CA USA) to generate paired-end 150 bp reads. Sequence reads were quality trimmed using fastp v0.23.2 and assembled using SPAdes v3.15.4. Sequence reads were aligned to reference genome sequence 148 (GCA_004919595.1) using bwa v0.7.15 and single nucleotide variants were called using bcftools v1.9. Whole genome phylogenetic analysis was performed using IQ-TREE 2.2.0.

Results:

A total of twenty cases were identified, of which 16 were from two neonatal units from same city in Punjab, three isolates were from Karachi and one from Badin. Seven specimens had concomitant growth

of either bacteria or other *Candida* species. All patients were children aged <7 years of age (14 neonates, 4 infants, 2 children). Fluconazole MICs ranged from 2-64 ug/mL, MIC_{50} was 4 ug/mL and MIC_{90} was 8 ug/mL, whereas voriconazole MICs ranged from 0.015-0.5 ug/mL, MIC_{50} was 0.06 ug/mL and MIC_{90} was 0.12 ug/mL. MICs for amphotericin ranged from 0.12-0.25 ug/mL with MIC_{50} and MIC_{90} of 0.12 ug/mL. Although caspofungin MICs ranged from 0.06->8 ug/mL with MIC_{50} of 1ug/mL and MIC_{90} of >8 ug/mL but MIC ranges for anidulafungin and micafungin were 0.5-1 ug/mL and 0.25-1 ug/mL respectively. After WGS, four isolate sequences were excluded for low read coverage. One isolate sequence was found to be from a mixed culture of *K. ohmeri* and *Pichia kudriavzevii* and excluded. Whole genome phylogenetic analysis of the remaining 8 isolate sequences grouped the isolates into 2 major clades consisting of 6 and 2 isolates, respectively. Isolates differed by an average of 9 pairwise SNVs (range 3 – 14) within clades, but by an average of 53,538 SNVs (range 49,318 – 59,720) between clades.

Conclusion:

The emergence of sporadic cases and outbreaks of *K. ohmeri* emphasize the need to investigate the factors contributing to its occurrence and to develop prevention and management strategies. Higher fluconazole MICs are concerning and mandate use of amphotericin or echinocandins empirically. WGS can be used to reveal the clonal structure of *K. ohmeri* isolates in a region.

P213

First case report of Talaromycosis in an HIV patient living in Pakistan

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Objectives:
To describe a case report of talaromycosis in a human immunodeficiency virus (HIV) infected patient who was living in Pakistan.
Talaromycosis (formerly Penicilliosis) is caused by *Talaromyces marneffei* (formerly *Penicillium marneffei*), a dimorphic fungus. *T. marneffei* affects predominantly immunocompromised patients, especially people living with HIV. *T. marneffei* causes deep seated infections which may be localized or disseminated. It may also infect immunocompetent individuals, but less frequently, who get exposed to it either because they live in or travel to endemic areas. It is found in many Souteast Asian countries and cases have been reported from China, Northeast India, Thailand, Hong Kong, Vietnam and Taiwan.

Materials & Methods:
It was a retrospective review of a case of talaromycosis who presented to Aga Khan University Hospital, Karachi, Pakistan. Case was initially identified during laboratory reporting of patient's bronchoalveolar lavage (BAL) specimen and then it was followed up with the treating physician.

Results:
This was a 32-year-old male patient who travelled from China to Pakistan for employment. There were no known co-morbidities when he presented to our hospital's emergency department with chronic diarrhea, cough and fever. Following initial fluid resuscitation, laboratory tests were sent. His chest X-ray revealed scattered reticular infiltrates in bilateral lung fields, suggestive of bilateral pulmonary infection. His HIV test results came positive and his absolute count of CD4+ T-helper lymphocytes was found to be 7 (normal range 492-2014). He underwent bronchoscopy and a bronchoalveolar lavage specimen was obtained and sent to laboratory. Bronchoalveolar lavage fluid was positive for *Pneumocystis jirovecii* by PCR. He was also found to have cytomegalovirus viremia detected by PCR. No significant bacteria were found on culture of bronchoalveolar fluid.
He was managed accordingly and discharged after four days to be followed up in clinic. However, he was lost to follow-up.
Colonies of mold started growing on SDA incubated at 25°C, while SDA incubated at 37°C showed yeast like growth. After a week, a deep red pigment started diffusing from growing colonies of fungus. Lactopheonol cotton blue preparation from SDA at 25°C revealed septate hyphae and structures typical of *penicillium*. A successful mold to yeast conversion was performed and fungus was identified as *Talaromyces marneffei*. It was confirmed by sequencing of Internal Transcribed Spacer (ITS) region as *T. marneffei*.

Conclusions:
This describes first case report of *T. marneffei* infection in an HIV-positive patient living in Pakistan.

P214

INCIDENCE OF CANDIDA AURIS CANDIDEMIA IN COLONIZED PATIENTS IN A TERTIARY HOSPITAL OF ATHENS

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Objectives:

Candida auris is regarded as a critical nosocomial pathogen due to its long persistence in healthcare environments and inter-patient spreading. Moreover, *C. auris* infections provide a noteworthy challenge for healthcare professionals because of their adverse resistance profile and the potential high mortality rate. The aim of the study was to assess the cumulative incidence of *C. auris* candidemia in colonized patients.

Materials & Methods:

We conducted a retrospective study including 102 patients admitted to our hospital, with isolation of *C. auris* in any non- sterile body site – skin (combined axilla and groin skin swab), urine and respiratory tract specimens - between January 2021 and April 2023. Colonization with *C. auris* was defined by the isolation of *C. auris* from at least one of the above sites in the absence of clinical signs or symptoms of infection. Specimens were cultured on mycological media such as Sabouraud dextrose agar (SDA) and chromogenic media (Brilliance Candida agar, Oxoid, Thermo Scientific, UK) in an optimum growth temperature of 42 ^{o}C.

C. auris was identified in clinical and screening samples with Vitek II, Biofire® Filmarray® system (bioMerieux, Marcy l'Etoile, France) and eazyplex® *Candida auris* (Amplex Diagnostics GmbH, Germany). Antifungal susceptibility testing was carried out by broth microdilution method (BMD), using the Micronaut-AM (Bruker Daltonics, Germany). Minimum inhibitory concentration (MIC) values were determined for azoles, echinocandins, and amphotericin B. Since no species-specific susceptibility breakpoints are currently available for *C. auris*, results were interpreted according to the tentative breakpoints proposed by the US Centers for Disease Control and Prevention.

Results:

Out of 102 patients colonized with *C. auris*, the quota among male/female was 62(60,7%)/40(39.3%), respectively. The clinical features of the study population are summarized in Table 1. Overall, 15 patients (14,7%) developed *C. auris* candidemia. All *C. auris* blood isolates were resistant to fluconazole and susceptible to echinocandins, while one isolate (6,7%) was resistant to amphotericin B.

Conclusions:

Candida auris candidemia may occur approximately one in seven colonized patients. Multisite colonization is the most common. The higher incidence of colonization was observed in Internal Medicine and Neurosurgical departments. Multisite colonization appears to be a significant risk factor for the development of candidemia.

These findings show that *C. auris* colonization can occur without infection, and those patients can serve as reservoir for nosocomial transmission. Furthermore, it is crucial for routine laboratories to provide rapid and accurate diagnostic services in order to detect *C. auris* in clinical specimens.

Therefore, understanding the local epidemiology and transmission patterns is essential for establishing effective infection control strategies to prevent *C. auris* colonization and, hence, infection.

Site of *C. auris* colonization	No. of patients (%) (Total = 102)
Urinary colonization	55 (53,9%)
Skin colonization	34 (33,3%)
Respiratory colonization	22 (21,6%)
Multisite colonization	60 (58,8%)
Department	
Internal Medicine	45 (44,1%)
Neurosurgery	30 (29,4%)
Intensive Care Unit	19 (18,6%)
Other Surgical Departments	6 (5,9%)
Correlation between *C. auris* candidemia and its origin	No. of candidemia (%) (Total = 15)
Urinary colonization	10 (66,7%)
Skin colonization	7 (46,7%)
Respiratory colonization	3 (20,0%)
Multisite colonization	12 (80,0%)

P215

Antifungal susceptibility, biofilm forming and genetic features among Candida auris isolated from blood in patients with candidemia

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Objectives: The objective of this study was to estimate the *in vitro* antifungal susceptibility, biofilm formation and genetic features among *Candida auris* isolated from blood in patients with candidemia.

Materials & Methods: In the prospective multicenter study, we analyzed bloodstream isolates of *C. auris* (N=23) from patients with candidemia during the period from 2018 to 2022. All patients were in the ICU at the time of the first isolation of the fungus from blood. First *C. auris* obtained from blood were included in the study and were subsequently transferred to the reference center - The Department of Microbiology and Antimicrobial Therapy of the National Medical Research Center for Hematology (Moscow). All isolates were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany). The minimum inhibitory concentrations (MICs) of anidulafungin, caspofungin, fluconazole and conventional amphotericin B were assessed using broth microdilution method according to Clinical and Laboratory Standards Institute methodology (CLSI M27M44S, 2022) with RPMI-1640 broth medium (Sigma Aldrich, USA). All isolates were classified as susceptible and resistant to each antifungal drug according Centers for Disease Control and Prevention technique (CDC, 2020). Quality control strains (*C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 90028) were included in each experiment. Biofilm forming of *C. auris* was assessed with XTT assay. *C. auris* were classified as biofilm-forming if the optical density was ≥ 0.1 and non-biofilm-forming if optical density was < 0.1. Sanger sequencing of rDNA loci (D1/D2 and ITS regions) was used to differentiate between the major phylogeographic clades of *C. auris*. To investigate azole and amphotericin B resistance, *ERG11* and *ERG6* genes sequencing was conducted, respectively. Sequence analysis was carried using Clustal W/Mega software and Phylogenetic tree was constructed. A boot value of 1000 was used for constructing tree by neighbor joining method.

Results: A total of 23 *C. auris* were evaluated. Antifungal MICs are shown in **Table**. Based on the tentative CDC MIC breakpoints for fluconazole (≥ 32 mg/L), amphotericin B (≥ 2 mg/L), caspofungin (≥ 2 mg/L) and anidulafungin (≥ 4 mg/L), 100% of isolates were fluconazole resistant (MICs ≥ 64 mg/l) and 30.4% (7/23) were resistant to amphotericin B (MICs = 4 mg/L).

All strains were susceptible to anidulafungin (MICs 0.007-1 mg/l) and caspofungin (MICs 0.007-1 mg/l). *ERG11* sequences exhibited amino acid substitution K143R in 100% of strains, whereas no mutations in *ERG6* of amphotericin B resistant isolates were found. All *C. auris* had identical nucleotide sequences in ITS and D1-D2 regions and clustered with strains of South Asian clade I (**Fig.**). Biofilm production was observed in 100% *C. auris*.

Conclusions: All *C. auris* in ICU patients were resistant to fluconazole, and 30.4% of them were multidrug-resistant strains as they were additionally resistant to amphotericin B. Translated *ERG11* sequences harbored K143R amino acid substitutions, which mediates resistance to fluconazol. The phylogenetic analysis of ITS and D1-D2 regions revealed clustering of studied strains with isolates of South Asian clade I lineage. All *C. auris* were biofilm-forming. The data obtained reveal spread of *C. auris* belonging to clade I in Russia.

Table. Antifungal susceptibility of *C. auris* clinical isolates

Antifungal	Susceptible, n (%)	Resistant, n (%)	MIC_{50}	MIC_{90}
Fluconazol	0	23 (100)	128	128
Voriconazol	0	23 (100)	64	64
Caspofungin	23 (100)	0	0.032	1
Anidulafungin	23 (100)	0	0.032	1
Amphotericin B	16 (69.6)	7 (30.4)	1	4

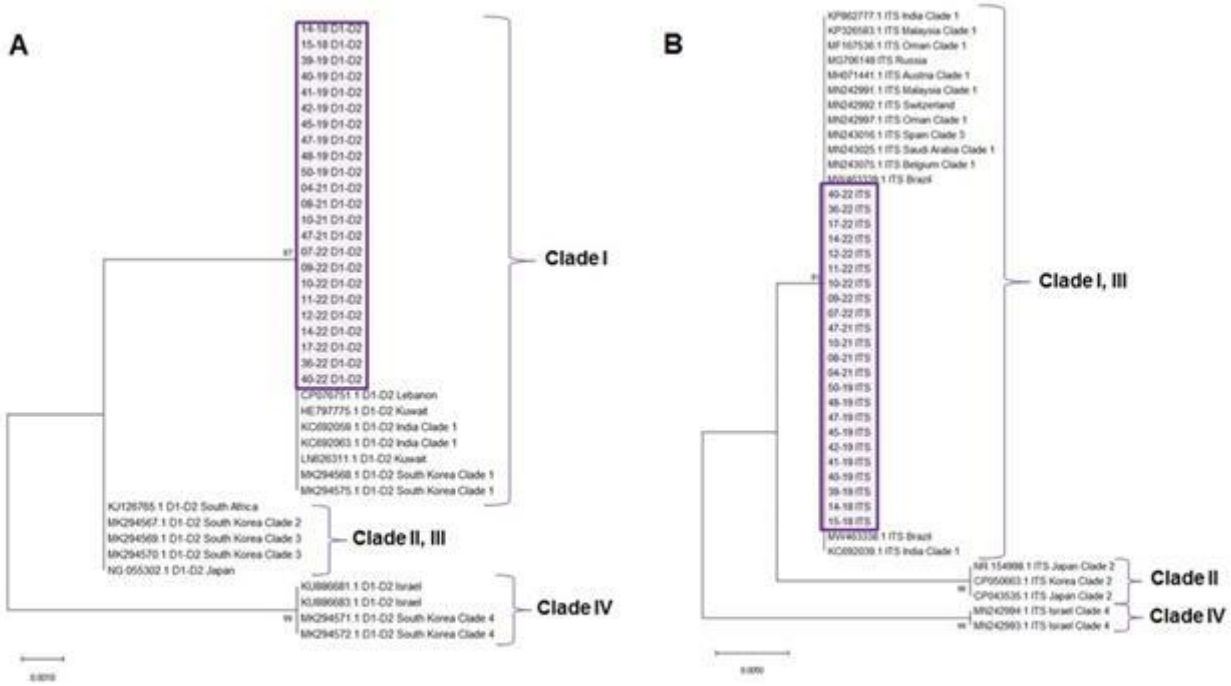

Fig. Evolutionary relationships based on the (A) ITS and (B) D1/D2 nucleotide sequences of investigated *C. auris* isolates (purple box) and other strains belonging to clades I to IV

P216

An emerging species of Cryptococcus gattii sensu lato in the American Southwest: C. decagattii (VGVI)

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Objectives
We investigate the apparent emergence of *Cryptococcus gattii* VGVI (aka *C. decagattii*) in the American southwest following the detection of multiple human cases infected with this rarely described species. Four major molecular types, or species, of *C. gattii* sensu lato (s.l.), referred to as VGI-VGIV, had been identified in tropical, semi-tropical and temperate regions around the globe, and a fifth, VGV, has been described only in sub-Saharan Africa. Here we report the likely endemic presence of a sixth species/molecular type, VGVI, following its identification associated with human and feline cryptococcosis cases.

Methods
C. gattii s.l. isolates were collected from three confirmed cryptococcosis patients in Arizona, where *C. gattii* s.l. was not previously known to be endemic. Additional active surveillance and investigations identified one historical veterinary *C. gattii* sample (2012) and two recent *C. gattii* infected cats (2023). A fourth *C. gattii* infected cat from 2019 had no isolate available for sequencing. Sequencing was conducted on three human clinical isolates and three veterinary isolates collected in Arizona from 2012 to 2023. Phylogenomic analyses were conducted on the sequenced genomes, along with the three previously known VGVI genomes, two from Mexico cases patients with uncertain clinical history, reportedly isolated in 1961 and 1965, and a third from a Mexican patient isolated in Spain in 1987. A single 2017 VGVI isolate from Argentina was also sequenced. The NASP mutation analysis pipeline provided phylogenetic high-confidence variant data used with the R package Phangorn to produce Maximum parsimony inference trees. FineStructure was used to study the genetic composition of the group in relation to other molecular types.

Results
All Arizona *C. gattii* VGVI genomes were genetically related and grouped with historical Mexican isolates which were termed *C. decagatii* and later as VGVI, establishing VGVI as a clinically important species/molecular type, from the Arizona/Mexico region. No epidemiologically useful information was available for the three clinical cases beyond that they all had with limited to no travel history and lived in central or southern AZ, typified by arid Sonoran Desert biome, that can experience extreme heat conditions (>45C). All four cats were also from southern AZ, and reported no travel. Local specific ecological and animal exposure investigation is ongoing. The VGVI lineage appeared to be most closely related to, and shared the most gene content with the VGIII lineage. The three Mexican VGVI genomes appeared to be clonal being separated by an average of 105 SNPs. Conversely, all AZ VGVI genomes were separated by approximately 100K SNP mutations, demonstrating a deep evolutionary history, establishing a likely long-term, cryptic presence in the region.

Conclusions
The previously proposed *C. decagattii* species (VGVI), although only originally based on three historical lab isolates, is confirmed to be a distinct species within *C. gattii* s.l., is causing human and veterinary infections in the American southwest, and appears to be inhabiting a novel habitat niche that is uncharacteristic of other *C. gattii* s.l. locales in the Western Hemisphere.

P217

Invasive Infection due to Trichosporon asahii, Associated Risk Factors And Outcomes In Tertiary Care Center

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Objectives: Trichosporon species are found widely in nature, *Trichosporon asahii* is the most common pathogenic species among invasive Trichosporon yeast infection and it can cause invasive fatal trichosporonosis, especially in immunocompromised hosts. The objective of this study is to access the risk factors and outcome due to invasive *Trichosporon asahii* infection.

Materials & Methods: : This was a prospective study. All patients with a culture positive for Trichosporon species from blood and percutaneous urine by nephrostomy samples were included in the study. Routine Mycology work up done like microscopy, culture and phenotypic identification was done and the Trichosporon species were confirmed by MALDI-TOF-MS (BioMerieux).

Results: A total of 19 *Trichosporon asahii* isolates were found during the study; 68.4% (n=13) were hospital acquired infections. We accessed the risk factors and found that 63.15% (n=12) had renal disease and 68.4% (n=13) had undergone surgical intervention. Mortality was seen in 15.7% (n=3) patients; these were patients with invasive Trichosporon blood stream infection with 100% mortality. The other patients recovered from the infection and are on regular follow up.

Conclusions: *Trichosporon asahii* are known to cause invasive fatal infections, urinary trac infections etc. In this study it was documented in patients with associated risk factors mostly those with kidney diseases and post-surgery. Hospital acquired infection are common with Trichosporon species and this agent is known to be resistant to echinocandins, with reported with breakthrough infection in patient treated with caspofungin and micafungin. The drug of choice is triazole; voriconazole therefore, clinicians should be aware of this infection which is known to cause fatal invasive blood stream infection.

P218

Emergence of phaeohyphomycosis due to Cladophialophora bantiana in France

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Objectives To describe autochthonous cases of *Cladophialophora bantiana* infections and its recent trends in France

Methods and Materials
All cases were diagnosed and/or confirmed at the National Reference Center (NRCMA) between June 2004 and June 2023. Case report forms were completed in each center with missing data collected at the NRCMA. Brain images available were centralized for unified reading. The number of cases was connected to the average annual outdoor temperature recorded in France.

Results
There were 23 patients included in the study, median age was 60 years. Every year until 2021, the number of notified cases was between 0 and 2. In contrast, there were 7 cases notified in 2022-mid 2023 which was the hottest period ever recorded since 1900 in France; half of patients were diagnosed in the southern part of France; a much hotter area. Cerebral involvement was observed in 15/23 (65%) patients (including 3 cases with dissemination); skin and soft tissue in addition to osteoarticular infections in 7/23 (30%) and isolated lung infection in 1 case. Any immunodepression was observed in 17 cases and diabetes in 4. When considering patients with cerebral involvement, 4 had major neurosurgical procedures including resection and drainage in 2 cases each. Three out of the latter survived. Of note, 11/15 (73.3%) were not operated or underwent only diagnostic surgery (8/11 died before month 9). Patients treated according to NRCMA recommendations consisting in liposomal amphotericin B + posaconazole and flucytosine versus any other strategy, had higher survival.
When considering the 7 patients with non-disseminated skin and soft tissue involvement, 3 had major surgical procedures, 4 were not operated and 6/7 survived.

Conclusion
To the best of our knowledge, we report the largest series of original cases of *Cladophialophora bantiana* infections at a country level. The incidence increases in France with a parallelism with average annual outdoor temperature (Global Health). We also add controversies when comparing our data and the following items reported in the literature: clinical presentation and underlying medical conditions are diverse with 65% of brain involvement; most of the patients were aged with any immunodeficiency; prognosis is more favorable when a triple antifungal therapy is administered and finally again contrasting with prior data reported by others, only few patients were managed with a surgical approach.

P219

Oral histoplasmosis in an elderly immunocompetent male patient - A commonly missed out diagnosis by dental surgeon.

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Objectives:

Histoplasmosis is caused by a dimorphic fungus i.e. *Histoplasma capsulatum.* It is rare disease as occasionally encountered in the hospital setting, however, out of all dimorphic fungi, it is the commonest one in India. Invariably it's causative organism is confused histopathologically with other diseases like toxoplasmosis, leishmaniasis, talaromycosis, Emergomycosis and cryptococcosis. The diagnosis is often missed in non-endemic regions like India due to lack of suspicion especially when it involves unusual sites like oral cavity and that too in individuals without any immunocompromising background. To increase awareness among medical/surgical staff of this group of emerging infections and to emphasize importance of an early diagnosis and treatment of oral histoplasmosis, we report a rare case of oral histoplasmosis with ulcerative lesion over the labial mucosa. The patient is a retired elderly male, resident of Panchkula (Haryana) in northern India. He presented with chief complaint of gradual bleeding over the gums and labial mucosa on touch. The detailed history of the patient was taken, clinical presentation, underlying illness and risk factor were noted. He is patient of controlled diabetes mellites and hypertension and there is no other significant underlying disease.

Materials & Methods:

A 74-year-old male patient presented to the Dental Clinic with ulcerative lesions in the oral cavity for last eight months. It was accompanied by bleeding on touching the local areas. On examination, there were multiple ulcerative lesions over both the lips. There was a diffuse ulceration on the upper labial mucosa. The right palatal mucosa was mildly tender on palpation with tendency to bleed profusely. The cytology of the lesion was inconclusive. **The** biopsy tissue taken as incisional / upper labial mucosa of soft tissue section exhibited surface stratified squamous epithelium along with chronically inflamed connective tissue. Ulcerated surfaces were noticed in few areas. The connective tissue showed immense lymphohistiocytic infiltration along with plasma cells. Russell's bodies were also noticed.

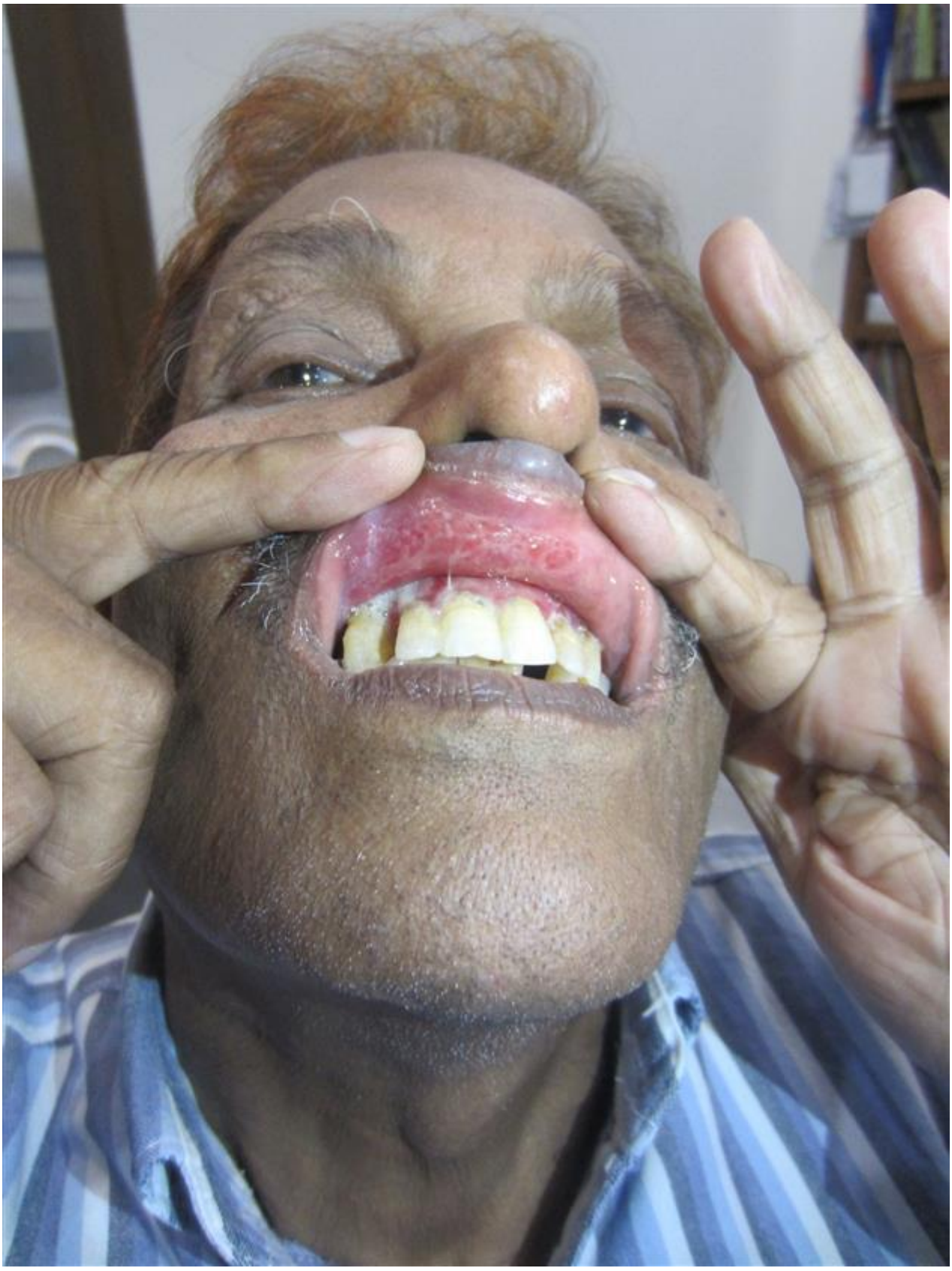
Results:

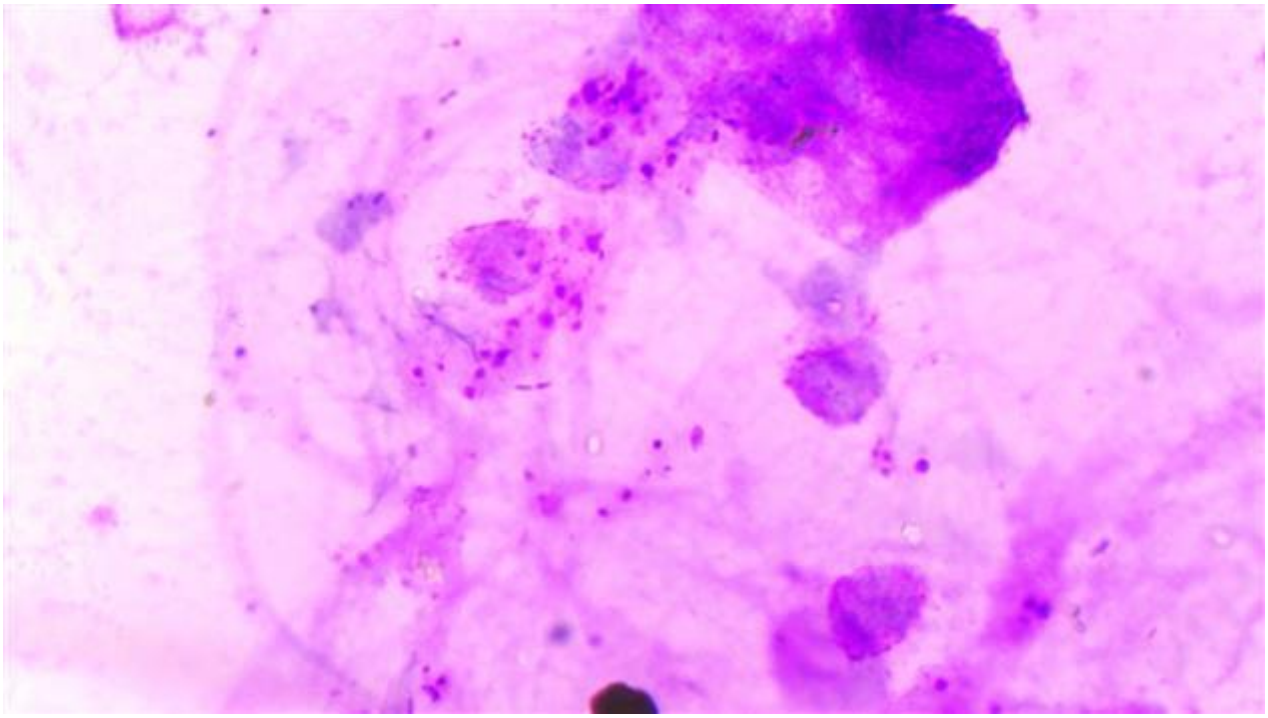
There were collections of histiocytes with intracellular and extracellular small bodies were noted. Some of these stained with PAS stain correlating clinically with the histopathology of lesions suggestive of oral histoplasmosis. Direct microscopic examination revealed budding yeast cells measuring 2-4 µm in size in KOH/CFW wet mount as well as histopathology of stained sections with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stainings. The diagnosis of oral histoplasmosis was made. Fungal cultures were put up on standard fungal culture media like Sabouraud dextrose agar and brain heart infusion agar for isolation and further species identification at 37°and 25°C, the growth is still awaited. His HIV status was negative. He was put on itraconazole 200 mg twice a day. He got improved in two weeks' duration and recently came for follow up and was fine.

Conclusions:

The present case highlights a rare presentation of histoplasmosis that mimicked oral malignancy. Hence a high index of suspicion both at the dental surgeon/clinician and microbiologist end is prerequisite for timely detection of rare fungal infection. There is an urgent need for keeping high

index of clinical suspicion of histoplasmosis thereby taking early FNAC and/or biopsy of affected site so that benefits of prompt diagnosis and appropriate therapy may be initiated. Such type of ulcerative lesions are invariably found as oral histoplasmosis. To avoid further aggression of the disease, the key is the establishment of an early diagnosis and prompt antifungal therapy.





P220

CNS blastomycosis: The first reported case in a Kuwaiti graduate student from America.

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Objective

Blastomycosis is a systemic fungal disease endemic in North America. Clinical presentation ranges from asymptomatic illness to severe disseminated disease. It can involve lungs, skin, bone and central nervous system. Because infection is geographically restricted, many clinicians may not be familiar with the disease. Here we describe the first case of Blastomycosis in Kuwait.

Case summary

A 24 years old Kuwaiti man was admitted with a history of generalized seizure, few months after returning from Tennessee, USA, for graduate study. During his hospital stay, MRI brain showed left round shaped temporal occipital lesion. Albendazole, steroids and Phenytoin were started on assumption of neurocysticercosis. However, serology was negative. The patient had travelled to France for further management. As routine microbiology and radiology investigations were not conclusive, elective craniotomy was performed and a stereotactic brain biopsy was taken. Macroscopically, the lesion looked more like an abscess. The whole lesion was removed without bleeding and a pus sample was sent to microbiology laboratory. Culture grew a mould which was identified as *Blastomyces dermatitidis* by gene sequencing. The patient was started on voriconazole 300mg BD. As a result, the patient had improved clinically and radiologically. He completed the course in Kuwait and voriconazole trough levels were closely monitored. Treatment was continued for 12 months with good resolution of brain lesions.

Conclusions

CNS blastomycosis is a life-threatening disease. Diagnosis requires high degree of suspicion with great emphasis on travel history to endemic areas. CNS lesions should be aspirated to perform microbiological and mycological investigations.

P221

Multiple forms of Histoplasmosis in Immunocompetent patients from Delhi and around Delhi

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Objectives: Invasive mycoses kill more than one million people every year. The disease burden of mycoses has increased over the last 20 years and the importance of fungal disease is expected to keep increasing in the years to come. One of these mycoses, Histoplasmosis, a disease caused by species of dimorphic genera, *Histoplasma* and is endemic in USA, Asia, and Africa with sporadic cases reported from India. In the United States alone, 3.4 cases per 100,000 people occur yearly. In India, prevalence of histoplasmosis is seen in eastern part of the country and few cases are reported from north India. Here we present four cases of classic progressive disseminated histoplasmosis from non-endemic central and northern part of India.

Material & Methods

Cases: Two cases, firstly 51-year-old male resident of Delhi, came with complains of fever, generalized weakness and weight loss for past 6 months and another 55-year-old in an apparently Immunocompetent female from a non-endemic state in central India. The disease was diagnosed by bone marrow aspirate incidentally. Another cases, like one 25-year-old female and a pediatric patient, with chief complaints of multiple skins colored swellings on her face, neck, chest, and back and also on both limbs. Physical examination revealed multiple skin coloured papules and nodules coalescing to form large plaques on face, neck, upper back, chest which had overlying yellowish-brown crusting. Entire face was involved with depression of nasal bridge and madarosis. Multiple discrete 0.1 to 0.5 mm papules and nodules on bilateral upper and lower limbs. Patient started on the treatment of cutaneous Histoplasmosis started with Inj. Liposomal Amphotericin –B 100 mg Intravenous (around 3 mg/kg/day) after pre-medication for 10 days. For both the cases, capsule Itraconazole 200 mg BD started for 4 days. Patient responded well to the treatment and follow up showed improvement in the condition of the patient.

Results

In all the cases, fine needle aspirate cytology of the lesions showed plenty of inflammatory cells and macrophages in pathological microscopic examination. Microscopic examination of skin tissue revealed that macrophages comprising of yeast forms of *Histoplasma* having pericellular halo around. Moreover, on long incubation of biopsy sample on SDA media plate at 27˚C, white/buff colonies with yellow tan on back were observed. Furthermore, microscopic examination of grown fungal culture showed mycelial septate hyphae bearing round to pear shaped, smooth-walled broadly elliptical microconidia or tuberculate macroconidia. In both the cases clinical suspicion, histopathological and mycological findings (microscopy, culture and post culture sequencing) leads to a confirmatory diagnosis of progressive disseminated Histoplasmosis by *H. capsulatum var. capsulatum* was made.

Conclusions

Three of these cases are of progressive disseminated histoplasmosis in an apparently immunocompetent patient from non-endemic state of north India, On contrary one of the case was HIV positive with mucocutaneous lesions. Disseminated Histoplasmosis in Immunocompetent Individuals is not a so rare entity, in India. High clinical suspicion and awareness regarding the pathogen is required. From the point of good patient care, an accurate diagnosis and timely management in cases of Histoplasmosis is warranted.

TIMM
Trends in Medical Mycology

P222

Disseminated Histoplasmosis in an immunocompetent lady. The first reported case in Kuwait.

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Objectives:
Histoplasmoasis is a fungal disease caused by *Histoplasma capsulatum*. It is AIDS defining illness, where it causes severe infection, but it can also infect healthy individuals though with mild disease. Histoplasmosis was once thought to be geographically restricted to America. With increased interest in epidemiology of fungal diseases and more advanced diagnostics, it became obvious that histoplasmosis is a global fungal disease. Some challenges that mask diagnosis include lack of awareness, clinical similarity with other common infectious diseases such as tuberculosis, and lastly technical difficulties in laboratory diagnosis of *Histoplasma*. Here, we describe, for the first time in Kuwait, a case of disseminated histoplasmosis in an immunocompetent individual.

Case description
A 52year old Bangladeshi lady had developed multiple head and neck swellings for 3 months duration, associated with fever, and weigh loss. A fine needle aspirate from cervical lymph node revealed granulomatous lymphadenitis. However, pus culture did not show any bacterial or mycobacterial growth. Other laboratory investigations showed marked eosinophilia and high Ig E. Despite being empirically treated with 1st line anti-TB drugs, her swellings became deeply ulcerated and the scalp bone was slightly exposed. A new pus sample was sent to mycology reference laboratory, and subsequently, there was a growth in cultures after 10 days incubation, which was later identified as *Histoplasma capsulatum var. capsulatum*, based on typical microscopic morphology, confirmed by PCR-sequencing of rDNA. An MRI brain has showed multiple parenchymal abscesses. In addition, PET/CT scan has shown widespread bone involvement including skull, femur, ribs, iliac spine, and humeral head. Accordingly, patient received L-amphotericin B. As a result, she showed a remarkable improvement both clinically and radiologically. She was discharged home and continued he antifungal in form of oral voriconazole. On follow up visit, she was doing well and brain imaging revealed regressive course.

Conclusions:

Histoplasmosis is underrecognized fungal disease especially in our region. It can mimic common infectious diseases such as tuberculosis. Failure to diagnose such disease can be fatal. Awareness of the epidemiology of histoplasmosis and its global distribution, and direct communication with clinical microbiologists facilitate early diagnosis.

P224

In vitro synergy of amphotericin B and flucytosine against Talaromyces marneffei: Implications for combination therapy against an endemic mycosis

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Objectives:
Talaromyces marneffei (Tm), a fungus endemic to Southeast Asia, southern China, and northeastern India, causes talaromycosis, a leading HIV-associated opportunistic infection. Despite potent induction therapy with amphotericin B, mortality on therapy is still up to 30%. In a similar disease cryptococcal meningitis, combination therapy with flucytosine has been shown to enhance fungal clearance and reduce mortality compared to amphotericin B alone. The concept of combination therapy is supported by *in vitro* evidence of partial synergistic activity of these two drugs against *Cryptococcus*. While flucytosine is known to exhibit *in vitro* fungicidal activity against Tm, its potential synergy with amphotericin B has not been studied. Here, we investigate the potential of combination therapy for talaromycosis by evaluating the *in vitro* interaction between amphotericin B and flucytosine using our newly-developed, colorimetric antifungal susceptibility testing method for Tm.

Materials & Methods:
We used the checkerboard method to test the interaction between amphotericin B and flucytosine in 27 randomly-selected Tm isolates obtained from patients enrolled in the Itraconazole versus Amphotericin B for Talaromycosis (IVAP) trial. Our novel antifungal susceptibility testing method is based on Clinical and Laboratory Standards Institute (CLSI) guidelines for testing yeasts, but rather than rely on the subjective measure of visual turbidity, we use the fluorescent dye alamarBlue to accurately quantify inhibition of Tm growth. An inoculum of 1–5 x 10^3 CFU/mL Tm yeast was added to 96-well microplates prepared with two-fold serial dilutions of the drugs (0.016–1 µg/mL). *Candida krusei* ATCC 6258 was used as quality control in each experiment. The minimum inhibitory concentration (MIC) endpoint was defined as the lowest drug concentration that inhibits at least 95% of fungal growth. The fractional inhibitory concentration index (FICI) was calculated to characterize interactions between the drugs, with FICI ≤ 0.5 corresponding to full synergy, 0.5 < FICI ≤ 1 to partial synergy, 1 < FICI ≤ 4 to indifference, and FICI > 4 to antagonism.

Results:
The geometric means (GM) of the MICs of amphotericin B and flucytosine acting alone against 27 Tm strains were 0.41 µg/mL (range: 0.25–1) and 0.21 µg/mL (range: 0.06–1), respectively. The GM of the MICs in combination (0.15 µg/mL for amphotericin B; 0.07 µg/mL for flucytosine) were significantly lower than the MICs of the two drugs when tested alone (P < 0.0001 for amphotericin B; P = 0.0007 for flucytosine; Mann-Whitney test). Partial synergy between amphotericin B and flucytosine (i.e., at least two-fold reduction in MICs for each drug in combination) was observed in 21/27 (78%) strains while indifference was observed in the remaining 6/27 (22%) strains. No strains showed full synergy or antagonism.

Conclusions:
Our study provides the first evidence of partial synergy between amphotericin B and flucytosine against Tm. As flucytosine combination therapy with amphotericin B has been

shown to be safe and highly potent against cryptococcal meningitis, our study offers a strong rationale to test this combination therapy in talaromycosis clinical trials.

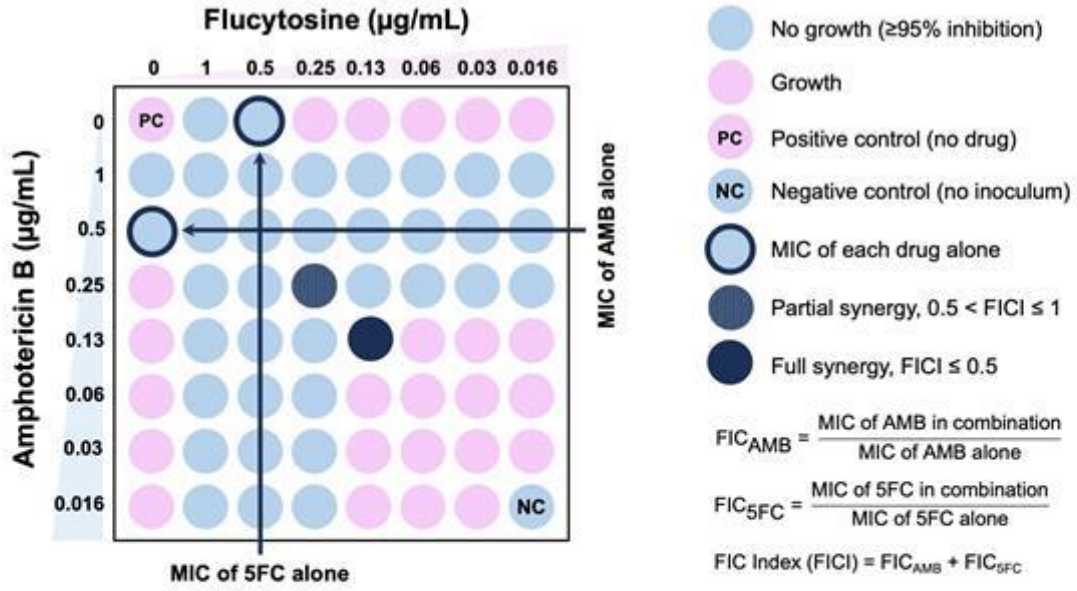

Figure 1. Layout of the checkerboard assay used to evaluate the interaction between amphotericin B and flucytosine against *Talaromyces marneffei*. The positive control well ("PC") contained no drug and allowed for uninhibited fungal growth. The negative control well ("NC") contained no inoculum. Wells showing partial and full synergy and the formulae for fractional inhibitory concentration (FIC) and FIC index (FICI) are also noted. Partial synergy is observed when the MICs of the drugs combined are at least one concentration (i.e., two-fold) lower than the MICs of the drugs alone. Full synergy is observed when the MICs of the drugs combined are at least two concentrations (i.e., four-fold) lower than the MICs of the drugs alone.

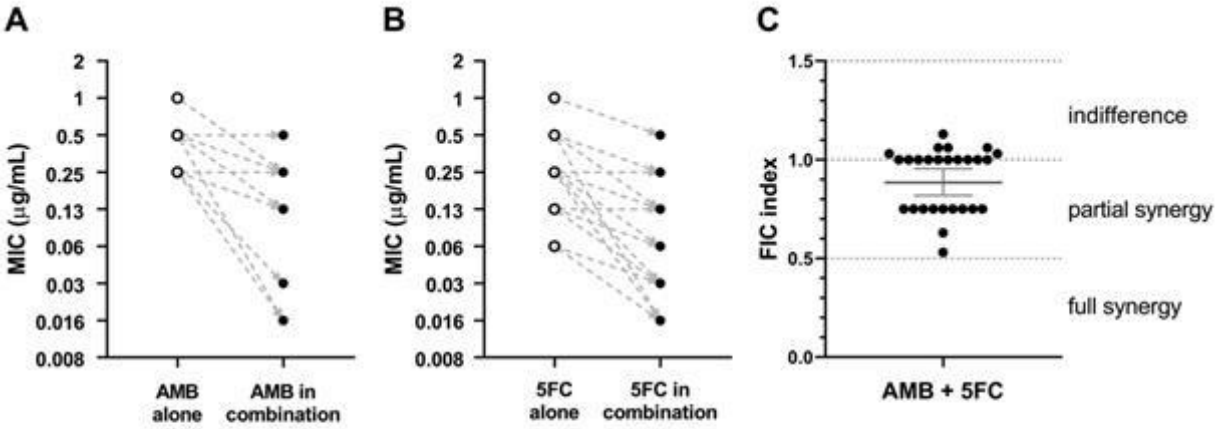

Figure 2. (A) Minimum inhibitory concentrations (MICs) of amphotericin B (AMB) tested alone and in combination with flucytosine (5FC) against 27 *Talaromyces marneffei* clinical isolates. Mann-Whitney test yielded $P < 0.0001$. **(B)** MICs of 5FC tested alone and in combination with AMB against 27 *T. marneffei* strains. Mann-Whitney test yielded $P = 0.0007$. **(C)** Fractional inhibitory concentration (FIC) index plotted for 27 *T. marneffei* strains treated with AMB-5FC combination. Geometric mean and error bars are noted.

P225

Improving the laboratorial diagnosis of endemic mycoses: a new RT-qPCR assay for the diagnosis of human sporotrichosis.

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Objectives: Sporotrichosis is an emergent fungal disease of public health concern in highly endemic countries. The conventional mycological diagnosis of sporotrichosis is based on culture, which is fastidious and may represent a biohazard for laboratory technicians. Furthermore, some clinical specimens have a low fungal burden and may present contaminants that may difficult *Sporothtrix* spp. growth in culture. Given the previously mentioned issues, molecular methodologies gain importance, as they are fast, accurate, and easily standardized, supporting the early diagnosis of this mycosis. The authors conducted a study to evaluate a new pan-*Sporothrix* quantitative reverse transcription PCR (RT-qPCR) assay, and then validated it on clinical samples from confirmed human sporotrichosis cases aiming to improve the laboratorial diagnosis of this neglected fungal diseases in endemic countries.
Materials & Methods: Molecular methods were designed and validated in the Mycology Laboratory, at Hôpital Saint-Louis, Paris, France. Sixty-eight human samples with culture-confirmed diagnostic of sporotrichosis were collected from 64 patients followed at a Brazilian reference center for infectious diseases and endemic mycoses. Whole nucleic acids extraction of the clinical specimens was performed, followed by the RT-qPCR protocol. The RT-qPCR targeted the mitochondrial small subunit ribosomal RNA (mtSSU) gene of *Sporothrix* sp. To assess the specificity of the RT-qPCR, DNA from 114 strains corresponding to 93 fungal species from the National Reference Center for Invasive Mycoses and Antifungals, Institut Pasteur, Paris, France, were analyzed in this study. The DNA from the three major clinically relevant species of the genus *Sporothrix*: *S. brasiliensis*, *S. schenckii*, and *S. globosa* were also tested. In addition, WNA from 25 clinical specimens collected from 22 patients diagnosed with other mycoses (histoplasmosis, n = 8; cryptococcosis, n = 6; aspergillosis, n = 5; fusariosis, n = 2; mucormycosis, n = 1) and tuberculosis (n = 3) were used as control samples. The data obtained in this study were analyzed using the Prism software version 9.0 (GraphPad, San Diego, California, USA).
Results: The assay herein evaluated presented a limit of detection of 244 fg, an efficiency of 1.919 (96%), and sucessefully amplified the genetic material of the three major clinically relevant species of the genus *Sporothrix*. Among the 68 samples analyzed, 62 were positive in RT-qPCR, showing an overall sensitivity of 91.18%, and varying according to the type of specimen: 96.72% in skin samples (n = 61), 100% in respiratory (n = 3), whereas all cerebrospinal fluid specimens (n = 4) were negative. The specificity of the assay was 100% when tested in 25 clinical samples of other mycoses (histoplasmosis, n = 8; cryptococcosis, n = 6; aspergillosis, n = 5; fusariosis, n = 2; mucormycosis, n=1), and tuberculosis (n=3). Furthermore, DNA from 93 fungal species did not yield positive results, confirming the high specificity of this test.
Conclusions: The RT-qPCR herein evaluated presented high sensitivity and specificity, representing an excellent tool for a fast and reliable diagnosis of human sporotrichosis.

P226

Evolution of Histoplasma fungal load by qPCR under treatment in HIV-patients with disseminated histoplasmosis under treatment with liposomal amphotericin B

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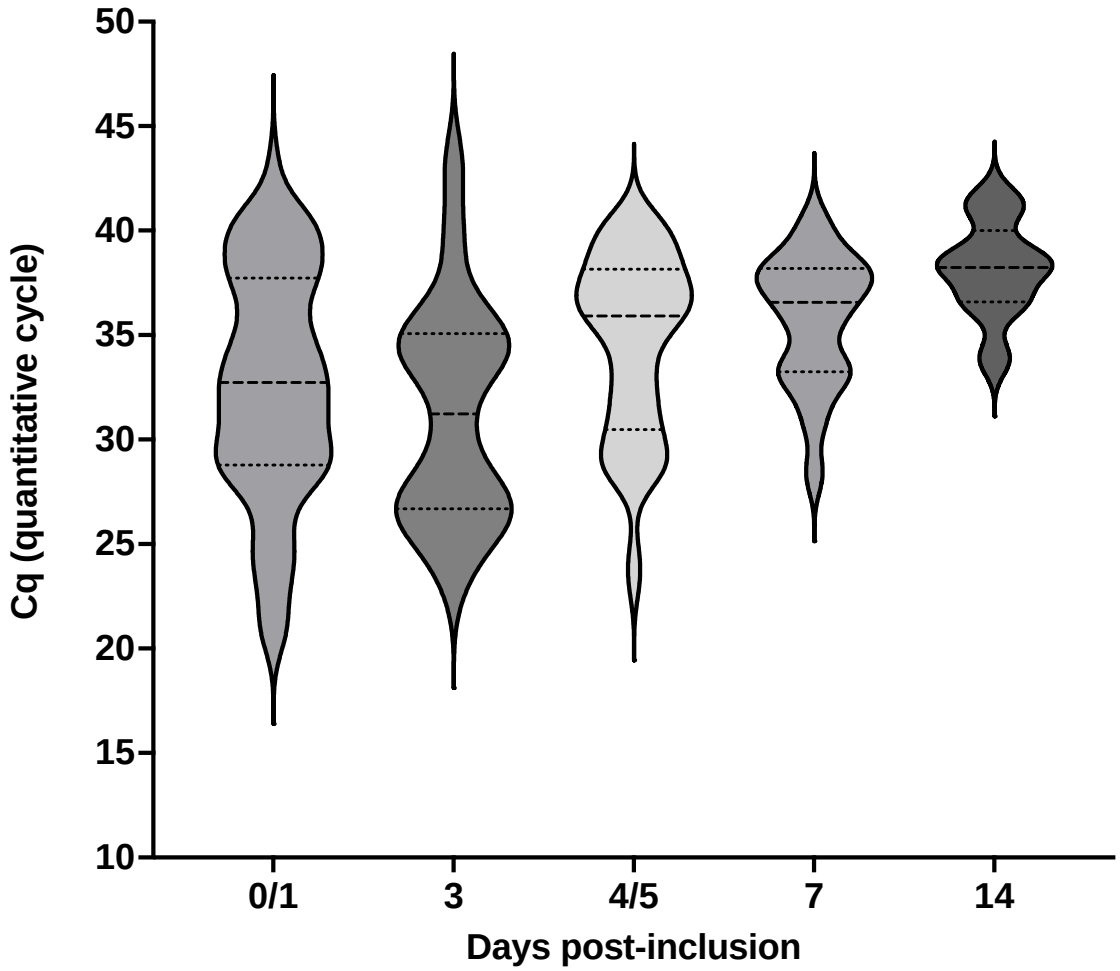
Objectives: Disseminated Histoplasmosis (DH) can affect patients with advanced HIV disease in regions where *Histoplasma capsulatum* is endemic. Quantitative PCR (qPCR) is an important tool that allows to determine the decrease in the *Histoplasma* load (HL) in patients under antifungal treatment. Only few centers perform qPCR for diagnosis. In this study, we aim to evaluate the behavior of HL in the blood of patients diagnosed with DH undergoing different liposomal amphotericin B regimens.
Methods and Materials: We obtained whole blood of 106 patients enrolled in a study that compared three different regimens of liposomal amphotericin B for DH treatment in HIV patients, cared in many centers across Brazil (Pasqualotto et al. CID 2023*). *Histoplasma* antigen titers were obtained in all patients at D0, D7 and D14. Blood was collected at D0/D1, D3, D4/D5, D7 and D14, frozen and stored until analysis. Nucleic acids were extracted from five hundred microliters of blood and qPCR was performed, as previously described (Alanio et al. JMolDiag 2021).
Results: 325 specimens were obtained and tested (98 at D0/D1, 34 at D3, 66 at D4/D5, 67 at D7, 60 at D14). The qPCR was positive at D0/D1 in 66.3% of the patients, but positivity increased to 84.6% in patients with pancytopenia, lung, skin and abdominal involvement; 15% were still positive at D14. The HL increased a little at D3 and then decreased overtime with median quantitative cycle (Cq) values of 32.7 at D0/D1; 31.2 at D3, 35.9 at D4/D5, 36.6 at D7 and 38.2 at D14. The initial HL correlated with the day qPCR became negative: higher initial HL was associated with a persistent qPCR positive at D7. Patients with initial negative qPCR had a median Ag titer of 6 ng/mL, as compared to those with low HL (Cq>30) [median Ag at 51.3 ng/mL], and with high HL (Cq<30) [median Ag at 110.2 ng/mL]. The initial fungal load was equivalent in all treatment arms, and an equivalent decrease in the % of qPCR-positive patients was observed in all treatment arms. No significant difference in the slope was observed among the 3 treatment arms. Death at first or second week was significantly associated with progression of the qPCR fungal load (negative slope). This association was not observed when analyzing antigen titers.
Conclusion: We found in this study a higher rate of qPCR positive patients associated with DH in the blood than in our validation cohort (66.2 vs. 43.1%). An increase of the fungal load overtime was associated with an increased risk of death at D7 or D14. This diagnostic tool should be evaluated prospectively to assess its usefulness in identifying patients with poorer prognosis.
* This was a collaborative study between the principal investigator (A. C. Pasqualotto) and Gilead Sciences. Gilead Sciences has provided researchers with the study drug, and has also given financial support to run the study.


Cq (quantitative cycle)
50
45
40
35
30
25
20
15
10
0/1
3
4/5
7
14
Days post-inclusion

P227

Atovaquone exposure, Pneumocystis jirovecii cytochrome b mutations and genotypes: French data and review of the literature

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Summary

Objectives. *Pneumocystis jirovecii* is a transmissible fungus responsible for severe pneumonia [*Pneumocystis* pneumonia (PCP)] in immunocompromised patients. Missense mutations due to atovaquone selective pressure have been identified on cytochrome b (*CYB*) gene of *P. jirovecii*. It was recently shown that atovaquone prophylaxis can lead to the selection of specific *CYB* mutants among organ transplant recipients. In this context, our objective was to assess the level of selective pressure exerted by atovaquone on *P. jirovecii* in France.
Methods & Materials. A total of 123 PCP patients (124 *P. jirovecii* specimens) from four metropolitan hospitals and two overseas hospitals were retrospectively enrolled. Fourteen patients had prior exposure to atovaquone whereas 109 patients did not, at the time of PCP diagnoses. A 638 base-pair fragment of the *CYB* gene of *P. jirovecii* was amplified and sequenced.
Results. A total of 10 SNPs was identified. Both missense mutations C431T (Ala144Val) and C823T (Leu275Phe) located at the active site of the enzyme, were significantly associated with prior atovaquone exposure ($P < 0.001$).
Conclusion. The aforementioned hospitals being representative of the national territory, the overall selective pressure exerted by atovaquone on *P. jirovecii* organisms in France remains low. Conversely, this selective pressure that leads to mutations in its target gene is common in PCP patients directly exposed to atovaquone.

P228

Healthcare burden of fungal infections in hospitalized patients

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Objectives:

Superficial and systemic mycoses represent a significant medical problem accounting for 10% of the total number of intrahospital infections. It is estimated that 25% of the global population suffers from superficial mycoses, while globally over 300 million people are afflicted with systemic mycoses. Fungal infections are responsible for >1.5 million deaths globally per year, primarily in those with compromised immune function. It has also been confirmed that invasive candidiasis and aspergillosis account for more than 90% of hospital-acquired fungal infections in adults and 60-65% in children. The aim of this study was to determine the prevalence of mycoses as well as the frequency of positive mycological findings of certain fungal isolates from different samples of hospitalized patients. Furtheremore, we aim to determine positivity rate of biomarker detection in suspected invasive fungal infections.

Materials & Methods:

In this retrospective study, adult and paediatric patients with positive mycological findings were included from June 1st, until December 1st 2019 hospitalized in University Clinical Centre Kragujevac. Various samples were taken from patients for the diagnosis of superficial and systemic mycoses, such as sputum, stool, skin and mucous membrane swabs, urine, blood, cerebrospinal fluid, aspirates, wound swabs, catheter swabs, etc. All samples were collected in accordance with the clinical diagnosis of the patient, in the acute phase of the disease and before the initiation of antimycotic therapy. All patient's data (clinical diagnosis, age, sex, department) and performed mycological analyses (specimen type, identified fungal genus/species, detected fungal biomarkers) come from the electronic medical records of the microbiology department of the University Clinical Centre Kragujevac. In order to diagnose the etiological agent of mycoses, conventional laboratory methods were used. Furthermore, to prove invasive fungal infections, serological methods were used for detection of *Candida* mannan antigen, and *Aspergillus* galactomannan antigen.

Results:

A total of 253 hospitalized patients were subjected for laboratory confirmation for suspected fungal infections. Out of them 102/253 (40.32%) were male, while 151/253 (59.68%) were female patients. The study included 234/253 (92.49%) adult and 19/253 (7.51%) paediatric patients younger than 18 years. Mean age of patients was 52,43, age range 0-89 years. In 176/253 (69.56%) patients laboratory findings confirmed fungal infection, while 77/253 (30.43%) had negative mycological result. Out of 176 patients with positive mycological result in 171/176 (97.16%) diagnosis was confirmed by conventional methods and isolation and identification of causative fungi. Among patients with positive fungal culture urine sample was found positive in 56/171 (32.75%), followed with positive stool 39/171 (22.81%), and sputum sample in 17/171 (9.94%). Majority of patients were positive for *Candida* spp. 165/171 (96.49%), while *Geotrichum candidum* was detected in 6/171 (3.51%). In 5/176 (2.84%) patients biomarkers were positive with 4 patients positive for *Candida* mannan antigen, while one patient was found to be positive on *Aspergillus* galactomannan antigen.

Conclusions:
The predominant fungal pathogen in our group of hospitalized patients was *Candida* spp. Identification of causative fungal species is necessary for determining the local epidemiology, and for optimising the antifungal stewardship for better patients' outcome especially in invasive mycoses.

P229

Malassezia infection in a scalp condition: About three zoonotic cases

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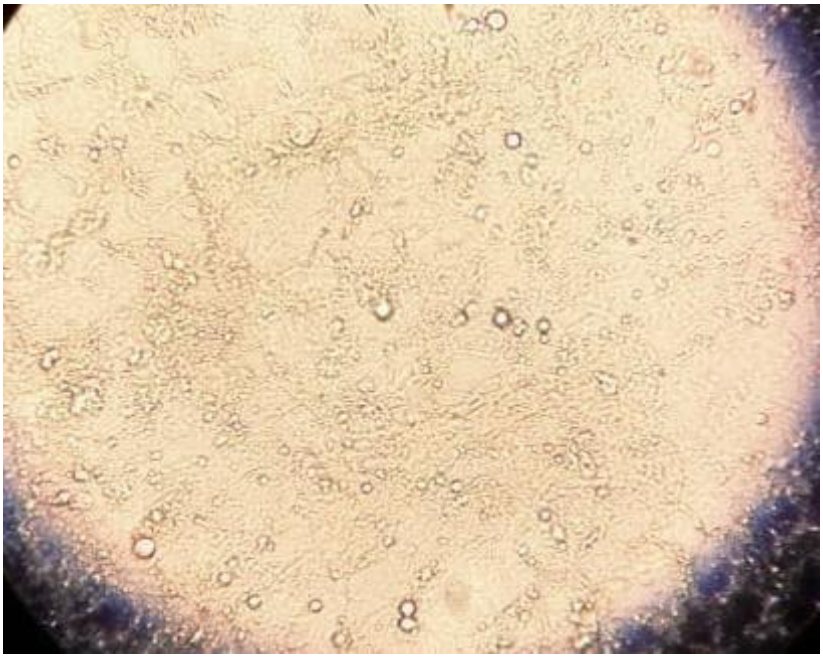
Objectives: The aims of this work were to: i) study the epidemiological and clinical features of three cases of *Pityriasis capitis* most likely of a zoonotic origin within three patients at the Parasitology-Mycology laboratory of the University Hospital Fattouma Bourguiba and ii) characterize the involved *Malassezia* species using both phenotypic and molecular methods.

Materials & Methods:
The first patient is an eleven year old child who presented with a non pruritic alopecic plaque in his scalp. The second is an eighteen year old teenager who had an irritant and a scaly plaque in the scalp. While, the third one is a fifth year child having a pruritic and scaly plaque-like lesion in his scalp. The first patient mentioned that he has had a poodle that has been recently showing an affected teddy. The second revealed his proximity to dogs keeping their farm animals. Whereas, the latter had a cat as a pet. Scales were taken from each patient by scraping the scalp lesions then they were inoculated in parallel on modified Dixon's medium (*mDXM*) and Sabouraud Chloramphenicol agar. *Malassezia* species were identified by phenotypic methods. This characterization was then confirmed using PCR-RFLP of the 26s rDNA using *Cfo I* enzyme.

Results: In all cases, direct microscopic examination in hydoxide potassium showed clusters of yeast cells, morphologically identical to *Malassezia*. In the three cases, phenotypic methods allowed to characterize the presence of *Malassezia* (*M.*) *furfur*. Whereas, PCR-RFLP allowed to detect a co-infection of *M. furfur*, *M. restricta* and *M. globosa* in the first case and a co-colonization of *M. furfur* and *M. restricta* in the second and third cases.

Conclusion: Molecular techniques remain faster and more accurate tools for the identification of *Malassezia* co-infections. Although *Malassezia* yeasts have been reported to be culprits in many scalp conditions, to the best of our knowledge this the first report describing a zoonotic source of scalp infection within humans.



P230

Prevalence of Bacterial Vaginosis and Candida Among Pregnant Women in Palestine

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Background: Vaginitis refers to any inflammation or infection of the vagina. This is a common gynecological problem found in women of all ages, with one third of women having at least one form of vaginitis at some time during their lives. The vagina is the muscular passageway between the uterus and the external genital area. When the walls of the vagina become inflamed, because some irritant has disturbed the balance of the vaginal area, vaginitis can occur. The most common types of vaginitis are: yeast infection and Bacterial vaginosis.
Objectives of Study: Therefore, the present study was carried out to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) of Palestinian women at the Palestine Medical Complex.
Methodology: Results of 2066 vaginal swabs were obtained between 2018 and the first quarter of 2023 investigated & diagnosed by the microbiology laboratory department at the Palestine Medical Complex for determining of vaginitis prevalence in the present study. Vaginal swabs from these patients were processed for detection of bacterial vaginosis and vulvovaginal candidiasis, based on microbiology guidelines.
Results & Discussion: The findings of the present study indicate that BV was the most prevalent infection with 71% followed by VVC 29%. *Escherichia coli* was found to be the most prevalent species with 28.7%. *Enterococcus spp*
. and *Streptococcus agalactiae* were found to be17.2% and 16%. The results of present study indicated that *Candida albicans* was most common with 30.2% caused by vulvovaginal candidiasis. Furthermore, the study showed the antibiotic sensitivity pattern for bacterial and yeast pathogens.
Conclusions: BV was the most prevalent vaginitis followed by VVC in Palestine Medical Complex. *Escherichia coli* was the most prevalent species in BV while among VVC species, *C. albicans* was found to occur at highest frequency. However, further studies are needed to assess specific diagnosis and role of clinical risk factors.
Keywords: *Candida*, Prevalence, Pregnant Women and Palestine.

P231

Hazardous Indoor Exposures to Mycotoxins and Microfungal Contamination Correlate with Different Immunodeficiencies

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Background:

Known immunosuppressants, macrocyclic Trichothecenes, exotoxins of *Stachybotrys* and *Trichoderma,* are extremely toxic, deeply respirable molecules (<0.01-0.03μ), smaller than COVID-19. They damage intracellular protein/DNA/RNA production and mitochondria, causing cell death: apoptosis. More toxic than *Fusarium*'s simple Trichothecenes contaminating food (e.g.T2-toxin, deoxynivalenol (DON, vomitoxin)), the macrocyclics are found in severely water-damaged or sewage-contaminated indoor environments. They remain toxic for decades despite disinfection or heat, attached to fungal debris, dust, mite-feces and can accumulate indoors. In humans, they spread rapidly via systemic circulation, especially with inhalation and damage phagocytes and lymphocytes.

To our knowledge, no prospective observational study combining comprehensive indoor contamination, exposure details with comprehensive medical evaluation of exposed humans has been done. With biostatistical analysis of real-world data and relevant real-world evidence, this study uses definite medical outcomes to determine which exposures and behaviors correlate with poor outcomes and immunodeficiencies after proven indoor macrocyclic trichothecenes exposure.

Objectives:

- Correlate immunodeficiencies with specific hazardous activities and indoor mold/mycotoxin exposures.
- Determine relationships between different immunodeficiencies, exposure variables (duration, intensity, specific molds and mycotoxins) and exposure markers based real-world data and relevant real-world evidence.

Methods:

Selection Criteria:
- Private practice patients with immune testing after indoor macrocyclic trichothecenes exposure:

Data:
- Professional Environmental Inspection including mold and mycotoxin testing.
- Comprehensive medical evaluation, testing with prospective follow-up.

Analysis:
- Ranked environmental hazardous conditions.
- Ranked hazardous exposure activities.
- Prospectively monitored and ranked severity of medical debility outcomes.
- Developed rating scales to correlate different cellular and adaptive immune deficiency combinations with exposure variables based on defined clinical outcomes using Pearson Correlation Coefficients.

Results:

Cohort: 44 patients: 13 children, 31 adults [17M,27F] exposed to 27 Trichothecenes-contaminated buildings.
Environmental fungal contamination is highly correlated with environmental trichothecenes and significantly correlated with mold/debris-exposing hazardous activities, immunoglobin deficiencies and combined cell-mediated immune (CMI) deficiencies.

Multiple indoor mycotoxins highly correlated with innate/neutrophil CMI deficiencies and multiple urinary mycotoxins.

Exposure duration highly correlated with combined CMI defects and significantly correlated with immunoglobulin deficiencies, and combined CMI-immunoglobulin deficiencies.

Disease severity highly correlated with exposure intensity ($p<0.00001$) and hazardous activities ($p=0.0001$), and significantly correlated with all CMI deficiency combinations and CMI-immunoglobulin deficiency combinations.

Urinary trichothecenes significantly correlated with CMI deficiencies.

Innate/neutrophil CMI deficiencies highly correlated with hazardous mold/debris-exposing activities, exposure duration and immunoglobulin deficiencies ($p=0.009$), and significantly correlated with disease severity and environmental mold contamination.

Multiple CMI defects [innate/neutrophil/lymphocyte] significantly correlated with disease severity, hazardous activities, neurological disease and urinary trichothecenes.

Immunoglobulin deficiencies highly correlated with CMI deficiency combinations ($p=0.009$) and significantly with exposure duration and environmental microfungal contamination.

Combined CMI-immunoglobulin deficiencies significantly correlated with neurological disease, disease severity, hazardous activities and exposure duration.

Conclusions:

Indoor mold/mycotoxin exposures may cause immunodeficiencies. Hazardous mold/debris-exposing activities and exposure duration significantly correlated with both cellular and adaptive immunodeficiencies, as well as disease severity and neurological disease. Excretion of macrocyclic trichothecenes significantly correlated with cellular immune defects.

Further epidemiological research is needed to determine the prevalence of such exposures and immunodeficiencies.

More markers for hazardous mold/mycotoxin exposures need to be developed.

TIMM
Trends in Medical Mycology

P232

Recurrent vulvovaginal candidiasis in Colombian women: Main clinical and microbiological characteristics

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Objective: To determine the main clinical and microbiological characteristics in a cohort of patients with diagnosis of recurrent vulvovaginal candidiasis (RVVC).

Material and methods: A cross-sectional study was carried out for 33 women, 17 with diagnosis of RVVC, and 16 healthy women as a control. Basic data of personal history as well as lower genital tract symptoms and signs were analyzed. Vaginal discharge or swabs were obtained from patients and controls, respectively, which were processed for culture, isolation, identification, and antifungal susceptibility of *Candida* spp. using selective culture media and the Vitek-2® system. Susceptibility to the following antifungals was evaluated: fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine. Additionally, from whole blood, and using antibodies for specific membrane markers, the different cell populations were identified by flow cytometry. Moreover, neutrophils and peripheral blood mononuclear cells (PBMCs) were isolated and purified to determine their fungicidal and proliferative capacity, respectively.

Results: The median age of the patients and healthy women was 30 (IQR 47–19) and 23.5 (IQR 44–20) years, respectively. All RVVC patients presented at least two of the following symptoms: discharge, irritation, or burning sensation. Seven (41%) patients presented more than four episodes per year while six (35%) presented more than eight episodes per year Of note, 14 (82%) patients used azoles as treatment for VVC during the last year. In all patients with RVVC, *Candida* spp. was isolated, of which 16 (94%) corresponded to *C. albicans* and one (6%) to *C. lusitaniae*. Two isolates of *C. albicans* resistant to fluconazole and voriconazole, and one isolate of *C. albicans* resistant to flucytosine were found. Additionally, one *C. albicans* isolate was sensitive dose-dependent to fluconazole and another presented intermediate susceptibility to voriconazole. Interestingly, in patients with CVVR, a significant decrease in the number of CD4 T cells and a significant increase in neutrophils were observed, but without alteration in the fungicidal capacity of the latter cells, while the PBMCs presented a lower proliferative capacity when compared to the healthy controls.

Conclusions: Contrary to what has been reported in the literature, the predominant species causing RVVC in this study was *C. albicans*. Of interest, no increase in the resistance of *Candida spp*. and a decrease in both CD4 T cell number and the proliferative response of the PBMCs were observed, which could suggest an alteration of the immune response.

Category: Epidemiology

P234

Epidemiological Trend, Species Distribution and Clinical Outcome of Invasive Candidiasis in a North-Eastern Italian Hospital: A Five Years Monocentric Experience

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Objectives:
To evaluate how the epidemiology, species distribution, antifungal susceptibility and outcome of candida bloodstream infection (BSI) have varied in the last five years in the Italian hospitals of the subregion of Friuli.

Materials & Methods:
In this retrospective monocentric observational study, we enrolled the overage patients hospitalised in the Friuli subregion with at least one positive blood cultures for *Candida spp* between 1st January 2018 to 31st December 2022.
Candida species were identified using both conventional and Maldi Tof mass spectrometry technology, while antifungal susceptibility was determined using broth microdiluiton dedicated panels.
All study data were recorded in a standard form and analysed with R-4.2.3 statistical software.

Results:
378 episodes of candidaemia were identified during the study period. Of these, six patients were excluded for lack of demographic or outcome information.
Demographic and clinical characteristics of the enrolled cases are summarised in Table 1, while profiles of sensitivity and stratification of the mortality rate according to *Candida* species are described in Table 2.
The most frequently isolated species of *Candida* was *albicans* (55.9%), followed by *glabrata* (16.1%), *parapsilosis* (15.9%) and *tropicalis* (8.9%).
Overall, 51.1% of patients died during the hospitalisation. Of these, invasive candidiasis was the main cause of death in the 9.6% of the cases and an additional cause in the 62.6% of them.
The overall survival (OS), calculated at 30 and 90 days from the first finding of blood culture positive for *Candida spp.,* was respectively 48.0% and 37.3%.
When data were stratified according to *Candida* species (the six rarer ones were grouped together), a statistically significant effects on outcome were observed (i.e., for death: χ^2_4=11.9, p=0.018; for 30-day survival: χ^2_4=14.6, p=0.006; for 90-day survival: χ^2_4=11.0, p=0.027; see Figure 1).
Specifically, *C. glabrata* BSI was associated with the highest mortality-rate (20.5%, OR=1.98, 95% confidence interval: [1.1, 3.7], p=0.024; with similar effect for 30-days survival). *C. parapsilosis*, instead, showed the lowest risk (11.6%, OR=0.51 [0.3, 0.9], p=0.023; also at 30/90-days).

Prompt adequate antifungal therapy administration considerably reduced the mortality-rate (OR=0.08 [0.014, 0.261], p<0.001). Surprisingly, *Fundus Oculi* execution displayed a protective role (OR=0.11 [0.1, 0.2], p<0.001); we interpreted this result as an indicator of correct invasive candidiasis management. In contrast, latency in removing endovascular devices resulted in a statistically significant increase of the death risk (OR=7.14 [2.6, 22.9], p<0.001; see Figure 2).

Conclusions:

As already shown in other studies[1,2,3], the incidence of candidaemia is increasing in the last five years and it still represents an important cause of morbidity and mortality, especially among elderly patients admitted to Medical wards[1].
Regular monitoring of local *Candida* species prevalence and antifungal susceptibilities is fundamental, since the isolation of *Candida non albicans* species with a reduced susceptibility spectrum to common antifungal, is becoming more frequent[2,3].

Unfortunately, this study present several limitations such as retrospectivity and monocentric analysis. Also inter-hospital differences in candidaemia diagnosis and management between hub and spoke centres, where an Infectious Disease Specialist is not always available, should be further investigated.

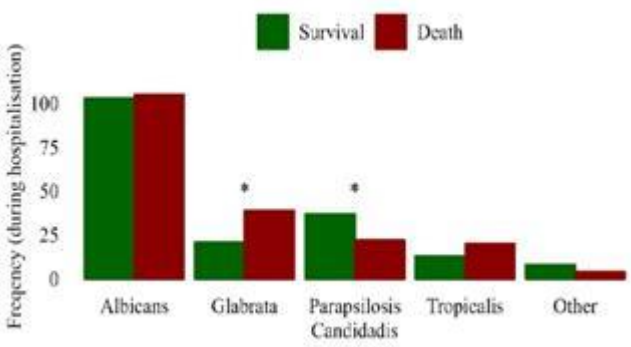
Bibliography:

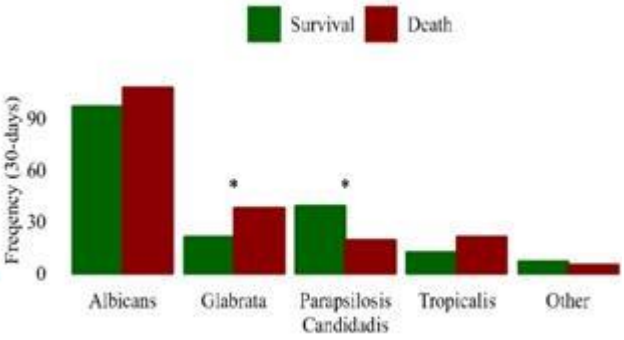
1. doi: 10.1371/journal.pone.0127534
2. doi: 10.1016/j.mycmed.2021.101210
3. doi: 10.1111/myc.13054

Patients	**372**
2018	19.6%
2019	21.0%
2020	13.4%
2021	24.5%
2022	21.5%
Sex (female)	41.4%
Age at candidaemia isolation	72.8 ±15.17 [6.2, 98.7]
[≤50] years-old	9.7%
[51, 60] years-old	9.1%
[61, 80] years-old	15.3%
[71, 80] years-old	33.6%
[81, 90] years-old	28.8%
[>90] years-old	3.5%
Area of admission	
Medical Area	68.3%
Surgical Area	18.3%
Intensive Care Unit (ICU)	13.4%
Hospitalization cause	
Infection	42.2%
Abdominal disease	11.7%
General deterioration of health	8.2%
Neurological event	7.4%
Oncological complication	6.5%
Orthopedic disease	4.1%
Other (Cardiac problem, elective surgical intervention, polytrauma, vascular disease, metabolic acidosis, etc)	19.8%
Charlson comorbidity index	5.8 ±2.99 [0, 14]
Intensive care Unit	27.2%
Days	17.8 ±22.16 [0, 115]
Surgery	39.5%
Intra-abdominal	51.4%
Orthopedic	16.7%
Heart surgery	6.9%
Urogenital system	6.2%
Brain	5.6%
Endovascular	4.9%
Invasive device	
None	22.8%
+1	32.0%
+2	33.6%
>2	11.6%
CVC/Midline	58.6%
IAP	6.2%
CV	60.8%
Other	23.2%

Concomitant antibiotics treatment	**84.0%**
Total parental nutrition (TPN)	38.9%
Candida colonization	13.6%
Concomitant steroid treatment	**9.3%**
Concomitant bacteremia	18.0%
Connextual SARS-CoV2 infection	10.9%
Species of isolated candida	
Albicans	55.9%
Glabrata	16.1%
Parapsilosis	15.9%
Tropicalis	8.9%
Lusitaniae	1.1%
Krusei	0.8%
Guilliermondi	0.5%
Dubliniensis	0.3%
Lambica	0.3%
Kefir	0.3%
Antifungal therapy	
None	10.8%
Echinocandins	62.4%
Other	26.8%
Blood cultures follow-up	
Not done	11.1%
Negative	63.0%
Positive	25.9%
Microbiological eradication, days	7.9 ±6.87 [0, 56]
CVC/Midline	
Removal	81.3%
Days for removal	3.3 ±3.82 [0, 27]
>2 days	46.3%
Positivity at cultural exam of the endovascular proportion of the catheter	54.9%
IAP removal	**61.1%**
Fundus oculi	**36.1%**
Echocardiography	**31.5%**
TE	16.1%
TT	83.9%
Positive	4.2%
Therapy duration in days	23.5 ±20.45 [1, 134]

	Triazoles												Echinocandine											
	Fluconazole			Voriconazole			Itraconazole			Posaconazole			*Anidulafungin*			*Micafungin*			*Liposomal Amphotericin B*			*5-Flucytosine*		
Species	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Albicans	100.0%	-	-	100.0%	-	-	100.0%	-	-	98.3%	-	1.7%	98.0%	-	2.0%	85.8%	-	14.2%	100.0%	-	-	100.0%	-	-
Glabrata	1.7%	93.3%	5.0%	100.0%	-	-	40.0%	33.3%	26.7%	100.0%	-	-	96.7%	-	3.3%	100.0%	-	-	96.6%	-	3.4%	100.0%	-	-
Parapsilosis	94.9%	-	5.1%	98.3%	1.7%	-	100.0%	-	-	100.0%	-	-	78.9%	21.1%	-	78.9%	21.1%	-	98.3%	1.7%	-	100.0%	-	-
Tropicalis	84.8%	12.1%	3.0%	87.9%	3.0%	9.1%	75.8%	21.2%	3.0%	95.0%	-	5.0%	96.9%	-	3.1%	100.0%	-	-	97.0%	-	3.0%	83.3%	16.7%	-
Lusitaniæ	100.0%	-	-	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Krusei	-	100.0%	-	No	No	No	No	No	No	No	No	No	100.0%	-	-	100.0%	-	-	100.0%	-	-	No	No	No
Guilliermondi	-	-	100.0%	100.0%	-	-	No	No	No	No	No	No	100.0%	-	-	No	No	No	No	No	No	No	No	No
Dubliniensis	100.0%	-	-	100.0%	-	-	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	100.0%	-	-
Lambica	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-
Kefyr	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-	-	-	100.0%	100.0%	-	-	100.0%	-	-





P235

Experience of Diagnosis of Chromoblastoma (2016- 2022): report from a clinical laboratory of Pakistan

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Objectives:
Chromoblastomycosis (CBM) is a chronic progressive disfiguring skin and subcutaneous tissue infection. It is caused by a group of dematiaceous fungi. A recent report published on global burden of CBM reported that Fonsecaea spp., Cladophialophora spp., and Phialophora spp. are the most common etiologic agent.
These organisms are ubiquitous and widely distributed in nature. Infection is acquired due to trauma. CBM has been reported from most of the continents in all climatic zones. However, it is more prevalent in humid tropical and subtropical countries. Majority of cases had been reported from South America and Africa, Central America and Mexico and Asia.1 Unfortunately, Pakistani data is scarce and only two case reports are published yet. Therefore, the objective of this study is to describe frequency of CBM cases diagnosed by histopathology and microbiology sections from 2016-2022 at a tertiary care hospital from Pakistan.
Materials & Methods:
This decriptive observational retrospective study was conducted at the Aga Khan University Hospital (AKUH) laboratory, Karachi, Pakistan. Seven years data (2016-2022), of skin and soft tissue biopsy specimens submitted for histopathology examination and culture was retrieved from hospital information system. Data was analysed to determine the frequency of diagnosis of chromoblastoma. Variables of statistical analysis were age, gender, site of lesion and geographical location of patient in the country.
Results:
During the study period 18 (n=432) specimens were reported as chromoblastoma. Histopathology division reported 14 cases from 230 requests for chromoblastoma as differential and microbiology section reported 4 from 202 specimens yielding dematiaceous fungi. Only one sample had been reported from both sections. Fourteen patients were below the age of 30 years and 4 were between 31-65 years. Most of the cases were daignosed among male (n=14, 78%) and (n=4, 22%) in female. Involvement of lower limb was 44%, face 39%, upperlimb 6% and no record was found for 11% cases. Majority of the cases were from northern parts of country which is hilly and green (13/18), and 5/18 were from southern part of country, which is dry and flat.
Oragnisms isolated were Alternaria species in 3 samples and Phialophora species in one case.
Conclusions:
CBM is not a commonly thought of when evaluating skin lesions in Pakistan. Though not very common, a high index of suspicion when assessing patients who may have a history of trauma and exposure to soil is warranted.

P237

Epidemiological trends of vulvovaginal candidiasis among symptomatic women in Greece

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Objectives: Vulvovaginal candidiasis (VVC) is a prevalent infection of the genitourinary tract, primarily affecting women during their reproductive lifetime. Its epidemiological features exhibit substantial variations, depending on the country, region and study population. To date, the epidemiology of VVC in Greece remains poorly reported and outdated. We therefore conducted a retrospective 2-year survey to assess the incidence of the infection, the species distribution and the antifungal susceptibility patterns of *Candida* spp. among symptomatic Greek women.

Materials/methods: High vaginal swab samples from adult women with clinically after gynaecologic consultation suspected VVC attending the private diagnostic laboratory "MycoLab" (Athens, Greece) between October 2019 and November 2021 were collected. Demographic and clinical data were obtained during the routine patient visits. VVC was confirmed by microscopic detection of yeast structures in vaginal fluid and *Candida*-positive cultures. Recurrent VVC (RVVC) was defined when the patient developed ≥3 symptomatic acute episodes/year. Species were identified by MALDI-ToF MS, while *in vitro* susceptibility testing was retrospectively performed according to the EUCAST-E.DEF7.3.2.

Results: Overall, 1,300 women were screened and 204/1,300 (16%) had confirmed VVC, whereof 11/204 (5%) were diagnosed with RVVC. All VVC patients were reproductive-age women of median (range) age of 28 (19-37) years. In total, 261 *Candida* isolates were recovered (13/248; 5% mixed infections); *C. albicans* was the most prevalent pathogen (127/261; 49%), followed by *C. parapsilosis* (94/261; 36%), *C. glabrata* (28/261; 11%), *C. krusei* (7/261; 2.5%) and *C. orthopsilosis* (5/262; 1.5%). Regarding the RVVC cases, 6 (55%), 3 (27%) and 2 (18%) were due to *C. albicans*-, *C. glabrata*- and *C. parapsilosis*, respectively. Fluconazole and itraconazole susceptibility patterns among all strains are summarized in **Table**. Fluconazole-resistant isolates were recovered from 4/11 (36%) RVVC patients, whereof one was infected by *C. albicans* (MIC 8-16 mg/L, itraconazole-susceptible MIC 0.03-0.06 mg/L) and three by *C. glabrata* (MICs 32->64 mg/L, two itraconazole-wild type MICs 0.5-1 mg/L and one itraconazole-non-wild type MIC >2 mg/L) and all were previously exposed to systemic (3 fluconazole, 1 itraconazole) and topical (1 econazole+miconazole, 1 clotrimazole+fenticonazole, 2 fenticonazole) antifungal therapy for ≥1 year. Among the remaining 7/11 RVVC patients infected by fluconazole-susceptible strains (all itraconazole-susceptible/wild type), 4 had repeated prior exposure to systemic (2 fluconazole, 1 itraconazole) and/or topical (1 clotrimazole+fenticonazole, 1 fenticonazole+miconazole, 2 fenticonazole) antifungal therapy for ≥1 year, while 3 (27%) did not have prior exposure to antifungals. All but one of the 7/193 (4%) non-RVVC patients infected by fluconazole-resistant strains (all *C. glabrata*, 3/7 itraconazole-non-wild type MIC >2 mg/L) had multiple courses of systemic therapy with fluconazole for ≥1 year. In the available follow-up of 7/11 patients

infected by fluconazole-resistant strains, all reported good control of symptoms and were culture-negative for a period of ≥1 year using boric acid (4/7), itraconazole+fenticonazole (1/7), clotrimazole+fenticonazole (1/7) or fenticonazole (1/7) suppression.

Conclusions: VVC is a frequent infection in our region, with *C. albicans* being the predominant species involved and resistance to fluconazole being low (5% of patients). Continued surveillance of local epidemiology regarding changes in species distribution and susceptibility to antifungals are necessary to guide treatment.

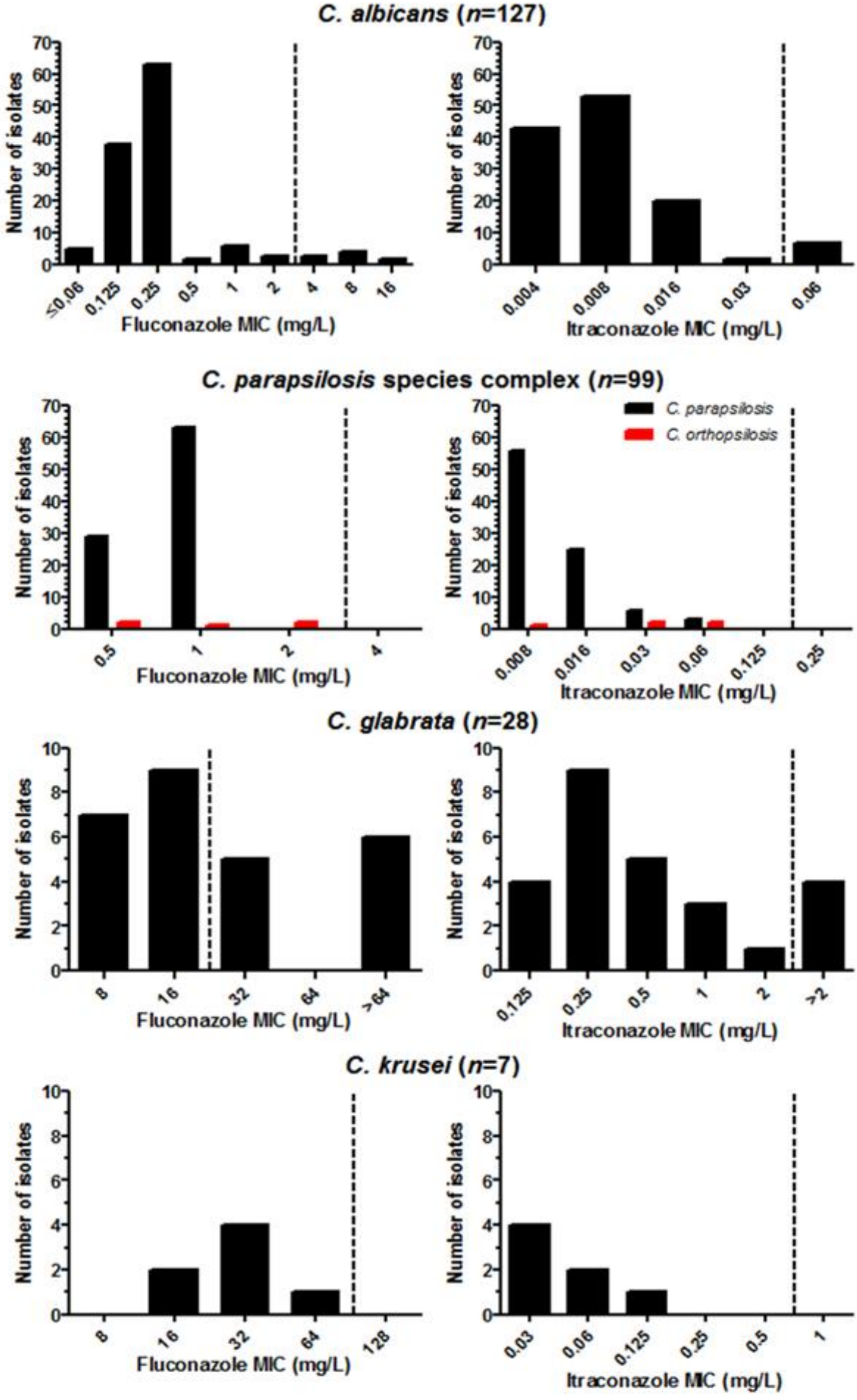

Figure. EUCAST fluconazole (left) and itraconazole (right) MIC distributions of Candida vaginal isolates. The broken lines indicate the

P238

Invasive Aspergillus flavus rhino-sinusitis in an immunocompetent patient using intranasal heroin

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Objectives:
Invasive aspegillosis typically occurs in immunocompromised patients such as patients with prolonged neutropenia, hematological malignancies, hematopoietic cell transplantation, receipt of high doses of glucocorticoids or other drugs / conditions that lead to chronically impaired cellular immune responses. However, immunocompetent patients have also been, rarely, reported to have chronic invasive fungal infections. Heroin and/or cocain intranasal use may lead to chronic rhinosinusitis as it can result in soft tissue and bone destruction, due to ischemia secondary to vasoconstriction, chemical irritation, trauma and secondary infections. We report a case of chronic invasive fungal rhinosinusitis due to *Aspefillus flavus,* with severe nasal damage and cutaneous involvement, secondary to heroin inhalation, in an immunocompetent patient.

Materials & Methods:
A 37-year-old female patient, user of inhaled heroin for the last 2 years, presented with intractable nasal and palate pain, and a history of progressive nasal congestion and epistaxis for three months. Her past medical history was negative for immunodeficiency or malignancy, and was otherwise unremarkable. Clinical examination revealed a nose deformity with necrotic lessions in the right anterior nare and nasolabial fold, nasal crusting and rhinorrhea. She had no focal neurologic deficits. Nasal endoscopy demonstrated heavy crusting with yellow-brown debris, areas of mucosal necrosis and erosions of the middle and inferior turbinates. The nasal septum cartilage was largely absent with areas of crusting and erosions that were extended to the nasal floor. Computed tomography showed an extensive erosive process involving the nasal septum, palate, nasal cavity, and right maxillary sinus without obvious intracranial involvement. A Magnetic Resonance Imaging confirmed the lack of intracranial disease, and again showed extensive erosions and tissue lose involving the nasal septum, palate, and right paranasal sinus.

Results:
Tissue biopsy revealed numerous branching septate fungal hyphae within the mucosa, with mixed inflammatory infiltrate and tissue necrosis. Intravenous liposomal amphotericin B was initiated, that was switched to isavuconazole, due to better tolerance, after the results of tissue culture via biopsy that grew *Aspergillus Flavus*. Voriconazole was not chosen for treatment due to significant drug interactions with the rest of phychiatric treatment that had been recommended by the phychiatric medical team. Extensive surgical debridement was conducted with removal of all necrotic debris. Upon evaluation two months after discharge and still on treatment with isavuconazole, she denied nasal discharge, or facial pain. The nasal mucosa was healthy in appearance with no crusting or tissue necrosis.

Conclusions:
Although invasive aspergillosis tends to infect immunocompromised patients, it should be included in the differential diagnosis when patients with a history of intranasal drug use present with chronic rhinosinusitis. We suggest that inhaled heroin abuse could be one of the predisposing factors of

invasive fungal infection in immunocompetent patients. Our patient was a user of inhaled heroin, which probably had caused bone and cartilage destruction, facilitating fungal penetration. Early biopsy of damaged mucosa is therefore warranted in this patient population for prompt diagnosis. Surgical debridement combined with long-term antifungal therapy are essential for favourable outcome.

P240

Epidemiological trends of fungemia due to rare moulds in a Greek tertiary care academic hospital

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Objectives: Although invasive aspergillosis and mucormycosis constitute the most commonly documented invasive mould infections (IMIs), mycoses attributable to rare moulds (RMs) are increasingly reported. Resistance of these emerging pathogens to various systemic antifungal agents remains a major challenge in empirical treatment. IMIs owing to RMs display geographical variety in terms of epidemiology and incidence globally (Hoenigl *Lancet Infect Dis.* 2021). To date, their epidemiological landscape in Greece remains unknown. We therefore conducted a retrospective 14-year survey describing the epidemiology of fungemia due to RMs in a Greek tertiary care teaching hospital.

Materials/methods: All microbiologically confirmed RM fungemia cases in patients hospitalized in "Attikon" university hospital during the period 01/04/2009-31/03/2023 were recorded. Patients' demographic and clinical characteristics were obtained from their medical records (where available). The incidence rate of the infection was expressed as the ratio of episodes per 1,000 hospital admissions and per 10,000 hospital bed days. The isolated strains were macro-/micro-scopically identified. If stored, they were retrospectively subjected to molecular identification by sequencing informative targets (ITS region, part of the β-tubulin gene for *Lomentospora* spp. and elongation factor α for *Fusarium* spp., Alastruey-Izquierdo *AAC* 2013) as well as *in vitro* antifungal susceptibility testing according to the EUCAST E.DEF 9.4 and CLSI M38Ed3 guidelines.

Results: During the 14-year period, 9 cases of RM fungemia were identified. Their overall incidence was 0.01/1,000 hospital admissions (0.03/10,000 bed days) accounting for the 1% (9/839) of total fungemic episodes. The vast majority (8/9; 89%) of the episodes occurred in patients admitted in internal medicine wards and 1/9 (11%) in ICUs. There were 7 (78%) male patients of median (range) age 70 (27-83) years. Haematological/oncological malignancy (5/9; 56%) and diabetes (4/9; 44%) were the most common underlying disorders. 7/9 (78%) bloodstream infections were due to *Fusarium* spp. (2 *F. dimerum* and 1 each of *F. verticillioides*, *F. chlamydosporum*, *F. solani*, *F. oxysporum* and *Fusarium* spp.), 1 to *L. prolificans* and 1 to *S. clavata* (8/9 molecularly identified). No bacterial bloodstream coinfection was recorded. Amphotericin B showed the highest *in vitro* activity against all the isolates tested. Voriconazole MICs were relatively higher, while the degree of activity of posaconazole was variable. Isavuconazole and itraconazole were only active against *S. clavata* (**Table**). Antifungal consumption data were available for 6/9 patients, whereof in 3/6 (50%) fungemia developed as breakthrough infection under micafungin (*S. clavata*), voriconazole (*F. dimerum*) or combination of isavuconazole+liposomal amphotericin B (*F. chlamydosporum*). 4/6 patients, all suffered by *Fusarium* fungemia, were treated with either liposomal amphotericin B (3/4) or voriconazole and survived, while the remaining 2 did not receive antifungal treatment since they died before notification of positive blood culture. The crude mortality rate within hospital stay was 44% (4/9).

Conclusions: This is the first epidemiological data on RM fungemia in Greece, reflecting the predominance of *Fusarium* spp. as causative agent and the significant proportion of

breakthrough infections. Clinical awareness and knowledge of the local epidemiology may help clinicians to refine the difficult diagnostic and treatment process of these potentially lethal IMIs.

Table. Antifungal susceptibility patterns among rare mould bloodstream isolates.

Species (number of isolates)	Antifungal agent	Median (range) CLSI MIC (mg/L)	Median (range) EUCAST MIC (mg/L)
Fusarium spp. (*n*=6)	AMB	1 (0.5-2)	0.5 (0.25-1)
	VRC	4 (2-8)	4 (2-8)
	POS	8 (2->8)	>8 (2->8)
	ISA	>8 (>8->8)	>8 (8->8)
	ITC	>8 (>8->8)	>8 (>8->8)
L. prolificans (*n*=1)	AMB	8	4
	VRC	>8	8
	POS	>8	8
	ISA	>8	8
	ITC	>8	8
S. clavata (*n*=1)	AMB	1	0.5
	VRC	0.25	0.5
	POS	0.5	0.5
	ISA	1	4
	ITC	0.25	0.25
	FLC	>8	8
	AFG	2	>4
	CAS	>4	>4
	MFG	4	>4

AMB: amphotericin B, VRC: voriconazole, POS: posaconazole, ISA: isavuconazole, ITC: itraconazole, FLC: fluconazole, AFG: anidulafungin, CAS: caspofungin, MFG: micafungin.

P241

Histoplasma seropositivity at the human-animal-environment interface in Upper River Region, The Gambia: A cross-sectional study using a household sampling approach

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Objectives:

Histoplasma species are globally distributed, thermally dimorphic fungi that cause human histoplasmosis. Recent designation as a high priority fungal pathogen by the World Health Organization (WHO) highlighted the need for more robust surveillance of *Histoplasma* spp. in endemic regions. Based on reported risk factors in the Americas, humans living in close proximity to domestic animals and potential environmental reservoirs of *Histoplasma* in The Gambia, may be at increased risk of exposure. Further research is warranted to ascertain the burden of *Histoplasma* in The Gambia and to explore context-specific risk factors for exposure at this human-animal-environment interface.

This study examined the human seroprevalence of anti-*Histoplasma* antibody in Upper River Region (URR) The Gambia and explored associations between seropositivity, demographic, clinical and environmental variables. This is the first study to systematically examine *Histoplasma* exposure in The Gambia.

Materials & Methods:

The study was prospective and cross-sectional, and used a cluster and household sampling approach. The population of interest was the general population in URR. Twelve Enumeration Areas (EAs) were randomly selected, using a probability proportional to size

approach stratified by urban/ rural classification. Sample size was determined based on current literature and a predicted between cluster variance of 5% at the EA level.

Within identified EAs, compounds and household occupants were selected randomly. Following informed consent in occupants ≥5 years, a clinical history, examination and venous blood sampling were performed, and participants completed a questionnaire addressing environmental factors translated verbally into the vernacular. Serum aliquots were frozen and stored prior to analysis using an IMMY Latex Agglutination *Histoplasma* test. Participants whose samples were assigned a two plus or greater reaction strength were considered positive for anti-*Histoplasma* antibodies.

Risk factors for seropositivity were explored by univariable logistic regression analysis.

Results:

The sample population (Table 1) demonstrated a seroprevalence of anti-*Histoplasma* antibody of 18.8% (*n*=56/298, 95% Confidence Interval (CI) 14.5-23.7%). On univariable logistic regression analysis, increased odds of seropositivity were measured in participants residing in rural as opposed to urban settlements (OR=3.32 95% CI 1.36-8.11, *p*=0.008), and in participants reporting domestic animal contact (OR=4.46 95% CI 1.04-19.15, *p*=0.04), contact with equids (OR=2.05 95% CI 1.04-4.02, *p*=0.04), and involvement with manure disposal (OR=3.81 95% CI 1.56-9.28, *p*=0.003) or collection activities (OR=4.25 95% CI 1.63-11.11, *p*=0.003) in the compound. A statistically significant association was measured between seropositivity and age (OR=0.97 95% CI 0.95-0.99, *p*=0.001). Increased odds of seropositivity were measured in participants reporting domestic work as a primary occupation or household role (OR=2.55 95% CI 1.28-5.09, *p*=0.008) and in participants identifiying as students or teachers (OR=2.04 95% CI 1.11-3.75, *p*=0.02). Using the latent variable approach, 12.6% variance in seropositivity was due to EA (variance=0.48, SE=0.32).

Conclusions:

This study provides evidence for frequent exposure to *Histoplasma* in a general population in URR The Gambia. Strong associations were measured between seropositivity and animal contact and management variables in the compound setting. Further examination of *Histoplasma* transmission dynamics at the human-animal-environment interface and public health implications is warranted, which would align with the WHO's priority areas for action.

Table 1. Baseline demographic and clinical characteristics of study participants (*n*=298).

Variable	Frequency, n (%), total N=298
Demographic	
Sex	
Male	133 (44.6)
Female	165 (55.4)
Age, years	
5-10	33 (11.1)
11-20	84 (28.2)
21-40	104 (35.1)
41-60	52 (17.4)
>60	25 (8.4)
Clinical	
'Do you currently have any of the following symptoms?'	
Cough	
No	239 (80.2)
Yes	59 (19.8)
Shortness of breath	
No	292 (98.0)
Yes	6 (2.0)
Chest pain	
No	280 (94.0)
Yes	18 (6.0)
Fever	
No	287 (96.3)
Yes	11 (3.7)
Night sweats	
No	297 (99.7)
Yes	1 (0.3)
Appetite loss	
No	291 (97.7)
Yes	7 (2.3)
Weight loss	
No	294 (98.7)
Yes	4 (1.3)
Skin lesions	
No	298 (100.0)
Yes	0 (0.0)
Oral lesions	
No	297 (99.7)
Yes	1 (0.3)
Myalgia	
No	285 (95.6)
Yes	13 (4.4)
Arthralgia	
No	277 (93.0)
Yes	21 (7.0)

Table 1 continued.

Variable	Frequency, n (%), total N=298
'Of the following options regarding smoking, what is your current status?'	
Active smoker	19 (6.4)
Ex-smoker	15 (5.0)
Never smoked	264 (88.6)
Clinical examination findings	
Temperature, degrees Celsius	
Median (IQR)	36.5 (36.2-36.9)
Respiratory rate, breaths per minute	
Median (IQR)	18.0 (17.0-19.0)
Chest pain	
No	281 (94.3)
Yes	17 (5.7)
Dyspnoea	
No	295 (99.0)
Yes	3 (1.0)
Cough	
No	294 (98.7)
Yes	4 (1.3)
Abnormal chest auscultation	
No	296 (99.3)
Yes	2 (0.7)
Palpable lymph nodes	
Cervical	
No	259 (86.9)
Yes	39 (13.1)
Clavicular	
No	258 (86.6)
Yes	40 (13.4)
Axillary	
No	259 (86.9)
Yes	39 (13.1)
Oral lesions	
No	296 (99.3)
Yes	2 (0.7)
Skin lesions	
No	295 (99.0)
Yes	3 (1.0)
'Are there any other important findings on general examination?'	
No	288 (96.6)
Hypertension	1 (0.3)
Hypertension and diabetes	2 (0.7)
Abdominal pain	3 (1.0)
Cardiac problem	1 (0.3)
Eye infection	1 (0.3)
Ulcerative skin lesion	1 (0.3)
No response	1 (0.3)

TIMM
Trends in Medical Mycology

P242

Predominance of Trichophyton tonsurans causing tinea capitis: A 12- years retrospective study in the north of Iran

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Predominance of *Trichophyton tonsurans* causing tinea capitis: A 12- years retrospective study in the north of Iran

Abstract

Objectives: Among different clinical entities of dermatophytosis, tinea capitis (TC) was considered a major public health challenge in the world, especially in regions with poor health and low income. Therefore, we aimed a retrospective analysis of the referred suspected patients to have TC to the medical mycology referral laboratory of Mazandaran a northern province of Iran.
Methods: A retrospective analysis of the referred suspected patients to have TC from July 2009 to April 2022 was performed. Hair roots and skin scrapings were collected. The laboratory diagnosis was confirmed by direct microscopic examination and culture.921/11095 (8.3%) patients were suspected to have TC.
Results: TC was confirmed in 209/921 patients (22.7%). Of 209 TC patients, 75.1% were male. The ratio of male to female in TC patients was 1:3.0. *Trichophyton tonsurans* (146/174, 83.91%) was the most etiological agent, followed by *T. mentagrophytes* (13/174, 7.47%), *T. violaceum* (9/174, 5.17%), *Microsporum canis* (3/174, 1.71%), *T. verrucosum* (2/174, 1.15%) and *T. rubrum* (1/174, 0.57%). Endothrix (77.0%) was the most prevalent type of hair invasion.
Conclusions: The predominance of *Trichophyton tonsurans* as a causative agent of TC was revealed. Despite being prevalent, the absence of appropriate consideration highlights that TC is a neglected complication among children.

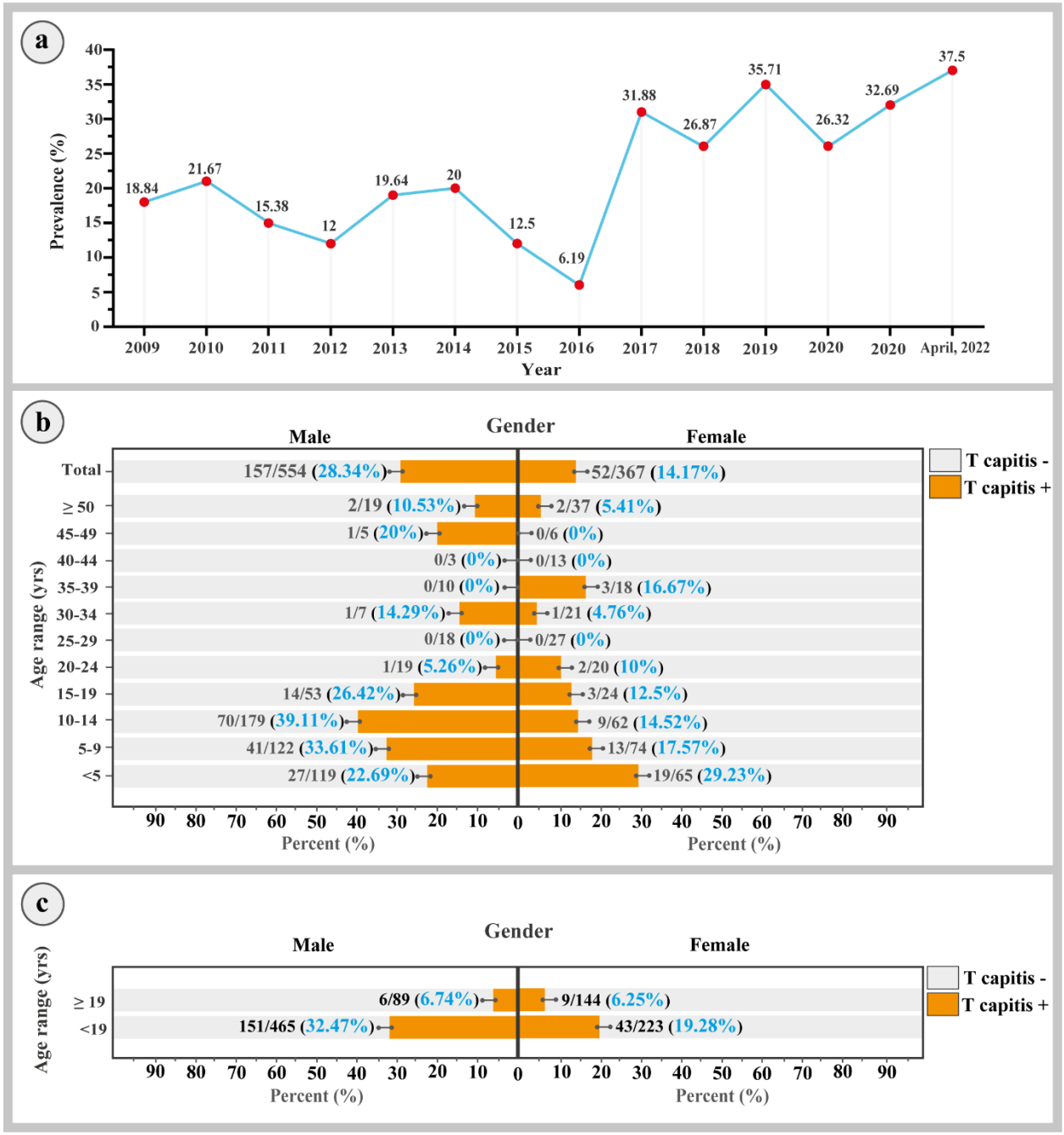

Figure 1. The frequency distribution of dermatophytes with respect to years (A) Dermatophytes isolation with respect to gender and age groups (B) Comparison of the frequency distribution of tinea capitis in two age groups < 19 years and ≥ 19 years old (C)

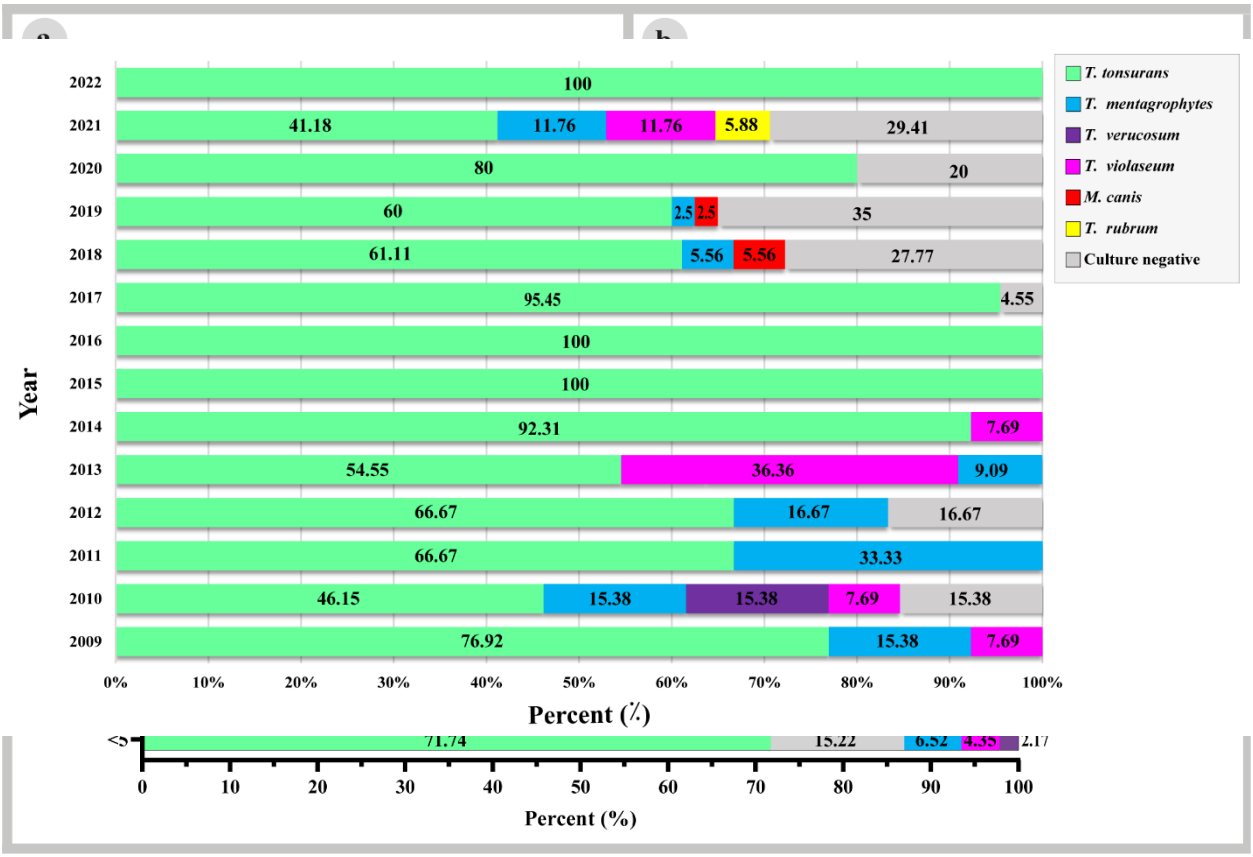

Figure 2. The frequency of the hair invasion classification of tinea capitis based on direct examination (A) The frequency distribution of various agents among confirmed dermatophytes(B) Dermatophyte species diversity according to age groups (C)

Figure 3. Comparing the dermatophyte diversity by the year

TIMM
Trends in Medical Mycology

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Invasive infections by non-albicans Candida in a fourth level hospital in Colombia: an approach to their epidemiology and antifungal susceptibility

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Objectives. We aimed to study the epidemiology of invasive infections caused by non-*albicans Candida* species in a fourth level hospital in Bogota, Colombia, and to determine the antifungal susceptibility profile of the etiological agents.

Materials & Methods. All non-*albicans Candida* isolates that were reported to be causing invasive infections between January 2022 and March 2023, were studied. The isolates were identified by conventional routine methods, including Vitek and MALDITOF. Patients affected by these yeasts had epidemiological data on sex, age, treatment and outcome. In addition, the antifungal susceptibility of the recovered yeasts to amphotericin B, flucytosine, caspofungin, micafungin, anidulafungin, fluconazole, itraconazole, voriconazole and posaconazole was determined using YeastOne broth microdilution. Statistical analyses were carried out to correlate epidemiological variables and to establish differences in antifungal susceptibility between species, per antifungal drug.

Results. In the studied period, 51 non-*albicans Candida* isolates, recovered from 42 patients, were identified. From the species, 22 (43,1%) were *Candida parapsilosis*, 16 (31,4%) *Candida tropicalis*, 9 (17,6%) *Candida glabrata*, 3 (5,9%) *Candida dubliniensis* and 1 (2%) *Candida guilliermondii*, recovered mainly from blood (56,9%) and other sterile sites (43,1%). From the patients, the majority were men (57,1%). Patient's age ranged between 18-day and 93-year old (average 62 years). Most patients were hospitalized in the ICU (61,9%) and treated with caspofungin (31%) followed by fluconazole (23,8%). A third of the patients did not receive any antifungal treatment. General mortality was 45.2%. However, patients affected by *C. glabrata* were found to have a higher mortality risk (89%) than patients affected by other species. Higher mortality was also associated with an older age. Antifungal susceptibility showed that *C. parapsilosis* isolates were the less susceptible to echinocandins, compared to isolates of other species, while *C. glabrata* isolates were the less susceptible to azoles followed by *C. tropicalis*. Amphotericin B and flucytosine susceptibility did not differ among species. From the isolates, one of *C. glabrata* was resistant to caspofungin, two of *C. tropicalis* were resistant to both voriconazole and fluconazole and three others of *C. tropicalis* were resistant to flucytosine.

Conclusions. In the studied hospital, *C. parapsilosis* predominates among non-*albicans Candida* species causing bloodstream infections, especially in patients older than 60 years, with significant mortality. In addition, less common species, such as *C. dubliniensis* and *C. guilliermondii*, were also reported causing invasive infection. This study contributes epidemiological data to the surveillance of non-*albicans Candida* species in Colombia and globally, which is very important considering the increase in immunocompromised patients or with other risk factors, who are more predisposed to develop an invasive fungal infection, specially associated with health care. Our study also shows that the therapeutic strategies used to treat these infections are not always adequate to contribute to better outcomes, since there are resistant isolates or with decreased susceptibility to certain antifungals, which could significantly increase morbidity and mortality rates. Further studies on the molecular identification of the isolates will be carried out by ITS sequencing, in order to compare species identification using conventional and molecular techniques.

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Monitoring of airborne fungi during the second wave of COVID-19 in the referral university hospital in southeastern Iran

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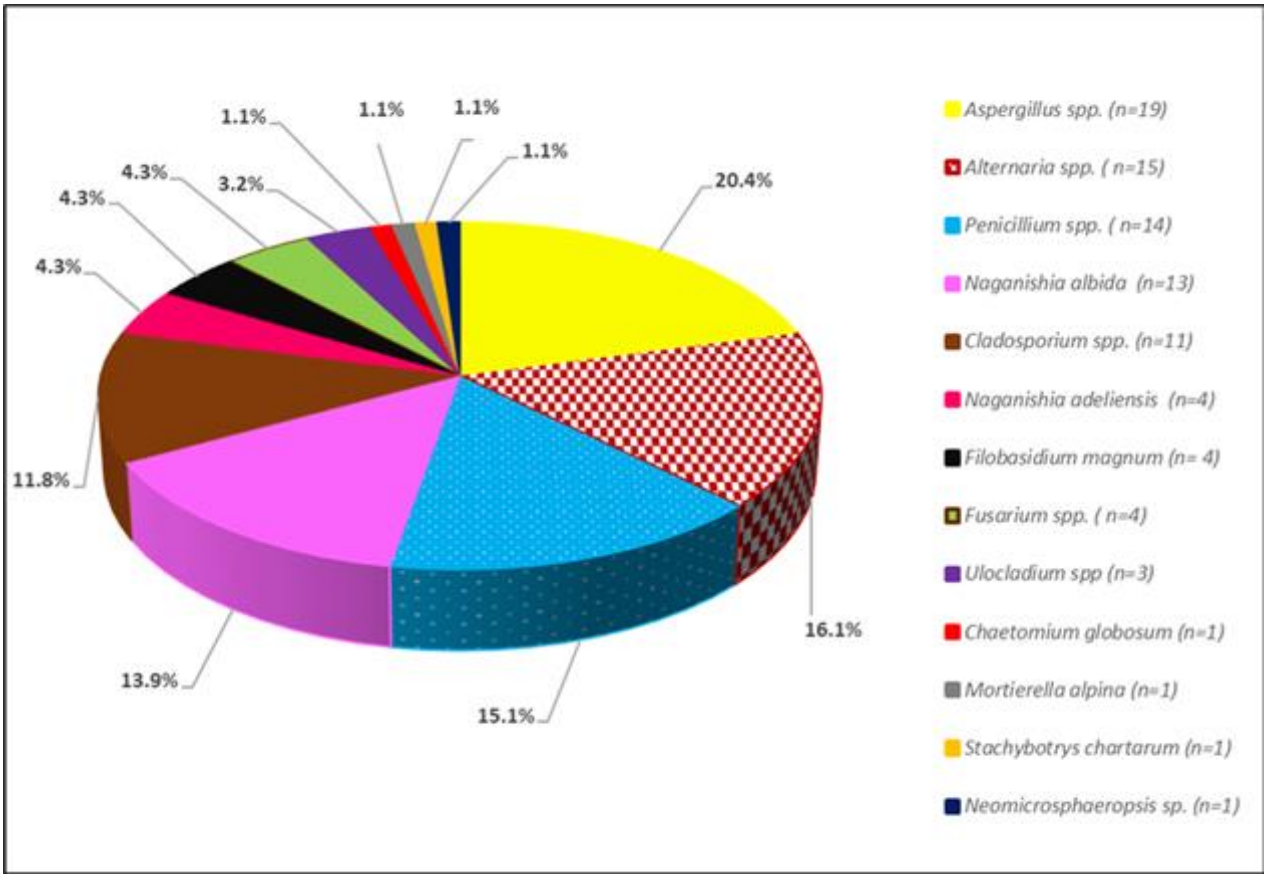
Objectives:
Microbiological monitoring of the air hospital is essential for prevention and control, due to the possible airborne route of infection transmission, especially in high-risk wards. This study aimed to monitor the airborne fungi during the second wave of the COVID-19 pandemic in selected wards of the biggest university educational hospital in Kerman, southeastern Iran.
Materials & Methods:
This study was conducted in 11 different wards, separated into the patient room and nursing station, of the Afzalipour Hospital, a referral university hospital, from May to August 2021. Fungal isolates were characterized to the species level by conventional and sequencing methods.
Results:
Out of 93 obtained fungal colonies, 70 (75.3%) isolates were filamentous and 23 (24.7%) isolates were yeast. *Aspergillus* species were the predominant fungal isolates among the filamentous colonies (n=19; 27.1%), and *Naganishia albida* (formerly *Cryptococcus albidus*) was identified as the most common yeast isolate (n=13/23; 56.8%). The infectious ward was the most contaminated unit (n=19/93), while the least contaminated units were the neonatal intensive care unit (n=3/93), and oncology (n=3/93). The statistical findings displayed that the number of fungal isolates in patients' rooms is significantly higher than in nurses' stations (p-value=0.013).
Conclusions:
Our study demonstrated the presence of diverse fungal species in all wards of the hospital. Considering the presence of airborne fungi in hospitals and related public health problems is one of the critical issues for health systems management. In this regard, efficient monitoring of airborne fungi might play an influential role in hospital infection control and surveillance, particularly in high-risk hospitalization patients in critical wards.


1.1%
1.1%
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20.4%
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3.2%
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4.3%
11.8%
16.1%
13.9%
15.1%
Aspergillus spp. (n=19)
Alternaria spp. (n=15)
Penicillium spp. (n=14)
Naganishia albida (n=13)
Cladosporium spp. (n=11)
Naganishia adeliensis (n=4)
Filobasidium magnum (n= 4)
Fusarium spp. (n=4)
Ulocladium spp (n=3)
Chaetomium globosum (n=1)
Mortierella alpina (n=1)
Stachybotrys chartarum (n=1)
Neomicrosphaeropsis sp. (n=1)

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Rapid assessment and containment of Candida auris transmission in a tertary-care hospital in Northern Greece.

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Objectives: *Candida auris* is an emerging, drug-resistant pathogen that causes nosocomial outbreaks with considerable mortality rates. The aim of our study was to demonstrate the assessment and containment of *Candida auris* transmission in our hospital.

Materials & Methods: In December 2022, the first case of *Candida auris* was detected at AHEPA University Hospital, in Thessaloniki, Greece. Upon its emergence, infection prevention and control (IPC) measures were applied including active surveillance of *C auris*, contact precautions, isolation of colonized patients and intense environmental cleaning. Of note, physical isolation was not feasible in the Intensive Care Units (ICUs). Active surveillance included combined axillary/groin swabs that were obtained weekly at the affected departments, and upon admission from patients hospitalized in the 3 ICUs (2 general and 1 surgical), the hematology and the pediatriconcology department. Swabs were inoculated on the chromogenic agar Brilliance™Candida (Oxoid, UK). Species identification of the isolates of either surveillance swabs or clinical samples that grew candida species, were performed on both MALDI Biotyper® sirius System (Bruker, USA) and Vitek®2 (BioMérieux, France). In cases that identification was not feasible by the aforementioned methods, samples were tested by isothermal LAMP(Loop-mediated AMPlification) method using the eazyplex® *Candida auris* assay (amplexDiagnostics GmbH, Gars-Bahnhof, Germany). Antifungal susceptibility profiles were performed by the MICRONAUT-AM (Bruker, USA) on the Multiskan® EX (Thermo scientific, USA). CLSI guidelines were applied for interpretation of antifungal susceptibility testing.

Results: Overall 388 axilla/groin swabs were obtained by 178 patients. Out of them, 14 patients were found to be colonized by *C auris*; 7 were female (median age was 64 years). *Candida auris* was recovered from 10 surveillance , 3 urine and 1 central line catheter cultures. Antifungal susceptibility data are displayed on the table. The susceptibility testing could not be performed on 2 isolates due to technical issues. Of the affected with *C. auris* patients, 2 were hospitalized in the 1st Internal Medicine Department, 1 in the 2nd Internal Medicine Department, 1 in the Cardiovascular ICU, 1 in the ICU A' and 9 in the ICU B'. It is of interest that all 5 patients hospitalized in the first 4 departments mentioned above were transferred from either Long-term Care Facilities (LTCFs) or other hospitals. No further transmission events were identified in these departments. Of the 9 patients in the ICU B' the first to be found positive was transferred there by a LTCFs. Mean of days to colonization was 9. 13 out of 14 patients were only found to be colonized with no signs and symptoms of candidiasis; 1 patient was treated for *Candida auris*. No deaths due to *Candida auris* infections were registered.

Conclusions: Infection control measures seemed to have been efficient towards the containment of *C. auris* in most departments provided that isolation was applied. LTCFs seem

to have been the main source of our cases and thus intensive screening of patients admitted from these institutions should be applied. Most of our cases were identified through active surveillance wich is essential for the early recognition of carriers and the timely application of IPC measures.

Table Antifungal susceptibility data of C. auris

Antifungal	MIC (µg/ml) BMD			
	50%	90%	Range	Susceptible %
FLC	>128	>128	32->128	0
CAS	0.125	8	0.125-8	66.7
MFG	0.015625	0.03125	0.015625-0.03125	100
AFG	0.03125	0.0625	0.015625-0.0625	100
AMB	1	1	0.5-1	100

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Recurrent candidemia: clinical and genetic analysis

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Objectives:
Candidaemia is a life-threatening invasive infection. In rare cases, the infection can occur on a recurrent basis with bloodcultures (BC) positive for the same species between different distant episodes. We aimed at better understanding the pathophysiology of such recurrent candidemia (RC).

Materials & Methods:
RC was defined when a delay of 15 days was seen between the last positive BC of a 1st episode and the first positive BC of a subsequent episode. We performed a retrospective analysis of RC diagnosed in our centre over a 7-year period (2015-2021). Demographic, clinical and therapeutic characteristics of patients were recorded. Isolates from the different episodes were subjected to genotyping by the mean of microsatellite length analysis techniques. Antifungal susceptibility testing was performed for strains isolated during the different episodes.

Results:
We retrieved 15 patients having presented 17 episodes of RC. The median delay between 2 episodes was at 39 days (range 17-890). 77% (10/13) of the patients were immunocompromised and 54% (7/13) had a vascular context (intravenous drug abuser, arteriovenous shunt, vascular prosthesis, recurrent vascular thrombus) at the time of the 1st episode. Species isolated were *C. albicans* (n=8), *C. parapsilosis* (n=3), *C. krusei* (n=1), *C. glabrata* (n=2) and *C. lusitaniae* (n =1). Patients were mostly treated with caspofungine (n=9) on a standard dosage and duration basis. Echinocandin resistance emerged in on case of *C. krusei* infection. Preliminary results (11 recurrent episodes analyzed) of molecular typing revealed that in all cases, the second episode was due to the same strain.

Conclusions:
Our results support the hypothesis that RC are due to relapse of infection rather than re-infection. “Vascular context” could play the role of a reservoir. The role of biomarkers to detect deep sites of infection and the possible adjustment of antifungal therapy in these cases should be further evaluated to avoid such recurrences.

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Gender differences and outcomes of allergic bronchopulmonary aspergillosis

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ABSTRACT

BACKGROUND: Whether gender differences influence outcomes of allergic bronchopulmonary aspergillosis (ABPA) remains unknown.

METHODS: We retrospectively included subjects with ABPA complicating asthma (2007-2019) and compared the immunological and radiological severity of ABPA and outcomes in men and women. We also performed a multivariate logistic regression analysis to determine whether women experience lesser ABPA exacerbations.

RESULTS: We included 810 ABPA subjects with a mean age of 34.9 years (49.4% women). There was no difference in the mean (95% CI) serum total IgE (8863 [8108-9619] vs. 9517 [8636-10398] IU/mL; p=0.27) and *Aspergillus fumigatus*-specific IgE (28.5 [26.1-31.0] vs. 26.4 [23.9-28.8] kUA/L; p=0.22) in women vs. men. Eosinophil counts were similar in the two groups. The mean (95% CI) number of segments involved by bronchiectasis (7.4 [6.9-7.9] vs. 7.6 [7.1-8.1]) was similar in the two groups. There was a trend towards higher frequency of exacerbations in women (172/400 (43.0%) vs. 152/400 (37.1%), p=0.09). On multivariate analysis, the only factor influencing ABPA exacerbation was the extent of bronchiectasis (adjusted odds ratio [aOR] 1.04; 95% CI [1.01-1.07], p=0.12) after adjusting for serum total IgE, high-attenuation mucus, and peripheral blood eosinophil count ≥1000 cells per microliter. Women did not experience lesser exacerbations than men (aOR [95% CI], 0.79 [0.59-1.05]; p=0.11)

CONCLUSION: We found no gender differences in the severity and outcome of ABPA-complicating asthma.

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Whole genome sequencing of Clinical isolates of C. auris from a tertiary-care hospital laboratory in Pakistan: strain diversity and evolution

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Introduction:
Candida auris is one of WHO's critically important fungal pathogens due to its potential to cause outbreaks, become multi-drug resistant and its association with mortality. Lately, our laboratory noticed the spread of *C. auris* beyond patients admitted to the Aga Khan University Hospital (AKUH). We aim to track the emergence and transmission of *C. auris* and its resistance profile using whole genome sequencing (WGS) to describe its spread through the population to supplement control efforts within Pakistan.
Methods:
Seventy-four non-duplicate *C. auris* strains were isolated from samples submitted at AKUH Clinical laboratories from Jan-Dec 2022. These strains were identified using Vitek 2 Yeast ID card (BioMerieux, France), antifungal susceptibilities performed on YeastOne Sensititre™ YO10 plates and interpreted according to CDC guidelines. The isolates were revived; DNA was extracted using QIAGEN DNA mini kit following standard protocol with an additional bead beating step during cell lysis, and 25ul lyophilised DNA was shipped to Northwestern University for WGS and bioinformatics analysis. Multiplex sequencing library was prepared on reconstituted specimens using PlexWell 24 kits (seqWell, MA USA) and sequencing performed on the NovaSeq platform (Illumina, CA USA) to generate paired-end 150 bp reads. Sequence reads were quality trimmed using fastp v0.23.2 and assembled using SPAdes v3.15.4. Sequence reads were aligned to reference genome sequence B8441 using bwa v0.7.15 and single nucleotide variants were called using bcftools v1.9. Whole genome phylogenetic analysis was performed using IQ-TREE 2.2.0.
Results:
Out of the 74 isolates, 53 (72%) were from AKUH and 23 non-AKUH, 76% were from adult patients, and 62% were male. Most of these were from blood (41%) or urine (36%) while other sites included ear swabs (12%), and miscellaneous sources (11%). Almost all (96%) isolates were resistant to fluconazole but none to echinocandins or amphotericin. One isolate each was excluded for WGS for low read counts post-sequencing, and for poor assembly. The median read count following adapter- and quality trimming was 11.5M (range 4.0M–22.0M) for an average fold coverage of 133x (range 46x–262x). Analysis of 18S rRNA gene sequences from assemblies showed all isolates were *C. auris*. The median assembly size for the remaining sequences was 12,354,768 bp (range 12,324,680–12,452,989 bp). Alignment against the reference genome sequence B8441 revealed all isolates belonged to geographical Clade 1 and differed by 0– 1146 pairwise SNVs. Phylogenetic analysis showed two major clades of isolates including one of very closely related sequences from multiple institutions and isolation sites and a second clade of more divergent isolates. Notably, the second clade consisted primarily of ear isolates (9 out of 10), whereas no ear isolates were found in the first clade.
Conclusions:

We demonstrated very little genetic variation among isolates infecting or colonizing most body sites across age ranges and institutions in the Karachi, Pakistan region. However, ear isolates were genetically divergent from other sources suggesting the ear may be a niche for *C. auris* strains distinct from other body sites. This study demonstrates the value of WGS for characterizing and contextualizing *C. auris* infections.

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Candidemia in COVID-19 pandemic: incidence and characteristics in COVID-19 versus non-COVID-19 patients in Northern Greece

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Objectives:
Covid-19 pandemic has resulted in significant healthcare challenges, particularly in hospitalized patients with secondary infections. Patients with SARS-CoV-2 are predisposed to secondary fungal infections mainly due to immunological and clinical risk factors. Reports for candidemias in hospitalized patients with COVID-19 are limited. We evaluated the incidence and characteristics of candidemias in COVID-19 versus non-COVID-19 hospitalized patients in a tertiary-care teaching hospital in Northern Greece.
Materials & Methods:
A single-center retrospective cohort analysis of all adult patients with candidemia hospitalized in the AHEPA University Hospital (Thessaloniki, Greece) with COVID-19 during three years of pandemic, from March 2020 to February 2023. The control group was comprised of patients with candidemia but without COVID-19 infection hospitalized for any reason from January 2019 to February 2023. Candidemia was defined as the first positive blood culture for Candida spp developed more than two days after admission. Diagnosis of COVID-19 disease was performed by real-time PCR using either the Abbott Molecular Real-Time or the NeuMoDx™ SARS-CoV-2 assays.Identification of Candida isolates and antifungal susceptibility testing were performed by Vitek2 (biomérieux, France) and Micronaut-AM (Bruker, Germany) respectively. Statistical analysis was performed with SPSS-v.23 (IBM, USA).
Results:
From January 2019 to February 2023, 137,468 patients were admitted in our hospital and 235 patients developed candidemia. From March 2020 on, 4,311 patients were admitted with COVID-19 in our hospital and candidemia was detected in 81 patients (48 males and 33 females with a mean age of 66.23 years) and incidence rate 18.78 cases/1000 admissions (Table). The average of interval between hospitalization date and development time of candidemia was 26.98 days. The in-hospital mortality in this group was 75.30%. Admission in ICU occurred in 54 out of 81 patients (66.66%) with an average time of staying in ICU of 29.92 days. The group of non-COVID-19 with candidemia included 154 out of 133,157 patients admitted during the January 2019 to February 2023 (80 males and 74 females with a mean age of 66.44 years) and incidence rate of 1.15 cases/1000 admissions. The incidence of candidemia in non-COVID -19 patients was similar comparing the periods before and after the onset of the pandemic in March 2020 (1.03 and 1.22 cases/1000 admissions respectively). In the group of non-COVID-19 patients the in-hospital mortality was 61.03%, 44 out of 154 (28.57%) were admitted in the ICU with an average time of staying in ICU of 28.57 days. *Candida parapsilosis* (53/235) predominated in both groups followed by *Candida albicans* (64/235). All Candida species were tested susceptible to amphotericin B and to newer azoles, posaconazole and itraconazole. Fluconazole resistance was high, 19.9% and especially for *C. parapsilosis,* 26.8%. Low resistance rates for voriconazole (1.99%) and echinocandin (1.08%) were observed.
Conclusions:

In conclusion, a significant increase of candidemia incidence was observed during the pandemic period in hospitalized COVID-19 patients compared to non-COVID-19 cohort either pre pandemic or during the three years pandemic era. COVID-19 patients were more likely to require ICU admission and had increased in-hospital mortality.

Table. Characteristics of the two group of patients COVID-19 versus non-COVID 19

	COVID-19 patients	NON-COVID 19 patients	p
Total hospital admissions, n (%)	4,311	133,157	
Patients with an episode of candidemia, n (%)	81	154	
Incidence per 1000 admisssions	18.78	1.15	
Age (years), mean	66.23	66.44	0.461
Gender, male, n (%)	48 (59.25)	80 (51.94)	0.285
Hospital stay (days), mean	43.03	43.42	0.464
Admission in ICU, n (%)	54 (66.66)	44 (28.57)	**<0.001**
ICU length of stay, days, mean	29.92	28.59	0.394
In-hospital mortality, n (%),	61 (75.30)	94 (61.03)	**0.028**
Hospital stay before candidemia onset, days, mean	26.98	27.00	0.497
ICU-stay before candidemia onset, days, mean	21.48	29.00	**0.048**
Days from diagnosis of candidemia until death, mean	12.16	14.22	0.492
Candida species			
C. parapsilosis	53	82	0.073
C. albicans	13	51	**0.005**
C. glabrata	8	10	0.354
C. tropicalis	5	8	0.755
C. krusei	0	1	0.467
C. lusitaniae	0	1	0.467
C. guillermondi	0	1	0.467
C. kefyr	1	0	0.167
C. utilis	1	0	0.167

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Epidemiological study of clinically human dermatophytosis and characterizing the causative agents using PCR-RFLP typing, in Golestan province, north of Iran

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Objectives: Determining the epidemiological status of dermatophytosis is necessary to identify changes in the causative agents and characteristics of the distribution of this infection. The aim of the present study was to describe the species spectrum of dermatophytes, isolated from patients in Golestan province, north of Iran, using PCR-RFLP typing by Mva1 restriction enzyme.

Methods: A cross-sectional descriptive study was conducted during 13-month period on all patients referring to medical diagnosis laboratories with clinically suspected dermatophytic lesions. 255 patients from 14 cities in all province diagnosed with dermatophytosis by direct examination and culture of samples of skin, hair, and nails. After obtaining consent to participate in the study, a research questionnaire including age, gender, marital status, occupation, level of education, living environment, contact with animal, location and duration of the lesions, and the results of laboratory diagnosis and culture was implemented for each of them. Dermatophytes species identification was performed by RFLP-PCR using *Mva*I restriction enzyme. In cases where this method was not able to detect the species of the organism, the sequence of the ITS region was used and the results obtained in the BLAST databases were compared and identified. Data obtained statistically analyzed using SPSS and Chi-square test was used to determine the association between two categorical variables. P values ≤ 0.05 at 95% confidence intervals were considered significant.

Results: Tinea cruris was the most common clinical type (38%) followed by tinea corporis (35.3%). *Trichophyton mentagrophytes/interdigitale* complex was the most common species (86.7%) followed by *T. rubrum* (5.1%) and to a lesser extent *T. tonsorens* (3.1%), *Microsporum canis* (2.7%), *T. benhamii* and *T. violaceum* each 0.8%, and *T. quinckeanum* and *M. ferroginum* each 0.4%. *Epidermophyton flucosum* was not isolated from patients. The female: male ratio was 1.6:1. In female, most of the patients were in the age group of 30-50 years and in male, 20-29 years. The most common type of tinea in female was tinea cruris (41.4%) and in male, tinea corporis (39.8%). Tinea unguium was seen only in age groups over 30 years old. There was a statistically significant relationship between location of the lesions and age groups, gender and dermatophytes species. Dermatophytosis showed a significant decrease with the increase in education level and dry weather. However, diabetes, living in rural/urban did not show any significant difference with the location of lesions and the dermatophytes species.

Conclusions: This study showed the importance of analyzing the epidemiologic profile of dermatophytosis in the region to allow proper preventive management of the condition. As the findings indicated, *T. mentagrophytes*/*T. interdigitale* species complex was the most common cause of dermatophytosis in this northern province of Iran, similar to the study conducted in Mashhad (Northeastern Iran) and *T. quinkeanum* was reported as the cause of human dermatophytosis in Iran for the first time.
Keywords: Epidemiology, dermatophytosis, dermatophytes species, PCR-RFLP molecular method, Golestan province, Iran

P251

Increasing number of cases of Candida auris in Greek healthcare facilities, 2019-2023

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Objectives:

Candida auris is an emerging and frequently multidrug-resistant yeast, which can spread in healthcare settings and cause invasive infections associated with high mortality rate. In 2019, the first isolation of *C. auris* was reported in Greece. Since then, more than 750 *C. auris* clinical isolates, responsible either for invasive infections or colonization, were referred to the Department of Microbiology, Medical School, National and Kapodistrian University of Athens. We therefore describe our experience on the source, antifungal susceptibility pattern and genetic variation of *C. auris* isolates recovered from Greek healthcare facilities over the past years.

Materials & Methods:

A total of 783 *C. auris* isolates were referred to the Department of Microbiology, Medical School, National and Kapodistrian University of Athens during the period 01/11/2019-31/03/2023. Clinical isolates were collected from patients who were hospitalized in 39 Greek healthcare facilities (30; 77% in the Attica region). All isolates were identified to species-level by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using two different platforms (Microflex LT platform, Bruker Daltonics, Bremen, Germany and Autof ms1000, Autobio, Zhengzhou, China). Minimum inhibitory concentrations (MICs) were determined for *C. auris* against antifungals by Sensititre YeastOne YO10 (SYO, ThermoFisher Scientific). Since *C. auris*-specific susceptibility clinical breakpoints do not currently exist, the interpretation of MIC values was based on the U.S. Centers for Disease Control and Prevention (CDC) tentative breakpoints for fluconazole (≥32 mg/L), amphotericin B (≥2 mg/L) and echinocandins (anidulafungin/micafungin ≥4 mg/L, caspofungin ≥2 mg/L). Molecular characterization of isolates was performed by multilocus sequencing typing of the ITS region of rDNA and *rpb1* gene.

Results:

Overall, 635 (81%) *C. auris* cases were characterized as colonization. Candidaemia was confirmed in 148 (19%) cases, whereof 82 (55%) episodes occurred during the COVID-19 pandemic (May 2020-June 2022) and in 7 (5%) cases SARS-CoV-2 coinfection was recorded. Antifungal susceptibility testing was performed in 116/148 (78%) bloodstream isolates. All isolates tested were resistant to fluconazole (SYO MIC range 32->256 mg/L) and susceptible to anidulafungin (SYO MIC range 0.03-0.5 mg/L) and micafungin (SYO MIC range 0.015-0.5 mg/L). For amphotericin B, 76/116 (66%) isolates had SYO MICs ≥2-8 mg/L and were categorized as resistant. Nevertheless, when a proportion of these isolates [10/76 (13%)] were retested by the CLSI reference broth microdilution method, all isolates had a CLSI MIC of 1 mg/L and were reclassified as susceptible. Multilocus sequencing typing showed that all bloodstream isolates clustered in clade I (South Asian) with high degree of relatedness.

Conclusions:

The current survey confirms that *C. auris* has emerged in Greek hospitals. All bloodstream isolates belonged to clade I and were resistant to fluconazole and susceptible to echinocandins. Resistance of *C. auris* to amphotericin B, as per the SYO colorimetric MIC and the CDC breakpoint of 2 mg/L, should be verified by a standardized broth microdilution methodology. Further prevention measures should be implemented in hospitals in cooperation with the respective infection control committee in order to restrain further spread of the pathogen.

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Distribution of Aspergillus Species and Prevalence of Azole Resistance in clinical and environmental Samples from a Spanish Hospital

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Objectives: Surveillance studies are a primordial tool for tracking antifungal resistance, being this one of the main causes of the increasing mortality rates for fungal infections, such as those caused by *Aspergillus spp.* Among this genus, the emergence and spread of azole-resistant *Aspergillus fumigatus* strains is one of the major concerns due to its association with treatment failure in patients with invasive aspergillosis. The aims of this study were to determine the *Aspergillus* species distribution and azole resistance prevalence in the isolates during this 3-year prospective surveillance study in a Spanish Hospital.

Materials & Methods: In this study, a collection of 365 *Aspergillus* spp isolates were analysed, being 307 clinical and 58 environmental isolates. These isolates were identified using microscopic identification or sequencing β-tubulin regions. All isolates were screened for azole resistance by using an expanded agar-based screening method for azole-resistant *A. fumigatus.* If an isolate showed growth in at least one of the triazoles used in the screening method (voriconazole, posaconazole and itraconazole), antifungal susceptibility testing was performed following the EUCAST reference method to determine the minimal inhibitory concentration. To confirm azole resistance, the *cyp51A* gene, including its promoter, was amplified and sequenced. To perform an epidemiological characterization, *A. fumigatus* strains were included in an extensive genotyping, using the previously described typing method TRESPERG.

Results: During the 3-year study period *A. fumigatus* was the predominant species recovered with a total of 196 isolates (53.7%), 156 were clinical and 40 were enviromental. The rest of *Aspergillus* spp were: 55 *A. niger* (15.1%), 33 *A. terreus* (9%) and 29 *A. flavus* (7.9%). Several other less frequent species were identified, including 19 *A. nidulans* (5.2%) and 12 *A. lentulus* (3%), among other *Aspergillus* species (6.1 %). All *A. fumigatus* strains, were TRESPERG genotyped showing a very diverse population (116 genotypes) with 59.8 % of the strains being represented as a single genotype. Eleven TRESPERG genotypes were common among clinical and environmental *A. fumigatus* azole susceptible strains, even some strains that were isolated months apart. We describe the occurrence of two azole-resistant *A. fumigatus* isolates, one from clinical origin (from an azole-naïve patient) and another from environmental surveillance inside the Hospital. Both isolates have the azole resistance mechanism consisting in TR_{34}/L98H mutation in the azole target Cyp51A. TRESPERG genotyping showed that the strain from the environment (t04Bm1.2c22be07) was not isogenic with the patient strain (t02m1.1c09e11), but they did not share genotype with any of the azole susceptible strains.

Conclusions: *A. fumigatus* is the most frequent isolated species recovered in this study. *A. fumigatus* genotypes showed a very diverse population. However, several genotypes were shared among clinical and environmental strains, suggesting that patients hospitalized in different parts of the same hospital can be infected with the same strain since every patient might inhale the same spore population. The isolation of azole-resistant strains from a patient and the hospital environment is an interesting finding, suggesting that an effective analysis of clinical and environmental sources must be done to detect azole resistance in *A. fumigatus*.

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Epidemiological study of onychomycoses in Athens, Greece: a five-year retrospective analysis (2018-2022)

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Objectives:
Onychomycosis is a common nail disorder caused by dermatophytes, yeasts or non-dermatophyte molds (NDM's). As other conditions may resemble onychomycosis and as the fungal infection requires a long-term systemic antifungal treatment, the accurate detection and identification of the causal agent is mandatory. The aim of this retrospective study is to determine the epidemiology of fungal species related to the onychomycoses in the area of Athens during the last five years (2018-2022)
Materials & Methods:
The retrospective analysis included outpatients who visited the "Andreas Sygros" Hospital (Athens, Greece), a tertiary referral hospital of Dermatologic Diseases covering more than four million people of the Greek capital (almost half of the national population). The study population included 7,894 patients (2,423 men and 5,471 women) with clinically suspected onychomycosis. Mycological nail investigation was performed by conventional methods (direct microscopy with KOH 20% and cultures on Sabouraud dextrose agar and Sabouraud dextrose agar with actidione).
Results:
Onychomycosis was confirmed in 2,056 (26%) patients (884 men and 1,172 women). In women, 669 onychomycoses (57.1%) were localized in toenails and 503 (42.9%) in fingernails. In men, 689 onychomycoses (77.9%) were localized in toenails and 195 in fingernails (22.1%). In 794 (38.6%) cases, dermatophytes were isolated, followed by yeasts 677 (32.9%) and NDM's 274 (13.3%). Most frequently yielded dermatophytes were *Trichophyton rubrum* in 701 clinical samples (88.3%) and *T. interdigitale* in 93 (11.7%). *Candida albicans* was the most common among yeasts, isolated in 655 (96.7%) samples. Regarding NDM's, *Acremonium* spp. (98; 35.7%), *Fusarium* spp. (72; 26.3%), *Scopulariopsis brevicaulis* (58; 21,2%) and *Aspergillus terreus* (23; 8.4%) were the most frequent isolates. 311 (15.2%) case were found microscopically positive but without fungal growth (NFF) in the culture mainly due to local or systemic administration of antifungal drugs.
Conclusions:
The most common pathogen involved in onychomycosis worldwide and in our study is the anthropophilic dermatophyte *T. rubrum* in toenail infections in both genders. Fingernails infections were most common in women while toenails infections in men. As for the fingernail infections in both genders the most frequent cause is the yeast *C. albicans.* Continuous monitoring should be performed in order to identify possible trends and shifts in species isolation rates especially nowdays that resistant dermatophyte isolates have been reported worldwide.

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The current state of laboratory mycology and access to antifungal treatment in the BeNeLux – preliminary results

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Objectives: Recently a large survey evaluating the capacity for the management of invasive fungal infections (IFI) in Europe was performed. However, no results of Luxembourg were available and since the countries were grouped by GDP it was not possible to evaluate results on country level. Therefore, it was estimated that a local spin-off in Belgium, The Netherlands and Luxembourg (BeNeLux) could provide additional insights. In addition, with the incidence of azole-resistance being rather high in Belgium and especially in The Netherlands, zooming in on diagnostics contributing to the detection of resistance and resistance mechanisms in this part of Europe is of importance.

Materials & Methods: A survey evaluating the diagnostic and therapeutic capacity for IFIs was performed in the BeNeLux. Data were collected via an online electronic case report form between March and April 2023. The survey was sent to a selection of Belgian hospital laboratories of interest. In The Netherlands, the survey was spread by the general newsletter from the Dutch Society for Microbiology. In Luxembourg, the survey was spread via professional contacts. Fisher's Exact Test was used to determine whether there were significant differences between Belgian and Dutch responses. Luxembourg was not included in this comparison as it was it was underrepresented.

Results: Until now, 20 hospitals from Belgium, 1 from Luxembourg and 8 from The Netherlands have responded to the questionnaire. Of the 29 responding hospitals, 22 (75.8%) report a very low to mild incidence of IFI, 5 (17.2%) a high incidence and 2 (6.9%) a very high incidence. The most important pathogens were as expected *Candida* spp. and *Aspergillus* spp. Availability of diagnostic possibilities are summarized in Table 1. There were no significant differences between available diagnostic tests in Belgium and The Netherlands apart from the possibility of DNA sequencing for species identification which was more accessible in The Netherlands (p=0.03). Interestingly, in 41.4% of the responding hospitals the *Aspergillus* lateral flow assay (LFA) and in 31.0% the *Aspergillus* lateral flow device (LFD) is available. *Aspergillus* PCR testing was available in 14 centres in Belgium (70,0%) and in all 8 centres in The Netherlands. Other antifungal susceptibility tests were available in 26 institutions (89,7%) (85,0% in Belgium and 100% in The Netherlands, p=0.5). Treatment

with Amphotericin B was only available in 82.8% of the responding centres. Anidulafungin was only available in 1 Belgian responding centre but in 6 Dutch centres (p=0.0005). Therapeutic drug monitoring (TDM) of voriconazole is possible in 26 centres (89.7%) in total and TDM of posaconazole in 24 centres (82.8%).

Conclusions: We have identified an improved access to diagnostic tools compared to previous reports. The availability of *Aspergillus* lateral flow tests (LFTs) is higher than previously reported in a study evaluating implementation of LFTs in Belgium (implemented in 11%) and in the European survey (reporting use of LFAs in 33% and of LFDs in 24%). There also seems to be a larger availability of antifungal susceptibility test technologies in The Netherlands compared to Europe in general. As the survey is still ongoing, additional responses must be awaited to draw final conclusions.

Table 1

Diagnostic possibilities in the Benelux (overall = Belgium + The Netherlands + Luxembourg, BE = Belgium, NL = The Netherlands, GM = galactomannan, LFA = lateral flow assay, LFD = lateral flow device).

	Overall		*BE*		*NL*		*p-value*
	n	% (of replies)	n	% (of replies)	n	% (of replies)	
Total	29	100,0%	20	100,0%	8	100,0%	
Microscopy	28	96,6%	20	100,0%	8	100,0%	1
Methodologies							
Calcofluor white	21	72,4%	16	80,0%	5	62,5%	0.3715
Giemsa stain	15	51,7%	11	55,0%	4	50,0%	1
China/India ink	18	62,1%	11	55,0%	7	87,5%	0.1937
Potassium hydroxide	17	58,6%	11	55,0%	6	75,0%	0.4188
Silver stain	7	24,1%	4	20,0%	3	37,5%	0.3715
Access to fluorescence dye?	25	86,2%	17	85,0%	8	100,0%	0.536
Direct examination in body fluids when cryptococcosis suspection?	23	79,3%	15	75,0%	8	100,0%	0.2808
Silver stain when pneumocystosis suspection?	6	20,7%	3	15,0%	3	37,5%	0.3107
Direct microscopy when mucormycosis suspection?	15	51,7%	9	45,0%	6	75,0%	0.2213
Culture and fungal identification	29	100,0%	20	100,0%	8	100,0%	1
Fungal culture methods							
Agar Niger	4	13,8%	2	10,0%	2	25,0%	0.5546
Chromogen	15	51,7%	10	50,0%	5	62,5%	0.686
Lactrimel Agar	2	6,9%	0	0,0%	2	25,0%	0.07407
Potato Dextrose Agar	7	24,1%	3	15,0%	4	50,0%	0.1423
Sabouraud dextrose agar	25	86,2%	16	80,0%	8	100,0%	0.2947
Sabouraud dextrose agar + Chloramphenicol	22	75,9%	15	75,0%	7	87,5%	0.6399
Sabouraud dextrose agar + Gentamicin	15	51,7%	10	50,0%	5	62,5%	0.686
Selective agar (Chloramphenicol + Cycloheximide)	17	58,6%	11	55,0%	6	75,0%	0.4188
Available tests for specific identification	29	100,0%	20	100,0%	8	100,0%	1
Automated identification (i.e. VITEK, other commercial tests)	12	41,4%	8	40,0%	4	50,0%	0.6908
Biochemical tests (classic mycology)	17	58,6%	12	60,0%	5	62,5%	1
DNA sequencing	14	48,3%	7	35,0%	7	87,5%	*0.03285*
MALDI-TOF	27	93,1%	18	90,0%	8	100,0%	1
Mounting medium	8	27,6%	5	25,0%	3	37,5%	0.6508
Available antifungal susceptibility test technologies	26	89,7%	17	85,0%	8	100,0%	0.536
CLSI	12	41,4%	10	50,0%	2	25,0%	0.401
EUCAST	12	41,4%	6	30,0%	5	62,5%	0.1998
E-test	12	41,4%	7	35,0%	5	62,5%	0.2309

VITEK	8	27,6%	5	25,0%	3	37,5%	0.6508
Serology	23	79,3%	15	75,0%	7	87,5%	0.6399
Aspergillus spp.	22	75,9%	14	70,0%	7	87,5%	0.6334
Candida spp.	17	58,6%	11	55,0%	6	75,0%	0.4188
Histoplasma spp.	19	65,5%	14	70,0%	5	62,5%	1
Antigen detection	29	100,0%	20	100,0%	8	100,0%	1
Aspergillus overall	28	96,6%	20	100,0%	7	87,5%	0.2857
Aspergillus GM (ELISA)	17	58,6%	11	55,0%	5	62,5%	1
Aspergillus GM (LFA)	12	41,4%	9	45,0%	3	37,5%	1
Aspergillus GM (LFD)	9	31,0%	6	30,0%	3	37,5%	0.609
Candida antigen	12	41,4%	7	35,0%	5	62,5%	0.2309
Cryptococcus overall	26	89,7%	18	90,0%	7	87,5%	1
Cryptococcus (latex agglutination test)	20	69,0%	16	80,0%	4	50,0%	0.172
Cryptococcus (LFA)	19	65,5%	11	55,0%	7	87,5%	0.1937
Histoplasma	18	62,1%	12	60,0%	6	75,0%	0.6692
Beta-glucan	25	86,2%	17	85,0%	7	87,5%	1
Molecular tests	29	100,0%	20	100,0%	8	100,0%	1
Aspergillus PCR	22	75,9%	14	70,0%	8	100,0%	0.1412
Candida PCR	18	62,1%	11	55,0%	7	87,5%	0.1937
Pneumocystis PCR	29	100,0%	20	100,0%	8	100,0%	1
Mucorales PCR	18	62,1%	12	60,0%	5	62,5%	1

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Epidemiology and susceptibility of Nakaseomyces (formerly Candida) glabrata bloodstream isolates from hospitalised adults in South Africa

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Objectives:
During 2016-2017, *Nakaseomyces glabrata* (formerly *Candida glabrata*) was the fourth most common cause of candidaemia in South Africa causing 14% of cases. We aimed to describe the characteristics of hospitalised adults with *N. glabrata* candidaemia and the antifungal susceptibility and mechanism of echinocandin resistance of bloodstream *N. glabrata* isolates.

Materials & Methods:
This study was nested within laboratory-based surveillance for candidaemia in South Africa. A case was defined as a person with any *Candida* species isolated from blood culture (an episode of infection was defined by an arbitrary 30-day period). We only included adults aged ≥18 years at 3 private- and 17 public- sector hospitals classified as enhanced surveillance sites in 2016-2017. We compared the clinical characteristics of adults with *N. glabrata* candidaemia to those with non-*N. glabrata* candidaemia at 20 enhanced surveillance sites. Isolates accompanied by a laboratory report with patient metadata were submitted by diagnostic laboratories to a reference laboratory for confirmation of identification by matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) mass spectrometry. Antifungal susceptibility testing was performed using Sensititre YeastOne YO10 broth microdilution plates. *N. glabrata* isolates with minimum inhibitory concentrations (MICs) in the intermediate or resistant range for ≥1 echinocandin were screened for mutations at four hotspot regions of the *FKS* genes.

Results:
N. glabrata accounted for 20% (172/917) of adult cases at enhanced surveillance sites. A higher proportion of patients with *N. glabrata* candidaemia were older (median age: 55 years [interquartile range (IQR): 41-65 years] vs. 49 years [IQR: 35-63 years]; p=0.04), female (87/164, 53% vs. 283/671, 42%; p=0.01), admitted to a public-sector hospital (152/172, 88% vs. 470/745, 63%; p<0.001), treated with fluconazole only (most with suboptimal doses) (51/95, 54% vs. 139/361, 39%; p<0.001), and had had surgery (47/172, 27% vs. 123/745, 17%; p=0.001) and a shorter hospital stay (median 7 days [IQR: 2-20 days] vs. 13 days [IQR: 4-27 days]; p<0.001) compared to patients with other causes of candidaemia. Ninety per cent (46/51) of patients with *N. glabrata* candidaemia who were treated with fluconazole only were given a daily dose of <800 mg while the remainder received ≥800 mg daily (5/51, 10%). Eight *N. glabrata* isolates (6%, 8/131) were screened for *FKS* mutations and had an R1377K amino acid substitution encoded by the hotspot 2 region of the *FKS2* gene. Only 11 isolates (8%, 11/131) were resistant to fluconazole. Ten of the 11 fluconazole-resistant isolates also had MICs in the non-wild type range for posaconazole and voriconazole and four had an MIC greater than the itraconazole epidemiological cut-off value.

Conclusions:

Our study provides insight into the clinical characteristics of patients with *N. glabrata* candidaemia in South Africa. We observed low levels of resistance to echinocandins and azoles. Most patients with *N. glabrata* candidaemia were treated with suboptimal doses of fluconazole but should ideally be treated with an echinocandin or polyene, thus further guideline training is required.

P256

Prevalence and association between exposure to aspergillus spores in indoor and outdoor air and sensitization among asthmatics-A case-control aero-mycological study

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Objective

To assess the prevalence and association between exposure to Aspergillus Spores in indoor and outdoor air and sensitization among patients with asthma

Methods

We used a case-control study design in which the asthma patients with Aspergillus sensitization (AS) formed the case and those without AS formed the controls. The study was conducted in the households of the selected cases and controls resident of rural and urban areas in northern India. We collected five samples (three indoor, i.e., bedroom, living room, kitchen and two outdoor, i.e., just outside the house and away from the house but in the same street) per household. We used a sieve air sampler (BioMe'rieux AESAP1076, Sampl'air™ Pro or high-performance microbial air sampler, Bruz, France) for sampling the ambient air. The spores were allowed to impact on 90mm petri plates of dichloran rose-bengal (DRBC) agar (BD Difco) with a flow rate of 100L/min for one minute. The plates were then transported to a microbiology facility and incubated at 28°C and 37°C for up to 48 hours. Any growth of Aspergillus molds was identified using standard mycological methods (gross morphology) and microscopic finding with Lactophenol Cotton Blue (LCB) stain.

Results

Of the total 325 air samples collected, 195 (60%) were indoor air samples and the remaining were outdoor air samples. Of the 141 indoor air samples from case households, 114 (80.9%), 99 (70.2%) and 57 (40.4%) showed the growth of Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus, respectively. The same for control households (n-54) were 46 (85.2%), 47 (87.0%) and 25 (46.3%) for

Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus, respectively. Similarly, the colony forming units (CFU/m^3) observed for Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus in the indoor environment of case and control were 8.0, 5.0, & 1.0 and 14.0, 6.5, & 2.0, respectively. The Aspergillus growth (presence and CFU) in the indoor air samples was not significantly different between case and control households except for the high prevalence of Aspergillus flavus in control households (p-0.015).

Of the 94 and 36 outdoor air samples in case and control areas, 78 (83.0%), 59 (62.8%) & 39 (41.5%), and 33 (91.7%), 27 (75.0%) & 14 (38.9%) had the growth of Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus, respectively. Similarly, the CFU/m^3 observed in ourdoor air samples of case and control areas was 7.0, 3.0, & 1.0, and 6.0, 4.5, & 4.5 for Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus, respectively. No statistically significant difference was observed between case and control areas for the presence and quantity of growth of various Aspergillus species in outdoor air samples.

Conclusions

We observed a high prevalence of Aspergillus spores in indoor and outdoor air samples in households of asthmatic patients with or without AS. However, no significant difference in the prevalence (except for Aspergillus flavus) and quantity of Aspergillus growth was observed between case and control households. A prospective follow-up study quantifying the aspergillus spores in indoor and outdoor environments and AS periodically among asthmatics is the need of the hour.

P257

Candida auris: Outbreak, surveillance and epidemiological monitoring in Northern Greece

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Objectives

Candida auris is an emerging, multi-drug resistant yeast. In October 2022, the first case of *C. auris* in Northern Greece was reported in Thessaloniki, almost two years after the first isolation in Greece (Athens 2019). The prompt and accurate identification of the fungus is crucial. However, its identification can be challenging and requires multiple and specialised laboratory methodologies. The Mycology Laboratory of the Medical School of Aristotle University of Thessaloniki stands as the reference laboratory for fungal identification and monitoring in Northern Greece. A meticulous search for the yeast, in plenty of suspicious samples, has been run till 2019 in the Lab and also a retrospective control of all its yeasts' collections, back to 2008, with negative results. Here, we present our findings concerning the outbreak and surveillance of *C. auris* in Northern Greece, mainly the region of Thessaloniki and the broader Central Macedonia, being the second more populated area of Greece, from October 2022 till the end of May 2023. To our knowledge, this is the largest continuous, regionally organised, epidemiological-surveillance study of *C. auris* in our country till now.

Methods & Materials

Sixty-two isolates of *C. auris* were referred, mainly from three tertiary regional hospitals. The identification and confirmation consisted of phenotypical methods such as microscopy, growth at 42^{o}C and chromogenic media, an in-house evaluated PCR and MALDI-TOF MS. MICs of various antifungal drugs were measured, by commercial and reference methods. Selected strains, according to the referring hospital, were sequenced for both D1-D2 and ITS1 ribosomal regions, for molecular confirmation and multilocus phylogenetic analysis. Alignment was performed by ClustalW and phylogenetic analysis by PAUP (v.4.0a) and MrBayes (v.3).

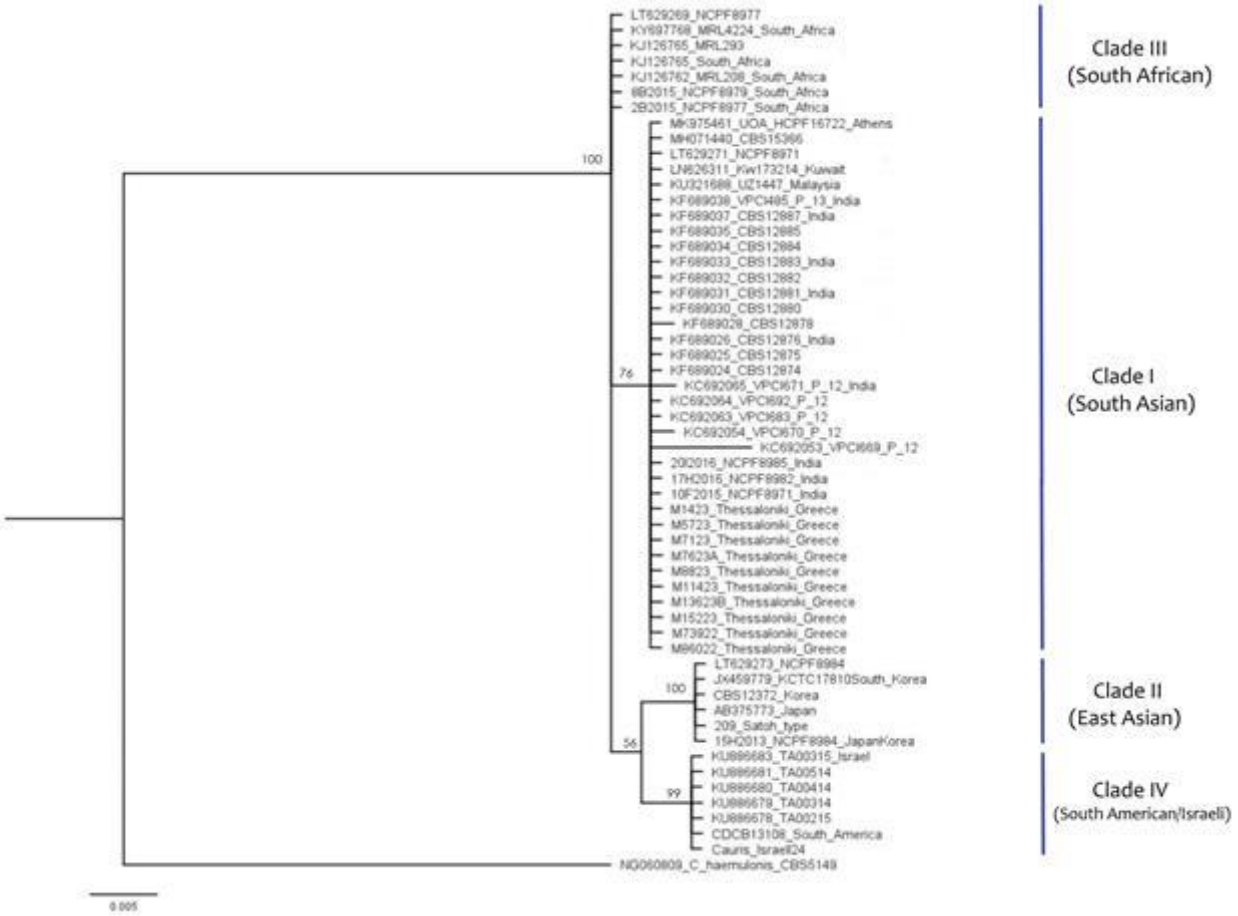
Results

The strains (36 from ICUs, 21 from Internal Medicine and Subspecialties Departments, 5 from Surgical Departments) were isolated from 61 hospitalised patients (37 men-24 women, mean

age: 64.24±17.02 years, 18-92) and one oximeter. According to the data, 32 isolates concerned colonisations and 13 deeper sites (among them, blood and urine, two/eight). There were 27 recorded deaths but only one was directly attributed to *C. auris*. All isolates were resistant to fluconazole (geometric mean: 108.24 μg/mL, 32-256, modal 128) with a sporadic tendency for higher MICs for voriconazole (gm: 1.50 μg/mL, 0.13-4, modal: 4) and sensitive to all other drugs (amphotericin-B, 5-flucytosine, isavuconazole, itraconazole, posaconazole, echinocandins). The phylogenetic analysis (figure 1) revealed that the isolates of our region cluster with the South-Asian (Clade I) strains, similarly to other strains from Greece.

Conclusion

Candida auris presents increased ability for colonisation and horizontal spreading and tends to affect seriously ill, more vulnerable hospital patients. It can be a direct threat for clinical prognosis and normal flow work in health system, and influences the health care infrastructure and finance. Although it seems for the moment that its spreading in our region is under a relative control and that there are still therapeutic alternatives, nothing is certain for the future. Well organised surveillance approaches and continuous monitoring are constantly needed in order to keep a high level of preparedness and efficient response.



P258

Prevalence and burden of chronic pulmonary aspergillosis in patients with post-tuberculosis lung disease: a community survey

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Prevalence and burden of chronic pulmonary aspergillosis in patients with post-tuberculosis lung disease: a community survey

ABSTRACT

Background: Post-tuberculosis lung disease (PTBLD) is the most common risk factor for developing chronic pulmonary aspergillosis (CPA). However, the prevalence of CPA in patients with PTBLD in India remains unknown.

Methods: In a cross-sectional study between November 2021 and August 2022, we identified subjects who had received anti-tuberculosis therapy (ATT) before November 2019 from the records of the 12 tuberculosis treatment centers attached to the national program. We used a structured clinical assessment form to record clinical and demographic details. We also performed computed tomography (CT) of the chest and estimated serum *A.fumigatus*-specific IgG. We categorized subjects as definite CPA, probable CPA, or diseased controls using a composite of clinical, radiological, and microbiological features. We further estimated the number of prevalent CPA cases in India that occurred following pulmonary tuberculosis treated in the year 2019 in India.

Results: We included 117 subjects with PTBLD, with a median time to enrolment after ATT completion being three years. The prevalence of CPA in PTBLD subjects was 22.2% (26/117). We diagnosed definite and probable CPA in 9/117 (7.7%) and 17/117 (14.5%) subjects, respectively. Using our results, we estimated 103,485-186,979 CPA cases who were treated in 2019 in India.

Conclusion: There is a significant burden of CPA in subjects with PTBLD. National TB program should incorporate screening for CPA in symptomatic subjects with PTBLD.

Key words: post-TB lung disease, bronchiectasis, aspergilloma, chronic cavitary pulmonary aspergillosis, CFPA

P259

Frequency of Candida sojae among other Candida species in paediatric haematooncology patients.

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Frequency of *Candida sojae* among other Candida species in paediatric haematooncology patients.

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Objectives: Invasive candidiasis (IC) is important cause of morbidity and mortality in immunocompromised host. We aimed to investigate *Candida sojae* occurrence described being involved in human infection. Our aim was to describe the frequency of *C. sojae* among the other Candida species in paediatric haematooncology patients and to characterize the *C. sojae* isolates.
Materials & Methods: We collected data about all Candida species detection at the Department of Paediatric Haematology and Oncology (DPHO) between January 2015 - December 2022. Due to epidemiological reasons, all patients are weekly monitored with GIT swabs during the haematooncological therapy. Detection of Candida species including *Candida sojae* was performed at Department of Medical Microbiology of Motol University Hospital. Cultured isolates were identified by MALDI-TOF and ITS2 sequencing. Only single Candida isolate per patient was calculated ignoring repeated detections. Whole genome sequence analysis was performed using short reads (Illumina) and combination of short reads and long reads (MinION) for selected strains.
Results: There were 3733 Candida species positive samples from 733 patients (representing 1042 of isolates). The majority of Candida species were cultured from GIT samples 89 % (rectal or oral cavity swab). The frequency of Candida species in patients was as follows: *C. albicans* (56 %), *C. dublinensis* (12 %.), *C. glabrata* (2.4 %), *C. krusei (2.3 %*), *C. parapsilosis* (3.9 %.), *C. tropicalis* (1.5 %), while *C. sojae* was detected in 22 patients representing 2.1 % of isolates.
Samples attributable to invasive candidiasis were positive for yeasts in 39 patients (5.3 % of patients with Candida detection). Candida species distribution in invasive samples was as follows: *C. albicans* (18 pts), *C. dublinensis* (2 pts.), *C. glabrata* (2 pts), *C. krusei* (3 pts), *C. parapsilosis* (8 pts.), *C. tropicalis* (1 pt).
All *C. sojae* isolates were detected in the rectal swab and oral cavity swab suggesting colonisation of GIT and in none patient invasive infection with *C. sojae* was observed. Thirteen isolates were available for further sequence testing, unfortunately the results were not available at the time of the abstract submission and will be presented at the conference.
During this period, there were also 7 isolates of *C. sojae* from patients outside of the DPHO including the first reported human invasive infection. The overall frequency of *C. sojae* in Motol University Hospital was estimated 0.15 %.

Conclusions: *Candida sojae* has been recently described in invasive human infection. It is very rarely detected in overall patient population, but there is no routine screening of GIT yeasts repartition among general patients. Observed frequency of *C. sojae* of 2.1% among all Candidas is similar to species detection of *C. krusei, C. parapsilosis* and *C. fabianii* in DPHO patients. No invasive *C. sojae* infection occurred yet in patients of DPHO. Further studies are needed to evaluate the frequency and impact of *C. sojae* in general patient population.

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P260

Natural history of allergic bronchopulmonary aspergillosis: a long-term follow-up study of 182 subjects

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Natural history of allergic bronchopulmonary aspergillosis: a long-term follow-up study of 182 subjects

ABSTRACT
Background: The natural history of allergic bronchopulmonary aspergillosis (ABPA) in terms of response and exacerbations is not well characterized.
Objectives: Our primary objective was to estimate the incidence rate of ABPA exacerbation. The secondary objectives included the: (1) frequency of patients experiencing exacerbation at various time-points; (2) percentage decline and increase in serum total IgE noted during response and exacerbation, respectively; (3) factors predicting exacerbation in subjects with ABPA.
Methods: We retrospectively included treatment-naïve subjects of ABPA-complicating asthma from three randomized controlled trials. All the subjects received oral prednisolone (0.5 mg/kg/day, 0.25 mg/kg/day, and 0.125 mg/kg/day for 4 weeks each. Then tapered by 5 mg every two weeks and discontinued after four months)**.** The patients were followed up every six weeks for six months, and then every six months. We defined treatment response as improvement (>75% of baseline) in cough and dyspnea, and clearance (≥50%) of chest radiographic opacities or improvement in FEV1 values compared to baseline. Exacerbation was defined as worsening patient symptoms and appearance of radiological opacities consistent with ABPA. The decline in serum total IgE (percentage) during response was calculated as: baseline IgE minus IgE values after six weeks of prednisolone therapy divided by the baseline IgE. Similarly, the percentage increase in serum total IgE during exacerbation was calculated as: exacerbation IgE levels minus IgE last recorded during clinical stability (pre-exacerbation IgE) divided by the pre-exacerbation IgE. We performed a multivariate logistic regression analysis to determine the factors predicting ABPA exacerbations.
Results: We included 182 subjects with ABPA-complicating asthma. Eighty-one patients experienced 120 exacerbations during 512 patient-years of follow-up. The incidence rate of ABPA exacerbations was 234/1000 patient-years. Majority of the subjects (62/81, 76.5%) experience ABPA exacerbation within two years of stopping therapy. The mean (range) time-to-first ABPA exacerbation was 608 (126-2279) days, while the mean (range) time-to-second ABPA exacerbation was 1013 (332-1928) days after stopping prednisolone therapy. The mean (95% CI) decline in IgE after six weeks was 49 (46-52) percent. The mean (95% CI) increase in IgE during exacerbation was 243 (190-296) percent. On multivariate logistic regression analysis, after adjusting for serum total IgE, *A.fumigatus*-IgE, and hyperattenuating mucoid impaction, only peripheral blood eosinophil count ≥1000 cells/μL (adjusted odds ratio [aOR] 2.68; 95% CI, 1.41-5.09) and the extent of bronchiectasis (aOR 1.10; 95% CI, 1.04-1.18) independently predicted the occurrence of an ABPA exacerbation.

Conclusions: We found ABPA exacerbations uncommon after two years. Peripheral blood eosinophil counts >1000 cells/µL and the extent of bronchiectasis predicted an ABPA exacerbation.

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Exploring the impact of the introduction of Elexacaftor/Tezacaftor/Ivacaftor for Cystic Fibrosis treatment and its potential impact on Aspergillus-Related Diseases

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Objectives

The introduction of the ETI combination, a triple therapy comprising elexacaftor, tezacaftor, and ivacaftor, has significantly broadened the therapeutic options for patients with Cystic Fibrosis (CF). Extensive double-blind, randomized, phase 3 trials have provided compelling evidence of the ETI combination's effectiveness in improving CFTR function and ensuring patient safety. These studies have also indicated a reduced frequency of pulmonary exacerbations and a rapid decline in both bacterial infections and the utilization of antimicrobial agents. However, the available data concerning the impact of ETI on fungal colonization and sensitization in CF patients remain limited. Therefore, our objective was to assess the influence of ETI therapy on colonization and fungal sensitization in individuals with CF.

Materials & Methods

A single-center retrospective observational pilot-study was conducted on 90 patients who received ETI therapy to evaluate its effect on colonization and fungal sensitization. Biological data retrieved, before and after initiation of ETI therapy, compiled results of sputum mycological cultures, anti-*Aspergillus* IgG antibody titers measured by ELISA, anti-*Aspergillus* precipitating antibodies by immune-electrophoresis, total IgE and anti-*Aspergillus fumigatus* IgE titers.

Results

In this study, a notable and swift reduction in the proportion of positive sputum cultures for Aspergillus spp. was observed for he CF patients included. Furthermore, there was a significant decrease in the levels of anti-*Aspergillus* precipitating antibodies measured through immune-electrophoresis, as well as a significant reduction in total IgE antibodies, following the initiation of the ETI combination therapy.

Conclusions

The findings from our pilot study provide support for the hypothesis that the initiation of ETI therapy leads to a rapid improvement in the clearance of *Aspergillus* in the lungs of CF patients. Should these encouraging results be substantiated by larger-scale studies, the implications could be transformative, leading to significant changes in the follow-up and treatment practices for individuals with CF.

P262

A prospective longitudinal study of chronic pulmonary aspergillosis in newly diagnosed pulmonary tuberculosis patients from diagnosis till end-of-treatment

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Objectives: Chronic pulmonary aspergillosis (CPA) and pulmonary tuberculosis (PTB) can co-exist together and may complicate the clinical course of each other. However, there is limited literature regarding *Aspergillus* co-infection in newly diagnosed cases of pulmonary tuberculosis and the incidence of CPA during and at end-of-therapy. Primary objective of this study is to estimate the frequency of CPA in a newly diagnosed PTB at baseline and at end-of -therapy (after six months of anti-tubercular therapy).

Materials & Methods: This was a prospective longitudinal study done over two years. Patients with newly diagnosed PTB were evaluated at baseline, third month and end-of-therapy with symptom assessment, anti-*Aspergillus* IgG antibody estimation (ImmunoCAP Asp IgG assay, Thermo Fisher Scientific Inc, >27 mg/L considered as positive) and radiological imaging of chest (chest x-ray and/or CT thorax). CPA diagnosis was done on the basis of clinico-radio-serological criteria (ERS/IDSA/ESCMID).

Results: A total of 256 patients were recruited at baseline out of which 156 (61%) patients completed their follow-up. At baseline ~ 50 % patients had microbiological evidence of PTB. Anti-*Aspergillus* IgG had been performed in 249 patients at baseline and was positive in 10.8% at baseline, 26.9% at third month and 31.8% at end-of-therapy. Overall, the criteria of proven CPA were fulfilled in 7%, 11% and 14% patients at baseline, third month and end-of-therapy respectively. Cough (23%) and loss of appetite (19%) were the most common symptoms seen in these patients at baseline and persistent shortness of breath (31%) and fatigue (24%) were most common symptoms noted at the end-of-therapy. The most frequently isolated species of *Aspergillus* was *Aspergillus flavus*. Radiologically, nodules (79%) and consolidation (62%) were the most commonly seen radiological findings at the baseline while fibro-parenchymal opacities (95%) and nodules (72%) were the most common radiological findings at the end of therapy. Cavity was found in 22% patients at baseline and 15% patients at the end-of- therapy. 6% patients had evidence of aspergilloma in CT chest at the end-of-therapy.

Conclusions: CPA can coexist in newly diagnosed PTB patients at the time of diagnosis and develop during treatment as well as at end-of-anti-tubercular treatment. Patients with persistent symptoms or developing new symptoms during treatment for PTB should be evaluated for CPA.

P263

The ReCap study: preliminary results of a nationwide French multicenter prospective study of Candida parapsilosis resistance to fluconazole

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Objectives:

Candida parapsilosis is one of the most common species implicated in invasive candidiasis and candidemia. It has also been implicated in nosocomial infections occurring in epidemic mode.

In recent years, fluconazole-resistant isolates of *C. parapsilosis* have emerged on a global scale, and are steadily increasing in number without any clear explanation for this sudden emergence. These isolates, which mainly carry the erg11 Y132F alteration that confers resistance to fluconazole, are also characterized by their ability to form clusters and to create local or locoregional outbreaks in hospitals.

In this context, we initiated the ReCap - Resistance of *Candida parapsilosis* to azole drugs - study. This is a prospective national multicenter study (France) that aims to describe the epidemiology of *Candida parapsilosis* resistance to fluconazole. The objectives were to determine the percentage of resistance among all *Candida parapsilosis* isolated in hospitals and to evaluate the existence of a relationship between resistance, clonality and clinical impact (infection vs. colonization, mortality, response to treatment).

Materials & Methods:

To achieve this objective, an exhaustive study of *C. parapsilosis* isolates identified in mycology laboratories of 16 hospitals throughout France was carried out. For a period of 9 months, each center includes all *C. parapsilosis* isolates routinely identified from clinical specimens received at the laboratory, covering all types of specimen and all clinical departments. To detect resistant isolates, a systematic screening of all isolates was carried out using the Etest®. Resistant isolates and a random selection of susceptible isolates are subjected to further analyses : i. determination of susceptibility using the EUCAST method, ii. genotyping (microsatellites) and iii. erg11 sequencing. Some clinical data were also collected.

Results:

To date, 1545 isolates have been included from 16 centers (range: 16-184/center). Among them **1265 isolates have been tested with a fluconazole** Etest strip. Of these, 122 isolates

obtained from 60 patients were resistant to fluconazole MIC > 4 mg/L. Resistance was confirmed by EUCAST. Overall, the percentage of resistant isolates ranged from 0 to 44%, depending on the center, with the percentage of patients carrying a resistant isolate ranging from 0% to 31%. Importantly, **genotyping analysis (available for 317 isolates)** indicate that 2 clusters are circulating in different hospitals of the Paris area. Erg11 sequencing available for some isolates indicated the presence of the Y132F alteration and will be carried out for the rest of the isolates. Investigations are ongoing to assess the potential impact of resistance on clinical form (infection versus colonization) and patient outcome.

Conclusions:
The preliminary results of our study indicate that in France, *C. parapsilosis* resistance to fluconazole concerns 9.8% of isolates but with very significant variations between regions. Paris area is affected by the circulation of resistant isolates forming clusters and involving a large number of patients.

P264

Species distribution and antifungals susceptibility of clinical isolates of Penicillium and Talaromyces from respiratory samples in a French University Hospital

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Objectives: *Penicillium* spp. and *Talaromyces* spp. are ubiquitous molds widely distributed in the environment and associated with food spoilage and mycotoxin production. Despite the ubiquity of these fungi in air, their clinical significance is not well understood. Although *Penicilliums* are generally considered to be non-pathogenic colonizers, or even laboratory contaminants, they are increasingly being reported as rare opportunistic pathogens in humans. The distribution of species isolated from human clinical specimens is not well known due to the difficulty of morphological identification of these fungi and the complexity of the current taxonomy. The aims of this study were to identify clinical isolates from respiratory samples of *Penicillium* spp. and *Talaromyces* spp. at the species level and to determine their antifungal susceptibility profiles.
Materials & Methods: One hundred and three morphologically fungi identified as "*Penicillium-like*" were collected from respiratory samples of patients of a Parisian University hospital during one year (2021). Demographic and treatment data were analysed using the hospital's information system database. Species identification was performed by Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using the online MSI-2 fungal database. The determination of antifungals susceptibility to 8 antifungals were performed by concentration gradient strips method (E-test®).
Results: Among the 103 "*Penicillium-like*" fungi, 87 (83.5%) were *Penicillium* spp. and 16 (15.5%) were T*alaromyces* spp. Isolates were collected from respiratory samples of 96 patients including bronchial alveolar lavages (n=61), bronchial aspirations (n=19), sputa (n=18), tracheal aspirations (n=3) and others samples (n=2). The majority of patients were immunocompromised, including hematological disorders (n=22), solid tumor (n=17), HIV (n=9), solid organ transplants (n=8), but also respiratory pathologies such as bronchiectasis (n=11) or asthma (n=3). Among the species, *Penicillium crustosum* was the most common with 13% (11/87), followed by *Penicillium rubens* (11%; 10/87) and *Penicillium chrysogenum* (10%; 9/87). Among the 16 *Talaromyces* spp. isolates, six species were identified, the most frequent being *Talaromyces amestolkiae* (25 %; 4/16), *Talaromyces columbinus* (25 %; 4/16) and *Talaromyces australis* (19%; 3/16). Two *Talaromyces* isolates caused probable invasive fungal infections in two patients with haematological disease. Regarding the antifungals susceptibilities, some species showed high Minimal Inhibitory Concentrations (MICs) to azoles or amphotericin B. Among these species, *P. citrinum* (n=5), *P. allii* (n=2), *P. hetheringhonii* (n=1), *P. roseomaculatum* (n=1), *T. amestolkiae* (n=4), *T.columbinus* (n=4), *T. australis* (n=3), *T. diversus* (n=2), *T. ruber* (n=1) and *T. rubrifaciens* (n=1) had MIC up to 32 mg/L to azoles. Furthermore, *P. rubens* (n=10), *P. chrysogenum* (n=9), *P. roquefortii* (n=6) and *P. griseofulvum* (n=3) had high MIC up to 32 mg/L to amphotericin B.
Conclusions: Although *Penicillium* and *Talaromyces non-marnefeii* infections in humans are rare, it is important to know the distribution of the species according to geographical areas. The resistance profiles observed in certain species highlight the importance both of identifying

these fungi at species level and of knowing their sensitivity profile to the antifungal agents used in clinical practice.

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Epidemiological landscape of fungemia due to rare opportunistic yeasts in a Greek tertiary care academic hospital

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Objectives: Fungemia due to rare yeasts (RYs) is an emerging but less investigated condition. Intrinsic resistance/reduced susceptibility of RYs to echinocandins or fluconazole constitutes a challenge in empirical treatment. Regional differences in the prevalence of RY invasive infections render knowledge of their local epidemiological patterns essential (Chen *Lancet Infect Dis.* 2021). Data on their epidemiological trends in Greece are currently scarce. We therefore conducted a retrospective 14-year survey describing the epidemiology of RY fungemia in a Greek tertiary care teaching hospital.

Materials/methods: All microbiologically confirmed other than *Candida* fungemias in patients hospitalized in "Attikon" university hospital during the period 01/04/2009-31/03/2023 were recorded. Patients' demographic and clinical characteristics were obtained from their medical records (where available). The incidence rate of the infection was expressed as the ratio of episodes per 1,000 hospital admissions/10,000 hospital bed days. The isolated strains were identified by Vitek2. If stored, they were retrospectively identified by MALDI-ToF MS and were subjected to *in vitro* antifungal susceptibility testing according to the EUCAST E.DEF7.3.2 and CLSI M27A4 guidelines.

Results: Over the 14-year period, 31 RY fungemias were identified. Their overall incidence was 0.04/1,000 hospital admissions (0.11/10,000 bed days) accounting for the 4% (31/839) of total fungemic episodes. Most (23/31; 74%) of the cases occurred in patients admitted in internal medicine wards, 6/31 (20%) in surgical wards and 2/31 (6%) in ICUs. The episodes were found in all units and were distributed almost equally during the years (no clusters were recorded). There were 20 (64%) male patients of median (range) age 69 (17-88) years. Haematological/oncological malignancy (15/31; 48%) and diabetes (10/31; 32%) were the most frequent underlying disorders. 12/31 (39%) bloodstream infections were due to *R. mucilaginosa*, 9/31 (29%) to *S. cerevisiae*, 6/31 (19%) to *T. asahii* and 4/31 (13%) to *C. neoformans* (25/31; 81% identified by MALDI-ToF MS, MALDI-ToF MS-Vitek2 agreement 100%). Bloodstream coinfection with multi-drug resistant gram negative pathogens was recorded in 5/31 (16%) patients. Amphotericin B showed high *in vitro* activity against all the isolates tested. Echinocandins were only active against *S. cerevisiae*, while the degree of activity of each azole was species-specific(**Table**). Antifungal consumption data were available for 21/31 patients, whereof in 5/21 (24%) fungemia developed as breakthrough infection while receiving either fluconazole (2 *R. mucilaginosa, 1 S. cerevisiae*) or anidulafungin (1 *S. cerevisiae*, 1 *T. asahii*). 16/21 patients were treated with liposomal amphotericin B [7 *R. mucilaginosa* (5 alive) and 2 *C. neoformans* (1 alive)], echinocandins [4 *S. cerevisiae* (2 alive)] or liposomal amphotericin B followed by voriconazole [3 *T. asahii* (1 alive)], whereas the remaining 5 did not receive antifungal treatment since they died before notification of positive blood culture. The crude mortality rate within hospital stay was 58% (18/31).

Conclusions: RY fungemia is infrequent in our centre, but it is significantly associated with the development of breakthrough infections and has a considerable mortality rate. Knowledge about its local epidemiology and heightened alertness for its timely recognition remain crucial in initial antifungal treatment pending identification to species level and susceptibility profile of isolated pathogens.

Table. Antifungal susceptibility patterns among rare yeast bloodstream isolates.

Species (number of isolates)	Antifungal agent	Median (range) CLSI MIC (mg/L)	Median (range) EUCAST MIC (mg/L)
R. mucilaginosa (*n*=8)	AMB	0.5 (0.5-1)	0.25 (0.25-0.5)
	VRC	2 (1-4)	2 (1-4)
	POS	2 (1-2)	1 (0.5-2)
	ISA	0.25 (0.25-0.5)	0.25 (0.25-2)
	ITC	1 (1-1)	0.5 (0.5-1)
	FLC	>128 (128->128)	>128 (64->128)
	AFG	>4 (>4->4)	>4 (4->4)
	CAS	>4 (>4->4)	>4 (4->4)
	MFG	>4 (>4->4)	>4 (>4->4)
S. cerevisiae (*n*=7)	AMB	0.25 (0.25-0.5)	0.25 (0.25-0.25)
	VRC	0.125 (0.06-0.25)	0.25 (0.25-0.25)
	POS	0.5 (0.25-1)	0.5 (0.25-1)/1 (0.25-1)
	ISA	0.25 (0.125-0.25)	1 (0.5-1)
	ITC	16 (1-16)	8 (0.5-16)
	FLC	8 (4-8)	16 (8-16)
	AFG	0.06 (0.06-0.125)	0.125 (0.06-0.125)
	CAS	0.06 (0.06-0.5)	0.5 (0.5-1)
	MFG	0.125 (0.125-0.25)	0.125 (0.06-0.125)
T. asahii (*n*=6)	AMB	0.5 (0.5-1)	1 (1-1)
	VRC	0.125 (0.06-0.125)	0.125 (0.06-0.25)
	POS	0.25 (0.125-0.25)	0.5 (0.25-0.5)
	ISA	0.25 (0.125-0.25)	0.125 (0.06-0.5)
	ITC	0.25 (0.125-0.25)	0.5 (0.125-0.5)
	FLC	4 (2-4)	16 (8-16)
	AFG	>4 (>4->4)	>4 (4->4)
	CAS	>4 (>4->4)	>4 (4->4)
	MFG	>4 (>4->4)	>4 (>4->4)
C. neoformans (*n*=4)	AMB	0.5 (0.25-0.5)	0.25 (0.25-0.5)
	VRC	0.03 (0.03-0.03)	0.06 (0.06-0.06)
	POS	0.06 (0.03-0.25)	0.125 (0.125-0.5)
	ISA	0.016 (0.008-0.016)	0.06 (0.03-0.06)
	ITC	0.06 (0.06-0.06)	0.125 (0.125-0.25)
	FLC	4 (2-4)	4 (4-4)
	AFG	>4 (>4->4)	>4 (>4->4)
	CAS	>4 (>4->4)	>4 (>4->4)
	MFG	>4 (>4->4)	>4 (>4->4)

AMB: amphotericin B, VRC: voriconazole, POS: posaconazole, ISA: isavuconazole, ITC: itraconazole, FLC: fluconazole, AFG: anidulafungin, CAS: caspofungin, MFG: micafungin

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Epidemiological characteristics of cryptococcosis cases at a tertiary care centre in India

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Objectives: Cryptococcosis is considered as one of the AIDS-defining illnesses and exhibits high morbidity and mortality. The reports of cryptococcosis in non-HIV patients globally prompted us to determine the epidemiological characteristics among cryptococcosis patients at our tertiary care centre in North India.

Methods and Materials: This was a single-centre retrospective study conducted at our tertiary care hospital during 2021-2022. All cases diagnosed with cryptococcosis based on combination of clinical presentation with either demonstration of capsulated yeast on microscopy (India Ink) or isolation of *Cryptococcus* in culture and/or patients with cryptococcal antigen positivity in CSF/serum, were included in the study. Antifungal susceptibility testing was done for isolates as per CLSI guidelines M27 4th ed. Data was analysed using SPSS version 29.0.1.0.

Results: A total of 137 cases were diagnosed with cryptococcosis (CSF-89.3%, blood-6.8%, brain abscess-2.9%, lung fine needle aspirate-1%) during the study period. A detailed clinical history was obtained in 103 cases. The mean (±SD) age of the patients was 36.16±14.3 years. Majority of the patients were males (80.6%). The most common predisposing factor was human immunodeficiency virus (HIV) (60.2%) followed by transplant (6.8%), liver cirrhosis (2.9%), malignancy (2.8%) and diabetes milletus (1.9%). Around 30% patients were apparently immunocompetent. The common symptoms included fever (78.6%), headache (59.2%), nausea/vomiting (39.8%), altered mental sensorium (32%), seizures (19.4%), visual disturbances (14.6%), neck stiffness (13.6%) and speech disturbances (9.7%). Other

symptoms included generalised weakness, loss of weight and appetite, cough, dyspnoea, chest pain, abdominal distension and pain. The mean (±SD) duration of symptoms was 27.93 (±43.7) days *C. neoformans* was the most common isolated species. Majority of the patients were managed using AMB alone (38.1%); combination of AMB and 5-FC (22.9%); combination of AMB and fluconazole (17.14%) and using fluconazole monotherapy (5.7%). The treatment regimen inclusive of induction {amphotericin B (AMB) with 5-flucytosine (5-FC)} and maintenance (fluconazole) therapy was used only in 7.6%. Antifungal susceptibility testing showed minimum inhibitory concentration as follows (µg/ml): AMB 0.25-4, fluconazole 0.12-2, voriconazole 0.03-0.06, itraconazole 0.03-0.12, posaconazole 0.03-0.12, caspofungin 0.06-8, anidulafungin 0.03-4 and micafungin 0.03-16 ug/ml. 74.8% had a favourable outcome, while 25.2% succumbed to the illness. The patients presenting with stiff neck (26.9% vs 9.1%, p=0.042) and altered mental status (53.8% vs 24.7%, p=0.008) had significantly higher mortality. The patients who were given 5-flucytosine had better survival (38.7% vs 13%, p=0.024). Comparing characteristics between HIV and non-HIV patients revealed significantly shorter duration of symptoms in the former group (19.69±18.25 vs 40.53±64.35 days, p=0.006) while all other factors did not show any significant difference.

Conclusion: The present study describes clinicoepidemiological characteristics of cryptoccoccosis cases from North India. Middle aged HIV positive males were noted to be at highest risk. We report a considerable number of cases in apparently immmunocompetent patients. *C. neoformans* is the most common species causing the infection in North India. The higher MIC against AMB (drug of choice) needs to be investigated. The administration of 5-FC in the regimen shows better survival and its inclusion in the management protocol needs emphasis particularly in developing countries with high burden like India.

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Comparison between candidaemia episodes caused by Candida albicans vs non-albicans in a 12-year cohort in a tertiary-care Spanish hospital

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Objectives: Candidaemia caused by non-*albicans* species (CNA) is an increasingly common fact. The rising incidence of fluconazole-resistant *Candida parapsilosis* infection is a major concern, with significant impact in the hospital setting and high associated morbidity and mortality. The objective of our study was to compare the main characteristics and frequency of candidaemia episodes caused by *Candida albicans* species (CA) and CNA.

Materials & Methods: A prospective observational study was conducted on a tertiary care hospital in Madrid. We included adult hospitalized patients diagnosed with candidaemia over a 12-year period (from 2010 to 2022). Study variables were collected using a predefined protocol. A comparison of main risk factors for candidaemia, clinical presentation, and prognosis was made between patients with CNA candidaemia and those with CA.

Results: A total of 535 candidaemia cases were analyzed, with 270 (50.5%) episodes of CA and 271 (50.7%) of CNA. There were 12 cases of mixed infections. The distribution of CNA species was as follows: *Candida parapsilosis* 42.9%, *Candida glabrata* 27.4%, *Candida tropicalis* 18.6%, *Candida krusei* 5.7%, and others 5.3%. No cases of fluconazole-resistant *C. parapsilosis* or *C. auris* were recorded. Figure 1 shows the frequency of CNA versus CA candidaemia episodes in the annual distribution. An increase in CA cases was observed in 2020, likely due to the higher incidence of episodes in the context of the COVID-19 pandemic. Table 1 describes the main clinical differences between CA and CNA candidaemias. A higher proportion of CNA candidaemia was observed in patients with liver disease (19.5% vs. 12.4%) and hematological diseases (8.4% vs. 1.2%). No differences in classic risk factors were observed between the two groups. Regarding the source of candidaemia, there were differences in the abdominal focus, which was more prevalent in CNA candidaemias compared to CA (17.9% vs. 8.9%). 1,3 β-D glucan (BDG) determination was performed in 123 patients, with 34 using the Fungitell® technique and 89 using Wako-FUJIFILM (implemented in the institution since July 2017). BDG was positive in 49/68 patients with CA candidemia (72.1%) and in 34/55 (61.8%) with CNA candidemia, p=0.23.

Although there were no differences in the choice of initial antifungal therapy, echinocandins were more commonly used in CNA episodes, while fluconazole was preferred for CA candidaemias. There were no differences in terms of prognosis, complications, overall mortality, or mortality within 7 days of the episode.

Conclusions: Our long-term cohort demonstrates that CNA candidaemias account for a significant percentage of episodes, but there was no observed increase compared to previous years. Candidaemias caused by non-*albicans Candida* species predominantly occur in patients with a history of liver disease and onco-hematological conditions.

Figure 1. Temporal distribution of candidaemia episodes *Candida albicans* and *Candida* non-*albicans*.

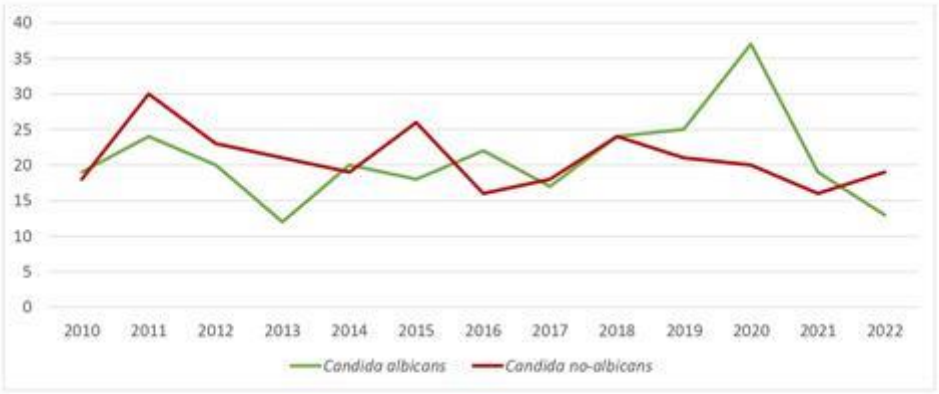

Table 1. Comparison between *Candida* non-*albicans* (CAN) and *Candida albicans* (CA) candidaemia episodes.

	CA N = 259 (49.6%)	CNA N = 263 (50,4%)	p
Comorbidity			
Cardiovascular	124(47.9)	93(35.5)	**<0.01**
Diabetes mellitus	89(34.4)	68(26.0)	**0.04**
Liver disease	32(12.4)	51(19.5)	**0.03**
Gastrointestinal disease	91(35.1)	95(36.3)	0.79
Chronic renal disease	58(22.4)	59(22.5)	0.97
Hematologic malignancy	3(1.2)	22(8.4)	**<0.01**
Solid tumor	130(50.2)	107(40.8)	**0.03**
HIV	9(3.5)	10(3.8)	0.83
Hemodialysis	18(16.4)	9(9.6)	0.15
Risk factors for candidemia			
Central venous catheter	187(72.2)	192(73.6)	0.73
Abdominal surgery	87(33.6)	85(32.4)	0.59
Parenteral nutrition	168(64.9)	149(56.9)	0.06
Previous colonization (6	49(53.8)	39(52.0)	0.81
months)	95(36.7)	85(32.4)	0.31
Corticosteroids	240(92.7)	233(88.9)	0.14
Broad spectrum antibiotics	40(44.9)	36(49.3)	0.58
Previous admission to ICU			
Origin of candidaemia			
Catheter	144(55.6)	136(51.9)	0.40
Abdominal	23(8.9)	47(17.9)	**<0.01**
Urinary	28(10.8)	18(6.9)	0.11
Primary	50(19.3)	49(18.7)	0.86
First antifungal therapy			
Echinocandins	54(59.3)	48(64.0)	0.54
Fluconazole	32(35.2)	19(25.3)	0.17
Complications			
Thrombophlebitis	20(17.2)	12(12.9)	0.39
Ocular involvement	26(13.8)	19(9.6)	0.29
Endocarditis	5(2.7)	5(2.7)	0.98
Outcome			
Overall mortality	119(45.9)	102(39.1)	0.11
Seven-days mortality	44(17.0)	42(16.1)	0.78

*Mixed candidaemias not included.

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Identification and characterization of cryptic species of Candida isolated from ICU patients

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Objectives: In this study, we wish to identify and characterize antifungal patterns of *Candida* cryptic species recovered from Portuguese intensive care units (ICUs) during a 3-day point-prevalence study on fungal colonization in Portuguese ICUs.

Materials & Methods: This multicentre prospective study was conducted during the period of January 2020 to December 2022. Axillar/inguinal swab patient samples were performed for 675, 203 and 110 patients at admission, 5th and 8th day of ICU stay, respectively. This investigation has been approved by the Institutional Ethical Board of all institutions enrolled. All samples showing growth of *Candida* species were included in this study. Identification of the isolates was done using phenotypic methods and by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF). For a definitive identification of cryptic species, the isolates underwent optimized polymerase chain reaction (PCR) assays. *In vitro* antifungals susceptibility tests (AFST) were performed for fluconazole, voriconazole, amphotericin B and anidulafungin, according to concentration gradient Etest® strip technique following the manufacturer's instructions. *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) standard strains were used as quality controls. Results were interpreted based on the clinical breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Results:
A total of 988 samples were received from ICUs during the study period, of which 355 yielded *Candida* species. At admission, 5th and 8th day of ICU stay, 27.3%, 42.8% and 52.7% of the patients, respectively, were found to have positive cultures. 52.1% were identified as *C. albicans* complex (185/355), 31.5% as *C. parapsilosis* complex (112/355), 10.1% as *C. glabrata* complex (37/355), 4.5% as *C. tropicalis* (15/355), 1.1% as *Clavispora lusitaniae* (*C. lusitaniae*) (4/355) and 0.8% as *Meyerozyma guilliermondii* (*C. guilliermondii*) (3/355). The MALDI-TOF (Vitek MS database) and PCR analysis for the identification of cryptic species revealed that only three isolates (0.8%) belonging to *C. parapsilosis* complex were cryptic, two were *C. orthopsilosis* and one was *C. metapsilosis*. *C. orthopsilosis* isolates were collected from patients at ICU admission and *C. metapsilosis* at 5th day of stay from a mixed culture with a non-*Candida* species. Amphotericin B, voriconazole and anidulafungin were the most susceptible drugs for all *Candida* species, respectively 100%, 99.7% and 97.5%. The overall rate resistant to fluconazole was 2.3%. All cryptic isolates were susceptible to the four antifungals tested.

Conclusions:

C. albicans is the predominant species colonizing Portuguese ICU patients. Currently, local available data on cryptic *Candida* species is very limited. Overall, the triazoles, both older and new compounds, and the echinocandins have good *in vitro* antifungal activities against *Candida* species recovered from Portuguese ICUs patients. We did not find significant differences in susceptibility pattern neither in azole resistance between *sensu* stricto and cryptic species. Based on these results, and although considering the limitation that the percentage of cryptic species isolated in this cohort is very low, it seems that the identification of the *Candida* isolates to its cryptic species is not a critical step on antifungal prescription.

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Candidaemia Incidence Soared during COVID-19 Pandemic: A Ten-Year Review from a Belgian Tertiary Hospital

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Objectives

Candidaemia is a significant cause of morbidity and mortality in hospitals and requires comprehensive epidemiological data for effective antifungal treatment. *C. albicans* is still the leading cause of candidaemia, but antifungal preexposure increased non-albicans candidaemia and antifungal resistance worldwide. This research aims to enhance understanding of local candidaemia epidemiology, contributing to improved patient cares.

Materials & Methods

This retrospective study was conducted at CHU UCL Namur Mont Godinne, a 386-bed Belgian university hospital. All patients presenting positive blood cultures for *Candida* spp between January 2013 and February 2023 were included. We collected comprehensive data on potential risk factors, including hospitalization unit, underlying medical conditions, exposure to antibiotics or antifungals, indwelling medical device presence, medical and surgical history, immune status, and immunosuppressive therapy. We further analyzed empirical and documented antifungal therapy and mortality rates. We also compared candidaemia in pre and per-COVID-19 periods. Kruskal-Wallis test, chi-square test, and logistic regression were done to investigate the associations between the variables of interest using “R software” with ‘TableOne’ package. *P* value under 0.05 were considered as significant.

Results

We identified 148 cases of candidaemia during the study period. The mean annual incidence was 1,17 cases/1000 admission/year. While the incidence of *non-albicans* candidemia remained stable, we noted a significant rise in *C. albicans* candidemia in 2020 (Figure.1). Most candidaemia cases were found in intensive care units (ICU) (45%), followed by surgical units (11%), oncology unit (9%) and haematology unit (8%). Table 1 shows the characteristics of patients presenting *C. albicans and non-albicans* candidaemia. The overall mortality at 30 days was 37,1%. *C. albicans* was the most common species identified (59%), followed by *C. glabrata* (19%). The haematology unit had the highest rate of *C.* non-*albicans* candidaemia (91%). No echinocandin resistance was observed, nor increased rates of fluconazole resistance (10%) throughout the study period. The species that showed higher rates of fluconazole resistance were *C. glabrata* and *C. tropicalis* (18.5% and 11.0%, respectively), while the rate in *C. albicans* remained low (2.3%). Units with the highest rates of fluconazole-resistant isolates were the haematology unit (33%), followed by pneumology (18%) and oncology unit (14%). Prior antifungal exposure was associated with non-*albicans* candidaemia and azole resistance ($p<0.05$).

Conclusions

Our study provides a comprehensive overview of candidaemia epidemiology at CHU UCL Namur Mont Godinne Hospital over a decade. A significant spike in the incidence of *C. albicans* candidaemia was observed during 2020, potentially linked to the COVID-19 pandemic, while other non-*albicans* species remained stable. There was no increase in antifungal resistance during the last ten years. We require in-depth analyses to comprehend the factors associated with this rise in candidemia caused by *C. albicans* during the early waves of COVID-19.

Table 1: Characteristics of patients with *C. albicans* and C. non *albicans* candidaemia

Characteristics	**Total**	***C. albicans***	***C. non-albicans***	***p***
Number of patients	148	85	63	
Age - yr (median)	64	63	65	0.714
Male sex n(%)	86 (58.1)	48 (56.5)	38 (60.3)	0.764
Underlying diseases				
Haematological malignancy n(%)	16 (10.8)	3 (3.5)	13 (20.6)	<0.05
Solid tumor n(%)	36 (24.3)	19 (22.4)	17 (27)	0.64
Solid organ transplant recipients n(%)	15 (10.1)	8 (9.4)	7 (11.1)	0.95
Gastrointestinal disease n(%)	47 (31.8)	30 (35.3)	17 (27)	0.37
Chronic pulmonary disease n(%)	44 (29.7)	28 (32.9)	16 (25.4)	0.41
Chronic cardiac insufficiency n(%)	13 (8.8)	6 (7.1)	7 (11.1)	0.57
Chronic liver disease n(%)	10 (6.8)	6 (7.1)	4 (6.3)	1
Chronic kidney disease n(%)	37 (25)	25 (29.4)	12 (19.0)	0.21
Neurological disease n(%)	9 (6.1)	2 (2.4)	7 (11.1)	0.06
Diabetes n(%)	42 (28.4)	24 (28.2)	18 (28.6)	1
Predisposing factors				
Immunosuppressive therapy n(%)	71 (48)	34 (40.0)	37 (58.7)	0.03
Neutropenia n(%)	23 (15.5)	8 (9.4)	15 (23.8)	0.03
Chemotherapy n(%)	36 (24.3)	15 (17.6)	21 (33.3)	<0.05
USI stay n(%)	101 (68.2)	65 (76.5)	36 (57.1)	<0.05
Prior surgery (3 months) n(%)	91 (61.5)	53 (62.4)	38 (60.3)	0.93
Presence of urethral catheter n(%)	93 (63.3)	58 (69.0)	35 (55.6)	0.13
Presence of gastric tube n(%)	81 (55.1)	53 (63.1)	28 (44.4)	<0.05
Presence of abdominal drainage tube n(%)	28 (18.9)	17 (20.0)	11 (17.5)	0.85
Presence of peripheral venous catheter n(%)	137 (92.6)	80 (94.1)	57 (90.5)	0.60
Presence of central venous catheter n(%)	118 (79.7)	72 (84.7)	46 (73.0)	0.12
Mechanical ventilation n(%)	67 (45.3)	46 (54.1)	21 (33.3)	<0.05
Presence of Porth-a-cath n(%)	41 (27.7)	20 (23.5)	21 (33.3)	0.25
Parenteral nutrition n(%)	49 (33.3)	29 (34.5)	20 (31.7)	0.86
Prior antifungal exposure n(%)	40 (27)	15 (17.6)	25 (39.7)	<0.05
Prior antibiotic exposure n(%)	124 (83.8)	71 (83.5)	53 (84.1)	1.000

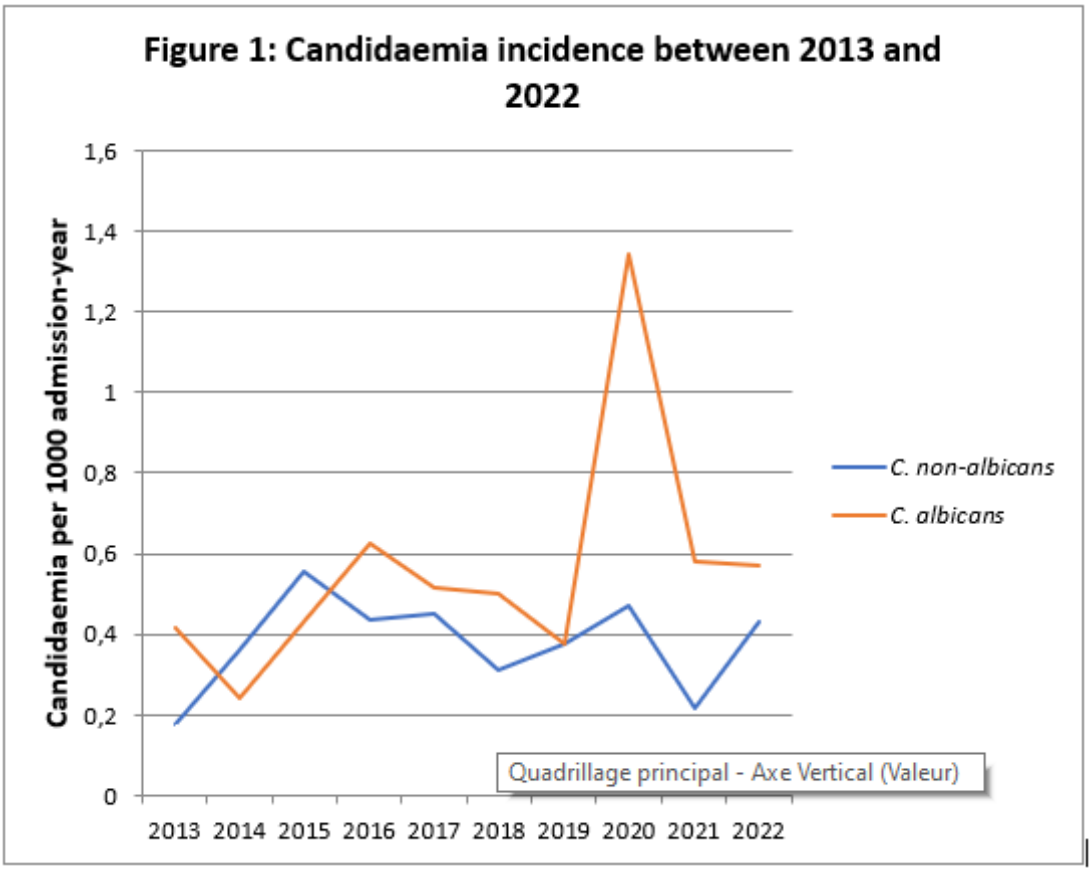
Figure 1: Candidaemia incidence between 2013 and 2022
Candidaemia per 1000 admission-year
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
2013 2014 2015 2016 2017 2018 2019 2020 2021 2022
C. non-albicans
C. albicans
Quadrillage principal - Axe Vertical (Valeur)

P270

Outbreak of single lineage Aspergillus flavus infections in a Danish hospital ward

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Objectives: To study the genetic relatedness amongst clinical *Aspergillus flavus* isolates from a suspected nosocomial outbreak in a pediatric onco-hematologic ward (POH) including isolates from potential indoor environmental sources and background clinical isolates from all over Denmark.

Methods: A total of 153 *A. flavus* isolates were included, of which 140 were clinical isolates from 120 patients, nine were isolated from air samples from the POH and four were control strains. The clinical isolates were sent from hospitals and general practitioners for identification and susceptibility testing at the mycology reference laboratory at Statens Serum Institut (SSI) from 1994-2023. Environmental isolates were sampled with a MAS-100NT Air sampler (MERCK) and collected in several areas of the affected ward and sites related to it. Identification was based on morphology, MALDI-TOF and for some (*N*=20) sequencing of ITS, BTUB and CMD. We used a slightly modified microsatellite-based typing scheme including nine distinct markers of short tandem repeats, previously described by Rudramurthy et al., Plos One, 2011.
Genetic relatedness among the isolates was estimated by Bruvo´s distance using the R package Poppr and visualized as a Minimum Spanning Network (MSN). To detect the number of groups (K) de novo, a DAPC analysis was conducted using the R package adegenet 2.1.15 (updated 2020). The optimal K was selected based on the lowest BIC value.

Results: Sequencing of ITS, BTUB and CMD provided 1587 concatenated base pairs, with POH-cluster isolates (*N*=6) being identical but differing 1-7 bp from other sequenced *A. flavus* isolates (*N*=14). Typing of all 153 isolates provided 116 distinct genotypes. We found a D-value of 0.9987 for all nine markers combined. The MSN showed that the POH-cluster was distinct from other isolates (Figure 1). The DAPC cluster analysis identified five clusters, with the POH cluster clearly differing from other isolates (Figure 2). The POH cluster contained 23 isolates from 16 patients and nine isolates from air-samples.

Conclusions: Microsatellite typing demonstrated a single lineage (the POH-cluster genotype) shared by all outbreak and environmental isolates. Inside Hospital-A, great diversity amongst non-POH lineages was observed and comparable to that of all included isolates. At the least, the POH-lineage has been present since 2008 and may have remained undetected, because of its limited ability to infect immunocompetent patients. Distinctiveness of the POH-cluster suggests a single-source outbreak, by a genetic and potentially novel

variant of A. flavus. We propose the actual contamination source is to be found inside the POH. Potential genetic traits of this lineage, however, remains to be discovered.

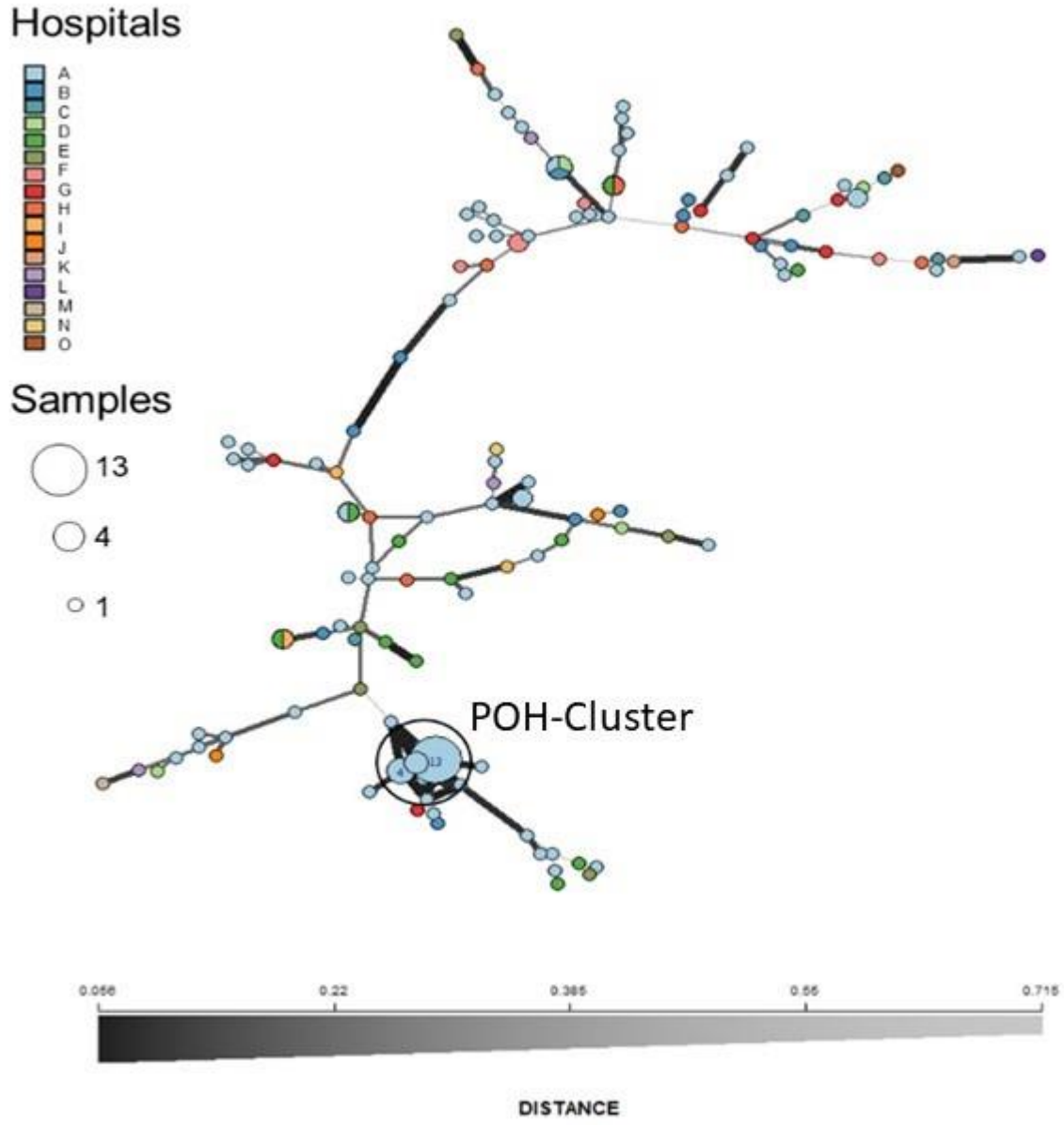

Figure 1 Minimum spanning network (MSNs) inferred using STRs from 153 Danish *Aspergillus flavus* isolates. Light blue nodes are from the same hospital (A). The POH-cluster is highlighted in a circle. The network is based on Bruvo's distance matrix. Each node represents a multi-locus genotype (MLG), with variable size depending on the number of isolates within that MLG. The distance between the nodes represents the genetic distance between MLGs.

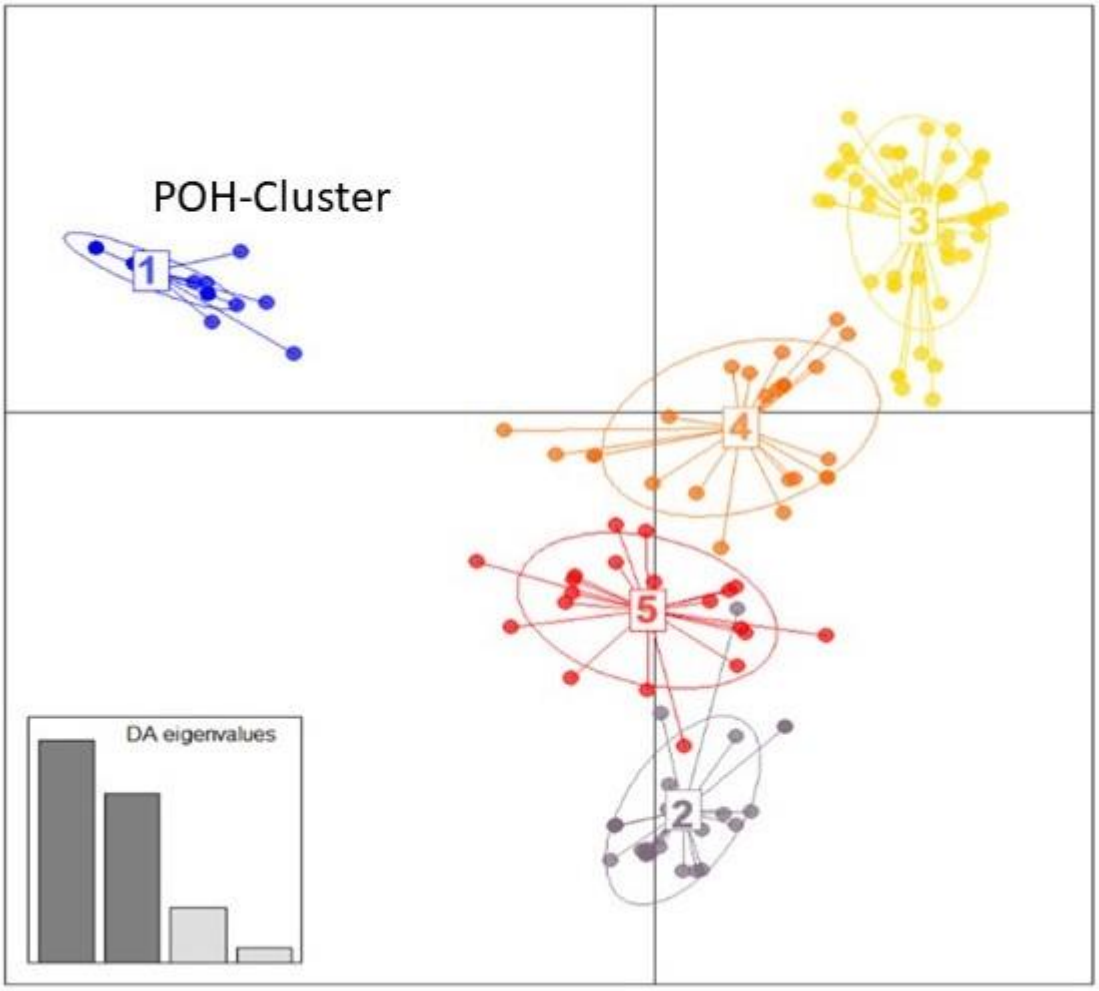

Figure 2 DAPC analysis was done to investigate the genetic structure of the genotyped *A. flavus* isolates. The blue group (1) represents the POH-cluster and is clearly distinct from all other groups, suggestive of a single lineage.

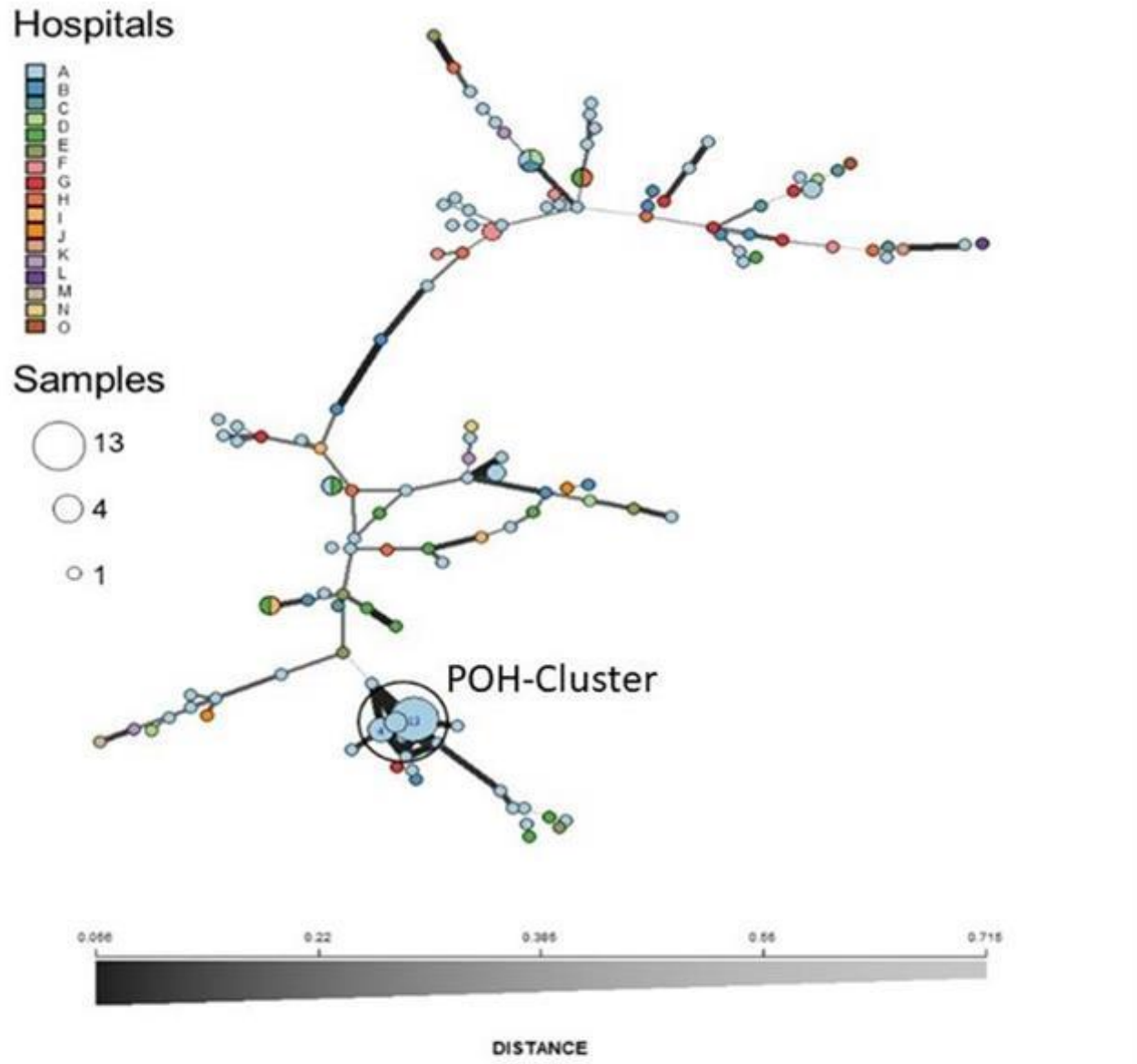

Figure 1 Minimum spanning network (MSNs) inferred using STRs from 153 Danish *Aspergillus flavus* isolates. Light blue nodes are from the same hospital (A). The POH-cluster is highlighted in a circle. The network is based on Bruvo's distance matrix. Each node represents a multi-locus genotype (MLG), with variable size depending on the number of isolates within that MLG. The distance between the nodes represents the genetic distance between MLGs.

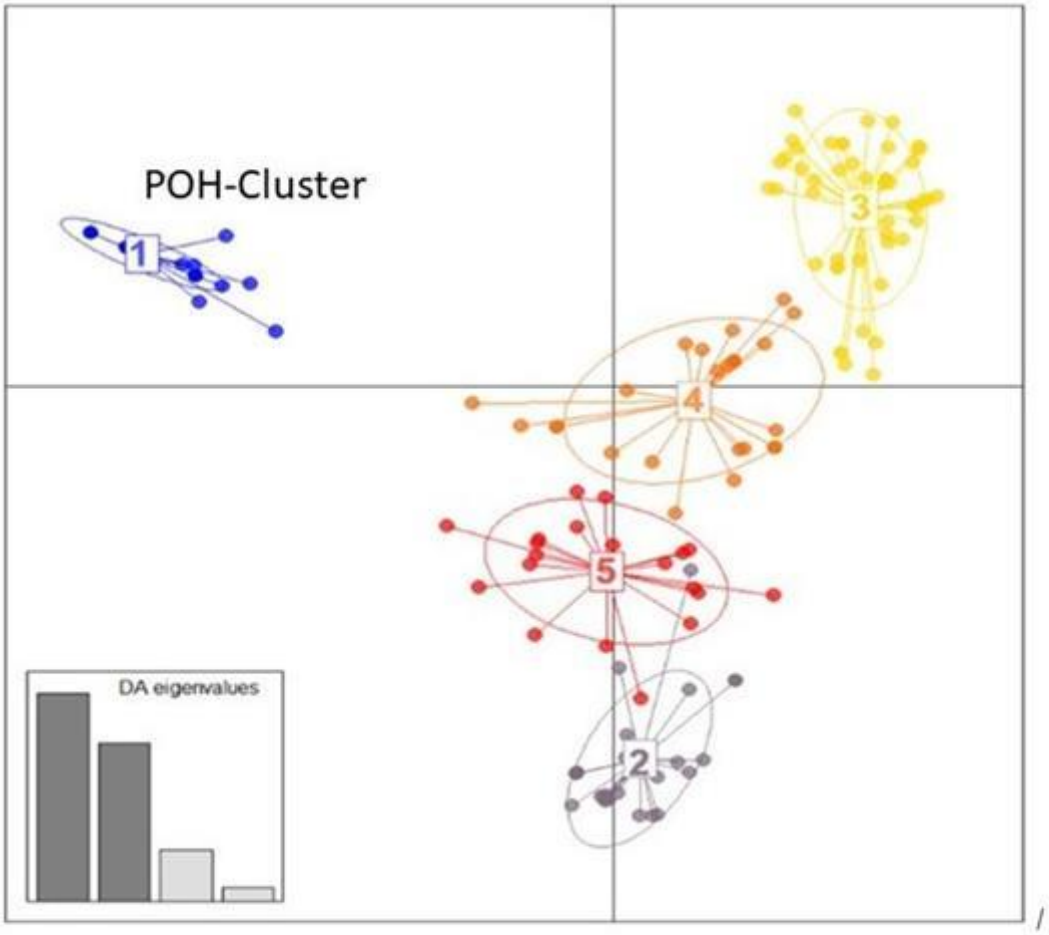

Figure 2 DAPC analysis was done to investigate the genetic structure of the genotyped *A. flavus* isolates. The blue group (1) represents the POH-cluster and is clearly distinct from all other groups, suggestive of a single lineage.

P271

A rare case of cervical hyalohyphomycosis caused by Fusarium species, in a middle-aged immunocompetent female patient.

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Objectives:

To increase awareness among medical/surgical staff of this group of emerging infections and to emphasize importance of an early diagnosis and treatment of cutaneous hyalohyphomycosis and/or phaeohyphomycosis. This disease is uncommon and is caused by various species of hyaline septate mycelial fungi reported among the immunocompetent as well as immunocompromised hosts. This predominantly involves the subcutaneous tissue and occasionally deep-seated infections in any of the visceral organ of the body. We report a rare case of cervical hyalohyphomycosis with swelling over the left cervical area. The patient is a home-maker 35-years old female, resident of district Fatehgarh Sahib (Punjab) in northern India. She presented with chief complaint of gradually increasing swelling over the left cervical area, which was approximately 5 x 5 cm in size. This was cystic in consistency and there was no nodularity or any open wound over the swelling. The detailed history of the patient was taken, clinical presentation, underlying illness and risk factor were noted. She is non-diabetic and as such there is no significant underlying disease.

Materials & Methods:

The Fine needle aspiration cytology (FNAC) was done from the local swelling. Direct microscopic examination revealed septate hyphae with branching in KOH/CFW wet mount as well as histopathology of stained sections with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Grocott's Gomori methenamine silver (GMS) stainings. The diagnosis of hyalohyphomycosis was made as the septate hyphae were hyaline and not brownish-black. Fungal cultures were put up on standard fungal culture media like Sabouraud dextrose agar and brain heart infusion agar for isolation and further species identification. Molecular techniques of the fungal strain identification were used.

Results:

Fungal culture media revealed yellowish-pink mycelial growth by the end of first week, which was identified as Fusarium species of LCB mount. The final identification of Fusarium species based on sequencing is still awaited. The total excision of swelling was done and draining gauge was put up in the wound. Empirically, anti-tubercular treatment (ATT) was initiated for period of about two weeks by the treating surgeon, however, it was stopped when fungal culture report received. Then she was put on itraconazole 200 mg twice a day. She got improved in the four weeks' duration and recently came for follow up and was fine. The drain wound also got healed completely.

Conclusions:

There is an urgent need for keeping high index of clinical suspicion of hyalohyphomycosis thereby taking early FNAC and/or biopsy of affected site so that benefits of prompt diagnosis and appropriate therapy may be initiated. Such type of swellings is invariably found as phaeohyphomycosis but it turned out to be hyalohyphomycosis. To avoid further aggression of the disease, the key is the establishment of an early diagnosis and prompt antifungal therapy combined with surgical excision of swelling including some healthy areas.


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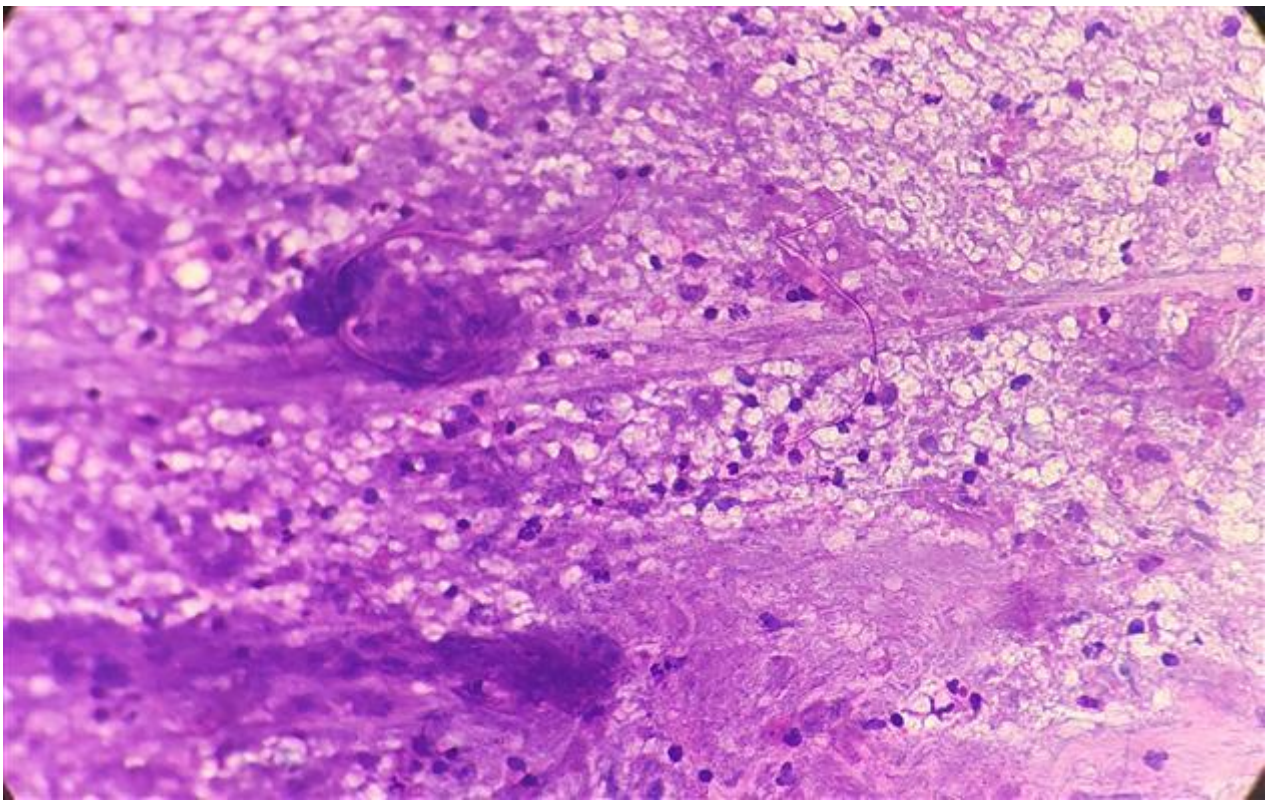

P272

Rapid Literature Review on the Epidemiology and Burden of Disease Caused by Non-Aspergillus and Non-Mucor Mould Pathogens in Europe, Asia and Australia

Malcolm Bain[1], Gill Karan[1], Mrs Debbie Cockayne
[1]Shionogi Europe, ,

Objectives:
Opportunistic invasive mould infections present a global concern due to high morbidity and mortality rates. Although these infections can be caused by a variety of moulds, the most common causative pathogens are *Aspergillus* spp. and *Mucormycosis* spp. Research has largely focused on these particular organisms, but it is also necessary to understand the epidemiology and burden of disease associated with the rarer mould pathogens that may be experiencing an increased prevalence in specific geographical regions and in particular infections. Therefore, this rapid literature review aims to summarize the current published literature on the epidemiology and burden of disease associated with infections caused by specific rare mould pathogens in Europe, Asia, and Australia.

Materials & Methods:
A rapid literature review was conducted using PubMed, Medline, and EMBASE libraries. Using the PRISMA (Moher 2009) methodology, the search was based on three research questions: 1. Epidemiology of disease. 2. Clinical burden of disease. 3. Economic burden of disease. The global geographies considered for this review were Europe (incl. Scandinavia), Asia and Australia. The pathogens selected were: *Lomentospora sp., Scedosporium sp., Fusarium sp., Rasamsonia* sp., Scopulariopsis sp., *Paecilomyces sp., Talaromyces sp., Penicillium sp. and Purpureocillum sp.* The Phaeohyphomycosis group of fungi were also included.
The studies considered for this review were published between January 2000 to March 2023 in English. Papers were screened in line with strict inclusion and exclusion criteria. Publications were included from the ongoing Fungiscope® Registry.

Results:
The initial search returned 6932 papers. Number of papers selected at each stage are summarised in the table below.

	Initial review	Abstract review	Data extraction
Epidemiology	1038	176	65
Burden of disease	936	192	89
Economic burden of disease	527	16	1

Papers selected by the literature search protocol – number of papers at each stage by research question

The focus of the 65 papers in the epidemiology section were fungal keratitis (n=32), systemic infection in cystic fibrosis patients (n=7) and adult and paediatric patients undergoing SOT or HSCT (n=10).
Incidence of infection within these groups varies by pathogen and geographical location.

89 papers were selected for data extraction regarding the burden of disease. These included the 2014 ESCMID/ECCMID and the 2021 ECMM Global guideline for the diagnosis and management of rare mould infections as well as systematic reviews focusing on these rarer mould pathogens in specific disseminated infections. The reviewers noted that very few of the papers(n=16) focussed on the burden associated with the rare pathogens only.

Conclusions:

Rare mould infections are a global concern, especially in the immunocompromised host. The incidence of these infections vary across different regions and populations, but this literature review has shown a scarcity of specific publications relating to the clinical and economic burden of disease associated with these pathogens. It is recognised that the lack of data is due to a low but increasing prevalence, a difficulty in diagnosis and the limited available treatment options. Further research is needed to improve our understanding of the epidemiology and burden of these infections and to aid regulatory authorities with reimbursement of new treatment options.

P273

Insights from a retrospective study at a South -East Rajasthan hospital on hematogenous affinity of Candida species in bloodstream infections

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[1]GMC, Kota, Rajastthan, India

Retrospective Study on the Hematogenous Affinity of Candida Species in Human Bloodstream Infections: Insights from a Tertiary Care Hospital in South-East Rajasthan, India.

Background

Fungal infections, particularly Candida bloodstream infections, pose a significant threat to immunocompromised and ICU patients, resulting in increased morbidity, mortality, prolonged hospital stays, and higher healthcare costs. Candidemia can manifest with a wide range of symptoms, from subtle to severe multi-organ failure, potentially leading to life-threatening invasive diseases. The changing epidemiology of Candidiasis, including the emergence of non-albicans Candida species as predominant agents globally, is a matter of serious concern, although its impact in India is currently underestimated. Recent years have witnessed a resurgence of Candidemia in specific regions of Rajasthan, India.

Objectives

1. Assess the prevalence and incidence of Candida species in human bloodstream infections at a tertiary care hospital in South-East Rajasthan, India.
2. Investigate the hematogenous affinity of different Candida species and their role in causing bloodstream infections.
3. Analyze the clinical outcomes associated with Candida bloodstream infections, including morbidity, mortality, and hospital stay length, providing valuable insights for patient management and healthcare planning in the region.

Materials & Methods

This retrospective observational analysis spanned three years (Jan 2020 - Jan 2023) and involved retrieving data from the hospital database of a tertiary care hospital in Kota, Rajasthan. A total of 5561 patients exhibiting signs and symptoms of suspected bloodstream infections were included in the study and underwent relevant testing. Candidemia diagnosis was determined using both conventional and automated techniques, while antifungal susceptibility testing utilized the modified Kirby-Bauer method (disc diffusion) and the automated Vitek2 method. Baseline clinic-epidemiological information was collected from the patients, and the data underwent statistical analysis.

Results

A total of 5561 patients suspected of having bloodstream infections were included in the analysis. Among them, 329 patients (age range: 3-80 years, mean+SD: 43.9+17.16) were identified as positive for Candida infection, accounting for 5.9% of the total cases (ranging from 1.7% to 21.5%). Of the Candidemia patients, 154 (47%) were male, and 175 (53%) were female. The majority of Candidemia patients required hospitalization (89%) while 11% of the patients recovered without the need for

hospitalization. Among the non-albicans group, Candida tropicalis was the most frequently isolated species responsible for the infection.

Conclusion

The study findings highlight the significant role of Candida infections in the development of bloodstream infections, emphasizing the need for improved antifungal therapy to avoid unnecessary use of antifungal drugs and prevent the emergence of drug resistance. Diabetes mellitus and bronchial asthma with steroid treatment emerged as common risk factors. The study underscores the importance of considering Candida infections in patient management and highlights the need for enhanced preventive strategies in this region.

TIMM Trends in Medical Mycology

P274

Increase of Invasive Aspergillosis caused by Aspergillus section Nigri in a General Hospital

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Objectives: *Aspergillus* section *Nigri* have been identified as colonizers of the nose and throat in immunocompetent patients with other predisposing conditions, such as chronic lung diseases. *Aspergillus* section *Nigri* is less likely to cause invasive aspergillosis (IA) than some other *Aspergillus* species. Rarely it is detected in IA of post-transplant patients, but patients with acute myeloid leukemia (AML) were particularly at risk. The present study aims to evaluate the incidence of invasive aspergillosis (IA) due *Aspergillus* section *Nigri* in our hospital over a 4-year period.

Materials & Methods: From January 2019 to February 2023, we included patients with IA caused by *Aspergillus* section *Nigri* who were admitted to the high risk units, haematology and pneumology with a diagnosis of respiratory failure. Demographic data, comorbidities, chronic pulmonary disease (COPD, asthma, and bronchiectasis), risk factors for invasive fungal infection, microbiological features, radiological data, treatments received in the hospitalization ward and the ICU, and clinical course and mortality were collected.

Results: A total of 137 patients with isolation of *Aspergillus* section *Nigri* were included in the study. Twenty-six patients (19%) were diagnosed with IA. The distribution of the number of patients with IA per year was as follows: 2019 (1), 2020 (12), 2021 (2), 2022 (9) and 2023 (2). Of the total of patients, 61.5% were male and 38.5% were female, with a mean age of 67 (33–91) years. The mean and range of weight was 71,2 (46-102) Kg. The global ICU mean length of stay was 32.9 (10–149) days.
In relation to comorbidities, 11 patients had (42.3%) chronic pulmonary disease, 10 (38.5%) SARS-CoV2, 5 (19.2%) diabetes, 2 (7%) hematological disease, and 2 (7%) solid organ transplantation. Five patients had no underlying disease (19.2%).
Regarding mechanical ventilation, 17 patients (65.4%) were treated with invasive mechanical ventilation, and 9 (34.6%) with non-invasive mechanical ventilation. Nineteen patients (73.1%) received corticosteroids. During admission, 9 patients with IA due to *Aspergillus* section *Nigri* died (34.6%), eight of them with azole monotherapy treatment.

Conclusions: During the study period there was an increase in the incidence of IA due to *Aspergillus* section *Nigri* in our hospital. Chronic pulmonary disease, SARS-CoV2, and diabetes seems to be the main comorbities present in patients with IA due to *Aspergillus* section *Nigri.* IA caused by *A.* section *Nigri* is associated with high mortality rate and azole monotherapy should be reevaluated.

P275

Candidaemia in a Neonatal Intensive Care Unit: a twelve-year period study (2010 - 2022)

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Objectives: *Candida* spp. are the third most common causative agents of bloodstream infections in the neonatal intensive care units (NICU). Candidaemia affects 10 to 20% of extremely low birth weight infants and 2 to 16% of very low birth weight neonates. Althgough candidaemia in the NICU are less frequent than that due to Gram-positive or Gram-negative bacteria, morbidity and mortality rates are higher, especially among premature neonates.
Materials & Methods: A retrospective study of candidaemia was performed in the NICU of the General and Maternity Hospital “Helena Venizelou”, Athens, Greece, between January 1, 2010 and December 31, 2022. All positive blood cultures with candidemia were registered. Demographic data and causative *Candida* species were collected and analyzed. Identification and antifungal susceptibility test of the yeast isolates was performed by Vitek 2 Compact (bioMerieux, France).
Results: A total of 19 candidaemic episodes were recorded in our NICU during the study period. 14/19 (73.7%) isolated strains were *C. albicans*. There were also 2 (10.5%) *C. parapsilosis*, 2 (10.5%) *C. glabrata* and 1 (5.3%) *C. lusitaniae*. All infected neonates were preterm (25.6 +/- 3.0 w) and among them there were 13 (68.4 %) with extremely low birth weight (ELBW) (850 +/- 430 g). During NICU admission, central venous catheter (CVC) and mechanic ventilation were applied to all neonates. Aminoglycosides, glycopeptides and β-lactams were administered to all neonates, prior candidaemia. 89.4% of the candidemic neonates were hospitalized >7 days in NICU. Ten neonates were fully recovered and were discharged, while three were transferred to other hospitals for further hospitalization. Six of 19 (32.5%) candidemic neonatess died.
Conclusions: Although, non-albicans isolates are outranking *C. albicans*, this remains the predominant yeast pathogen of bloodstream infections in our NICU. Prematureness and neonates with ELBW are vulnerable to candidaemia. Factors that are strongly associated with neonatal candidaemia are prolonged hospitalization, the usage of antimicrobials agents and the presence of CVCs.

P276

Incidence rate of fungal secondary infections in COVID-19 patients; retrospective data of infection control committee in a Turkish university hospital

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[1]Department of Microbiology, Gazi University Faculty of Medicine, Ankara, Turkey, Ankara, Turkey, [2]Department of Infectious Disease, Gazi University Faculty of Medicine, Ankara, Turkey, Ankara, Turkey, [3]Infection Control Committee, Gazi University Hospital, Ankara, Turkey, Ankara, Turkey, [4]Department of Public Health, Gazi University Faculty of Medicine, Ankara, Turkey, Ankara, Turkey

Objectives:

Fungal infections have been observed among novel coronavirus disease-2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) patients, especially in the intensive care unit (ICU) setting. Our study aimed to investigate the incidence rate of fungal secondary infections among ICU patients with COVID-19 and compare them with ICU patients without COVID-19 infection in the within the same time period

Materials & Methods:

Current retrospective study was conducted following ethical approval of Gazi University Ethics Committee among COVID-19 patients admitted to the Gazi University Hospital in Ankara, Turkiye. Data of eligible subjects were extracted from electronic medical records of Infection Control Committee of the hospital. All patients were aged ≥ 18 and tested for COVID-19 reverse transcription-polymerase chain reaction (RT-PCR) test during the period between April 2020 - May 2023. Blood cultures were incubated at 35°C anaerobically and aerobically for 5 days. Identification of significant growth within cultures is done through VITEK® MS (bioMérieux Inc.), an automated mass spectrometry microbial identification system based on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology. Microbiological identification of fungi was confirmed through growth of cultures recovered from blood using Sabouraud dextrose agar sub-culture, germ tube formation, corn meal agar morphology, and carbonhydrate assimilation assay named ID32C (bioMérieux Inc.).Crude overall incidence rate among the cohort, per 100 person was calculated with fungemia cases as the numerator and total number of COVID-19 patients as the denominator. 95%CIs for fungemia cases are calculated in both patient groups.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 26.0 (IBM, New York, USA). Pearson and Yate's Corrected Chi-square tests were used to compare the

number of fungemia cases among COVID-19 positive and negative cases. Statistical significance level (p) was set as 0.05.

Results:

Fungemia incidence rate for COVID-19 positive ICU patients was calculated for each year 2020, 2021, 2022 and 2023 (5 months) respectively. Out of 469 critically ill patients, 13 developed candidemia, with incidence rate 2,77% (CI 1,48-4,69%) in 2020. Candidemia incidence rate was 1,11% among COVID-19 positive 633 ICU patients in 2021 (CI 0,45-2,27%), while it was 1,69% among 472 patients in 2022 (CI 0,73-3,31%) and 5,17% among 116 patients in the first 5 months of 2023 (CI 1,92-1,92%) respectively. Total candidemia incidence rate was 1,97% (CI 1,37-2,75%) among COVID-19 positive ICU patients.

Candidemia incidence rate was 0,88% among 4320 COVID-19 negative ICU patients in 2020 (CI 0,62-1,21%), while it was 1,00% among 4707 patients in 2021 (CI 0,73-1,22%), it was 0,88% among 5579 patients in 2022 (CI 0,65-1,16%) and 1,14% among 2112 patients in the first 5 months of 2023 (CI 0,73-1,69%) respectively. Total candidemia incidence rate was 0,94% (CI 0,8-1,09%) among COVID-19 negative ICU patients. There was statistically significant difference between two groups (p=0,00005).

Conclusions:

Secondary fungemia among COVID-19 patients is with a predominance of *Candida* species. Incidence rate of candidemia among COVID-19 patients is slightly higher than that is seen in non- COVID-19 patients.

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Emergence of fungal colonization and infections in COVID-19 mechanically ventilated ICU patients

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Objectives: COVID-19 primarily affects the respiratory tract and can lead to acute hypoxemic respiratory failure and admission to the Intensive Care Unit (ICU). Although primary co-infections are rare, ICU patients are more vulnerable to develop secondary infections, including fungal ones. Aim of the present study was to evaluate fungal colonization and invasive fungal infections (IFIs) among COVID-19 ICU patients.

Methods: During the 15-month prospective observational study, 173 adult patients with confirmed COVID-19 admitted to the ICU were included. We registered date of symptoms onset, hospital and ICU admission, demographic data, co morbidities, disease severity, inpatient selected treatments, adverse events, ICU length of stay (LOS) and outcome, along with date and sample of fungi first isolation, colonization or IFI, susceptibility testing and corresponding treatment.

Results: Patients had mean age 67.8± 12.5 years old, APACHE II score at ICU admission 16.1± 6.7 and were mainly males (59.5%). Cardiovascular disease (58.9%), obesity (56.6%), diabetes (32.9%) and chronic obstructive pulmonary disease (COPD) (23.1%) were the most frequent comorbidities. Fungi were isolated from 47 clinical samples of 42 (24.3%) patients and represented colonization in 31 of them. Sixteen fungal species (34%) were isolated from urinary samples, 15 (31.9%) bronchial secretions or non-bronchoscopic lavage (non-BAL) samples, 8 (17%) peripheral blood samples, 7 (14.9%) catheter tip, 1 (2.1%) decubitus ulcer sample. The distribution of the isolated fungi were: *Candida albicans* 17(37%), *C.parapsillosis* 9 (19.6%), *C.glabrata* 5 (10.8%), *C.auris* 4 (8.7%), *C.lusitaniae* 1 (2.2%), non-

C.albicans 5 (10.8%), *Aspergillus fumigatus* 4 (8.7%), *A.niger* 1 (2.2%). The first isolation of fungal species was observed on ICU day 5 (2-11), whereas infection emerged on day 9 (5-20). Eleven patients suffered from IFI (6.3%) and their characteristics are depicted on Table 1.

Conclusions: Fungal colonization emerged early after ICU admission, in 17.9% of our Covid-19 patients, whereas IFIs were diagnosed in 6.3%, mostly attributed to Candida species. Patients with IFIs suffered also from bacterial co-infections (81.8%) and their crude mortality accounted for 72.3%.

TABLE 1. Characteristics of COVID-19 ICU patients with invasive fungal infections

Age/ sex	Co morbid	APA CHE II	Fungal agent	Sample	ICU day(at isolation)	Bacterial co-infections	LOS	Outcome
69M	Diabetes, hypertension	17	*C.parapsillosis*	Blood	40	*A.baumannii /Kl.pneumoniae*, bacteremia, day 14; *E.faecium*, bacteremia, day 33	52	Died
75F	Obesity, hypertension	15	*A.fumigatus*	Non BAL	3	*A.baumannii*, bacteremia, day 4	16	Died
62M	none	10	*A.fumigatus*	Non BAL	3	*A.baumannii*, pneumonia, day 9	21	Died
67M	Diabetes, hypertension	13	*A.niger*	Non BAL	5	*A.baumannii*, complicated pneumonia, day 39	60	Died
79M	Obesity, hypertension	21	*C.albicans*	Blood	5	*A.baumannii*, bacteremic pneumonia, day 11	15	Died
48M	Obesity, paraplegia	20	*C.albicans*	Blood	7	None	12	Died
77M	Diabetes,hypertension, malignancy	12	*C.albicans/ C.parapsillosis*	Blood	9	*E.faecium*, bacteremia, day 1	37	Died
71M	Hypertension	10	*C.paraspillosis*	Blood	11	*Kl.pneumoniae*, bacteremia, day 1	19	Survived
90M	Obesity, hypertension, diabetes	10	*C.glabrata*	Blood	12	*A.baumannii*, bacteremic pneumonia, day 1	29	Survived
70M	Obesity, hypertension diabetes, renal failure, immunosuppression	21	*C.paraspillosis*	Blood	23	*A.baumannii*, pneumonia, day 15	27	Died
49F	Obesity	5	*C.auris*	Blood	68	*A.baumannii*, pneumonia, day 8; *E.faecium*, pneumonia, day 58	128	Survived

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CANDIDA AURIS ISOLATION AMONG CRITICALLY ILL PATIENTS AND RISK FACTORS FOR CORRESPONDING INFECTIONS

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Objectives:
Candida auris has recently emerged as a multidrug-resistant yeast implicated in various healthcare-associated invasive infections and hospital outbreaks and poses a major concern for the immunocompromised and critically ill patients. Aim of the present study was to report a case series of *C. auris* isolation in Intensive Care Unit (ICU) patients and to determine potential risk factors for *C. auris* infections.
Materials & Methods:
During the 30-month retrospective observational study (November 1st 2020 to April 30th 2023), all patients with *C. auris* isolation confirmed with MALDI-TOF MS methodology (Microflex LT platform, Bruker Daltonics, Bremen, Germany) were included and divided into two groups, according to *C. auris* colonization or infection. We registered date of ICU admission, demographic data, co morbidities, disease severity, ICU length of stay (LOS) and outcome, along with date and sample of *C. auris* first isolation, colonization or invasive fungal infection, susceptibility testing using Sensititre YeastOne (ThermoFisher Scientific) methodology and interpretation of MIC values based on U.S.CDC tentative MIC breakpoints, as well as corresponding treatment. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquantile range). Continuous variables were compared with t-test and categorical variables were evaluated with Fischer's exact test.
Results:
Our case –series consisted of twenty-two critically ill patients (17 medical cases). Patients had mean age 65± 12 years old, APACHE II score at ICU admission 16.4± 6.8, were mainly males (59%) and had at least one central venous catheter and were mechanically ventilated. *C. auris* was isolated from 34 clinical samples and represented colonization in 28 of them. Eight patients (36.4%) were already colonized on ICU admission. *C. auris* was isolated from 5(14.7%)blood samples, 5 (14.7%) urinary samples, 8 (23.5%) non-bronchoscopic lavage samples, 3 (8.8%) catheter tip,2 (5.9%) decubitus ulcer sample and 11 (32.4%)groin and/or axilla samples. Infected patients suffered from bloodstream (5 cases) and skin and soft tissue infections (1 case) and in two cases, invasive fungal infection followed colonization after 19 and 40 days. All *C. auris* strains during the first isolation were resistant to fluconazole (MIC range 32 to 256μg/mL) and susceptible to echinocandins and amphotericin B. The clinical characteristics of study population are presented in Table 1.
Conclusions:
Prevalence of *C.auris* isolation accounted for 4.9% of ICU admitted patients during the study period and in the majority of cases represented colonization. No differences regarding comorbidities and other characteristics were identified among colonized and infected patients. All *C.auris* isolates were resistant to fluconazole and susceptible to echinocandins. Infected patients had a significantly longer ICU LOS and two-fold crude mortality.

Table 1. Clinical characteristics of study population[a]

Characteristic	Colonized patients (N=16)	Infected patients (N=6)	p-value
Gender (male)	9 (56.25)	4 (66.7)	1.0
Age (years)	67.3 ± 10.2	60.8 ± 16.3	0.275
APACHE II	16 ± 5.9	17.3 ± 9.5	0.694
Previous abdominal surgery	4 (25)	1 (16.7)	1.0
Diabetes mellitus	2 (12.5)	3 (50)	0.1
Chronic cardiovascular disease	5 (31.25)	2 (33.3)	1.0
Chronic respiratory disease	3 (18.75)	1 (16.7)	1.0
Chronic neurological/psychiatric disease	7 (43.75)	1 (16.7)	0.351
Malignancy	4 (25)	4 (66.7)	0.137
Covid-19 /post Covid-19	5 (31.25)	2 (33.3)	1.0
ICU day (1st isolation)	4.5 (2-15.5)	25.5 (21-54)	**0.012**
Multisite colonization	2 (12.5)	2 (33.3)	0.292
Previous antifungals	8 (50)	3 (50)	1.0
ICU LOS (days)	19.5 (13.5-34)	52.5 (44-100)	**0.008**
ICU mortality	5 (31.25)	4 (66.7)	0.178

[a] Data are no. (%) of patients unless otherwise stated

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Impact of liposomal amphotericin B treatment on serum creatinine levels in critically ill patients

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Objectives. Liposomal amphotericin B (L-AmB) has been successfully used to treat invasive fungal infections in the intensive care unit (ICU). Although substantially less nephrotoxic than conventional amphotericin B the information on the impact of L-AmB on renal function is limited (1, 2). The aim of this study was to assess the renal function impairment during L-AmB treatment in critically ill patients.

Methods: Observational, single center study in critically ill patients admitted to a multidisciplinary ICU and treated with L-AmB over three or more days. Demographic data, admission diagnosis and severity of acute illness at ICU admission, indication of L-AmB treatment, presence of renal impairment and daily values of serum creatinine were recorded.

Results: A total of 50 critically ill patients (mean age 56±18 years, 28 males), mean APACHE II and SOFA scores 22 (±7) and 11 (±3), respectively, consecutively admitted to the ICU were included. All patients were treated with L-AmB, at a dose of 2-4 mg/kg/day, for microbiologically confirmed invasive fungal infection (n=29) or as part of empirical treatment (n=21). The mean duration of treatment was 12±7 days (range 4-29). Admission diagnosis was surgical in 27 patients and medical in the remaining 23 patients. Sixteen patients had impaired renal function at the initiation of L-AmB treatment being treated with continuous venous-venous hemofiltration (CVVH). For all patients, creatinine levels did not increase during administration of L-AmB [from 1.46 (±1.1) at the start to 1.14 (±1.01) at the end of the treatment. The evolution of daily mean serum creatinine levels in patients under CVVH at the start of L-AmB and in those without kidney injury are shown in Figure 1. The overall ICU mortality was 66% (n = 33).

Conclusion. In critically ill patients, L-AmB treatment had not impact on serum creatinine levels. Therefore, L-AmB can be safely used for the treatment of invasive fungal infections in critically ill patients either with or without renal replacement therapy at the initiation of L-AmB treatment.

References: (1) J Chemother 2010:22:285, (2) Rev Esp Quimioter. 2012;25:206

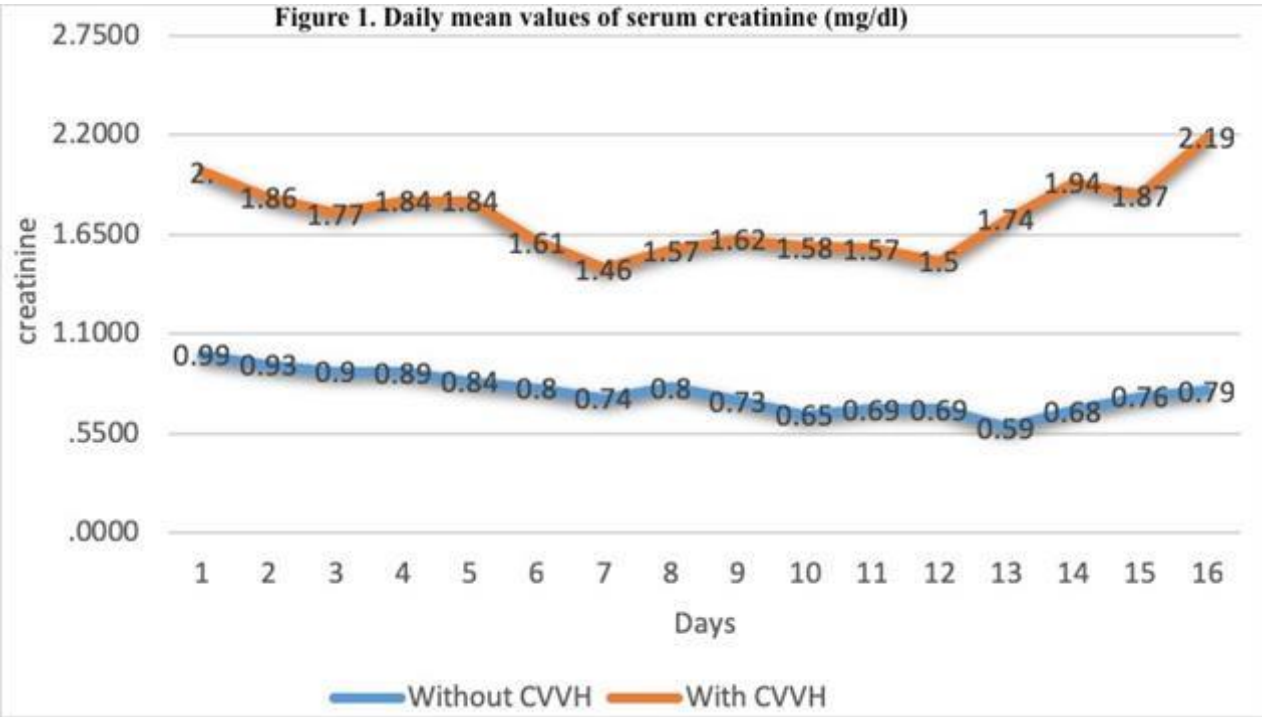
Figure 1. Daily mean values of serum creatinine (mg/dl)
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creatinine
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0.89
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10
11
12
13
14
15
16
Days
Without CVVH
With CVVH

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Hurrah Hand Hygiene - Be Aware, Wash with Care

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Objectives:
The Video abstract is to gain a better understanding of the transmission of *Candida auris (C.auris)* within the Intensive Care Unit (ICU) and the potential strategies to prevent its spread.

Methods:
Blood sample received from two of the patients admitted in the intensive care unit of our tertiary care hospital for automated culture yielded *C.auris* which were identified using Matrix-Assisted Laser Desorption ionization Time-of-flight mass spectrometry (MALDI-TOF MS). Owing to the multidrug resistant nature of this organism, we performed surveillance in the ICU in co-ordination with the HICC team.
Surveillance involved the following:
1.Collection of samples from the axilla, groin, oral cavity, and rectal mucosa, which were then cultured on Sabouraud's dextrose agar (SDA) plates.
2.Environmental sampling was done using moistened sterile swabs (soaked with sterile saline) on ICU high touch surfaces and were cultured onto SDA plates.
3.Testing of hand hygiene effectiveness by making the HCW's (Health care workers) wash their hands with 70% alcohol hand rubs. HCW surveillance samples were taken before and after hand washing. HCW's were asked to rub both hands in a sterile bag with Sabouraud dextrose (SD) broth containing chloramphenicol and gentamicin (50 mg/L). Handwashing bags were then incubated at 37^{O}C for 48 h. All subculture were done on SDA.
All isolates grown was identified using MALDI-TOF.

Results:
Patients from whom *C. auris* was isolated had a prolonged ICU stay for over two months. The patient's blood culture yielded *C.auris* and were found in various colonizing sites such as the axilla, groin, rectal area, and mouth of these patients. The study showed that samples taken from the patient bed railings and equipments such as monitor, ventilators also yielded *C.auris*. However hands of health care workers did not yield any growth.

Conclusions:
The persistent colonization in the hospital environment and various colonizing sites of the patient makes the infection highly transmissible and can cause prolonged outbreaks. Longer durations of ICU stay's increase the risk of acquiring the infection in the ICU, suggesting hospital transmission. Our strategies against *C. auris* included eliminating colonization through chlorhexidine washes, disinfecting the environment, and from the hands of healthcare workers through training and regular monitoring of the adherence to hand hygiene. Proper hand hygiene, following WHO recommendations, has helped in prevention of transmission of *C.auris* infection. The exact mode of transmission of *C. auris* in the hospital remains unknown, but it can persist in the environmental surfaces. Patients acquire it from the hospital environment and become colonized rapidly. Adherence to proper hand hygiene is effective against C. auris when used judiciously.

Colonisation study of Patient 1 :

Samples	Result
1) Axillary swab	**No growth**
2) Oral swab	**Candida auris**
3) Rectal swab	**Candida auris**
4) Groin swab	**Candida auris**

Environment sampling of Patient 1 :

Samples	Result
1)Nursing station	**No growth**
2)Monitor	**Candida auris**
3)Railings	**Candida auris**
4) Ventilator	**No growth**

Colonization study of Patient 2 :

Samples	Result
1) Axillary swab	**No growth**
2) Oral swab	**Candida auris**
3) Rectal swab	**No growth**
4) Groin swab	**Candida auris**

Environment sampling for Patient 2 :

Samples	Result
1)Nursing station	**No growth**
2)Monitor	**Candida auris**
3)Railings	**No growth**
4)Ventilator	**Candida auris**

Handwash samples from Health care workers (HCW's)

Samples	Handwash Samples
HCW 1	Ng
HCW 2	Ng
HCW 3	Ng
HCW 4	Ng
HCW 5	Ng
HCW 6	Ng
HCW 7	Ng

*No Growth - Ng

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A five year study of Candida bloodstream infections in a tertiary hospital

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Objectives: The purpose of the present study was to evaluate bloodstream infections due to Candida species and assess susceptibility to antifungal agents.

Materials & Methods: A total of 507 patients with bloodstream infection due to Candida were recorded between 01/01/2018 and 31/12/2022. The demographics of the patients, the nursing units and the candida species were studied. The study was non-invasive. Identification of isolates and susceptibility testing was performed by the Vitek 2 (Biomerieux, France). Identification of *C. auris* was confirmed by mass spectrometry MALDI-TOF MS (Microflex LT, Bruker Daltonics, Bremen, Germany Bruker Biotypes, Germany) at the Laboratory of Microbiology of the Medical School of the National and Kapodistrian University of Athens, when required.

Results: During our 5 year study, 507 patients were diagnosed with candidemia (311 men and 196 women, mean age 69 years). On the time of diagnosis 415 patients were hospitalized in ICUs, 52 in surgical wards and 40 in medical wards. All patients had a central catheter and all had previously received multiple antibiotics. Overall, 779 candidemic episodes occurred during the study period out of 8176 bloodstream infections. Identification of isolates yielded 424 (54,4%) *C. parapsilosis* species complex, 166 (21,3%) *C. albicans*, 80 (10,3%) *C. auris*, 50 (6,4%) *C. glabrata*, 38 (4,9%) *C. tropicalis*, 8 (1%) *C. lusitaniae,* 5 (0,6%) *C. krusei,* 5 (0,6%) *C. guilliermondii* and 3 (0,4%) *C. dubliniensis.* Susceptibility testing showed that all isolates were susceptible to echinocandins and amphotericin B. Resistance to fluconazole was detected in 28% of *C. parapsilosis* isolates and in 4% *C. albicans.* All *C. auris* isolates were resistant to fluconazole.

Conclusions: During the study, a significant number of cases of candidemia were recorded. The patients had multiple risk factors for fungal infection. The majority of cases were due to non-albicans Candida species, with *C. parapsilosis* being the predominant causative agent. After the introduction of *C. auris* there was a significant increase in *C. auris* candidemia cases that reached 22,4% of candidemias in 2022.

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Candida auris blood stream infection, Molecular characterization, Antifungal susceptibility and Analysis of risk factors in patients from Tertiary Care Hospital

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[1]sgpgims, lucknow, india, [2]sgpgims, lucknow, india, [3]sgpgims, lucknow, india, [4]sgpgims, lucknow, india, [5]sgpgims, lucknow, india, [6]sgpgims, lucknow, india

Objectives: To analyse the risk factors, molecular characterization and detection of drug resistance to fluconazole in patients with *Candida auris* candidemia.

Material and Methods: BACTEC blood culture positive for candida on Gram stain from were inoculated on SDA plate and incubated for at 37ºC for the isolation of yeast colonies. The isolates were subjected to phenotypic identification, Polymerase Chain Reaction and MALDI-TOF MS and antifungal susceptibility testing to fluconazole by Etest method. Demographic details of the patients were recorded and significant associated risk factors were analysed.

Results: A total of 59689 blood cultures were received during the study period from admitted patients. There was 623 episodes of candidemia during the study and 111 episodes was due to *C. auris*. Incidence of candidemia due to *C. auris* was 17%. Higher prevalence of candidemia among males (59.45%; n= 66) than in females (40.55%; n=45). The associated risk factors were diabetes mellitus (p <0.024), underlying respiratory illness (p <0.013), mechanical ventilation (p <0.009), dialysis (p <0.034), prolonged ICU stay (p <0.009), hypertension (p <0.035) and others included use of broad-spectrum antibiotics (94.5%) and steroids (23.4%). Only 9% (n=10) isolates were sensitive to fluconazole; 85.6% (n=95) were resistant and 5.4% (n=6) were sensitive dose-dependent. Study showed mortality in 36%.

Conclusions: Emergence of *Candida auris* infection has caused a significant threat in hospitalized patients as well those admitted to the ICUs. *C auris* is known to cause outbreaks in healthcare facilities. Strict precautions like barrier nursing, hand hygiene and proper infection control practices must be followed as well as use of appropriate antifungal therapy to prevent and control the spread of *C. auris.*

TIMM Trends in Medical Mycology

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A Retrospective Italian Analysis on the characteristics of Invasive Fungal Infections in the Intensive Care Unit setting: CHARTER-IFI study

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Objectives: Research and policy interventions to strengthen the global response to fungal infections are World Health Organization (WHO) priorities. Invasive fungal infections (IFI) are severe infections mainly occurring in critically ill patients in intensive care units (ICU). Among them, pulmonary aspergillosis and COVID-19 associated pulmonary aspergillosis (CAPA) are of increasing concern. To date, limited epidemiological and clinical data on IFI in ICU are available. This analysis aimed to assess demographics and clinical characteristics of IFI patients, type of IFI, length of ICU stay and mortality in Italy.

Materials & Methods: CHARTER-IFI is a retrospective analysis on a sample population of 78.220 individuals hospitalized in ICU from Italian Local Health Units databases, from Jan. 2012 to Sept. 2022.

Results: A total of 357 IFI patients discharged from ICU (incidence of 4.6 per 1000 ICU-hospitalized patients), were included (Table 1). Median age was 67 years, 215 patients (60%) were males, and the overall Charlson Comorbidity Index (CCI) was 2.2. Top-three diagnoses were candidiasis (N=269, 3.4/1000 ICU-hospitalized patients), aspergillosis (N=34, 0.4/1000), pneumocystosis (N=25, 0.32/1000). Among 17 patients with aspergillosis during the COVID-19 pandemic (2020-2022) 29% (5/17) were classified as CAPA. The most frequent drugs prescribed during 3 months before ICU admission were antiacids (43.7%) systemic antibacterials (37.8 %), and antithrombotics (35%). 52 patients (14.6%) had a previous hospitalization related to the respiratory system. The evaluation of the comorbidity profile in IFI patients revealed the presence of hypertension (57.4%), use of systemic GC/antibacterials (44% during 12 months before hospital admission and 15.4% during 3 months before hospital admission), cancer (26.3%), diabetes (23.5%) and cardiovascular diseases (23%) (Table 2). The mean (±SD) length of hospitalization in ICU was 20.4 ± 23.6 days (median 11 days), and deaths N (%) occurred in 127 (35.6%) of IFI patients (within 30 days from discharge). Mortality was tendentially higher in IFI patients with aspergillosis [16 (47.1%)].

Conclusions: In this retrospective analysis among ICU-hospitalized patients, the most frequent IFI was candidiasis followed by aspergillosis, including CAPA. Comedications and comorbidities were frequently detected. Length of hospitalization was nearly 3 weeks highlighting the severity of these fungal infections. Mortality was very high in IFI patients with aspergillosis, including CAPA. Understanding the burden of IFI in critical care units could be crucial to strengthen surveillance, investments in research and public health interventions as required by WHO.

TABLE 1: Characteristics of patients with IFI in the ICU setting

	Patients with IFI (N=357)
Age, mean (SD) at index date	65.3 (14.4)
Age, median	67
Male, N (%)	215 (60.2)
Charlson Comorbidity Index, mean (SD)	2.2 (2.2)
Type of infection (most three frequent):	
Candidiasis, n (%)	269 (75%)
Aspergillosis, n (%)	34 (10%)
Pneumocystosis, n (%)	25 (7%)

TABLE 2: Comorbidities in patients with IFI in the ICU setting

	Patients with IFI (N=357)
Bone disorder, N (%)	46 (12.9)
Burns, N (%)	0 (0.0)
Cancer, N (%)	94 (26.3)
Chronic Kidney Disease, N (%)	26 (7.3)
Cardiovascular disease, N (%)	82 (23.0)
Diabetes, N (%)	84 (23.5)
Dyslipidemia, N (%)	76 (21.3)
Hypertension, N (%)	205 (57.4)
Liver disease, N (%)	19 (5.3)
HIV, N (%)	11 (3.1)
Chronic obstructive pulmonary disease, N (%)	69 (19.3)
Obesity, N (%)	16 (4.5)
Sepsis, N (%)	29 (8.1)
HSCT/Organ transplantation, N (%)	8 (2.2)
Use of glucocorticoids (GC)/antibacterials (*12 months before hospital admission*), N (%)	157 (44.0)
Use of glucocorticoids (GC)/antibacterials (*3 months before hospital admission*, N (%)	55 (15.4)
Immunosuppression, N (%)	9 (2.5)
Asthma, N (%)	4 (1.1)

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Candida resistance in the ICU: the CandiRes study

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Objectives

1. Assess the impact of antifungal use on colonising and invasive *Candida* resistance epidemiology in adult ICU patients. 2. Evaluate biomarkers of fungal clearance in patients with invasive candidiasis(IC) and their relationship with antifungal exposure and resistance emergence.

Methods

CandiRes (ISRCTN14165977) is a prospective observational cohort study enrolling ICU patients on antibiotics with ≥1 risk factor for IC at 4 UK hospitals. Demographic, *Candida* risk factors, illness severity, antifungal prescribing and clinical outcomes are collected. Following enrolment, a baseline serum sample is saved for 1-3-β-d-Glucan(BDG) and *Candida* PCR and patients undergo twice-weekly mouth and perianal swabs for *Candida* culture and susceptibility testing, alongside any invasive isolates, throughout their ICU admission. Participants with invasive candidiasis undergo intensive sampling with serial blood cultures, serum BDG, *Candida* PCR, Mannan antigen and antibody and plasma (antifungal PK) over 7d of treatment. Antifungal exposed and unexposed groups were compared using Mann-Whitney-U test for continuous variables and Chi-squared/Fisher's Exact Test for proportions. Time-dependent modelling was performed using R package Stan.

Results

To date, 320 participants have been enrolled and 294 have completed the study. The most common risk factors for invasive candidiasis were an immunosuppressive drug (48%) and renal replacement therapy (39%). 47%(n=136) of participants were exposed to antifungals during ICU admission, most commonly echinocandins (n=99) and azoles (n=59). 73% of participants were colonised with ≥1 *Candida* species and 39% with ≥2, most frequently *C.albicans* (45%), *C.glabrata* (16%) and *C.parapsilosis* (13%). Preliminary findings suggest fluconazole exposure has a more pronounced impact on *Candida* flora (fig 1) and resistance profile: fluconazole MIC increased by ≥4-fold in 4/26(15.4%) serial colonising isolates in exposed vs 3/119(2.5%) in unexposed participants (p=0.02). The corresponding figures for anidulafungin were 5/55(9%) vs 3/90(3%) in those unexposed (p=0.16). For the subgroup of patients with ≥5d antifungal exposure, 4/25(16%) azole-exposed final isolates were resistant, compared to 2/29(7%) baseline isolates (p=0.34); anidulafungin exposure ≥5d resulted in 2 resistant and 2 intermediate isolates(/57) by end of study compared to 0/52 pre-exposure (fig 2, p=0.43)

19 participants had or developed IC, most commonly caused by *C.glabrata (*n=11) and *C.albicans* (n=6): genotyping will be performed to determine whether these are identical to their colonising flora. 11/19 episodes were positive on serum *Candida* PCR for the causative organism- all remained positive after blood culture conversion. We will present findings of time-dependent modelling, addressing colonisation dynamics of *Candida* spp and resistance

in relation to timing and duration of antifungal exposure, and results of linear mixed effects models of treatment response biomarkers in patients with IC.

Conclusion

Antifungal exposure for even short durations in ICU patients impacts colonising *Candida* resistance epidemiology. Our findings have important implications for antifungal stewardship and other interventions to stem the rising tide of *Candida* resistance globally.

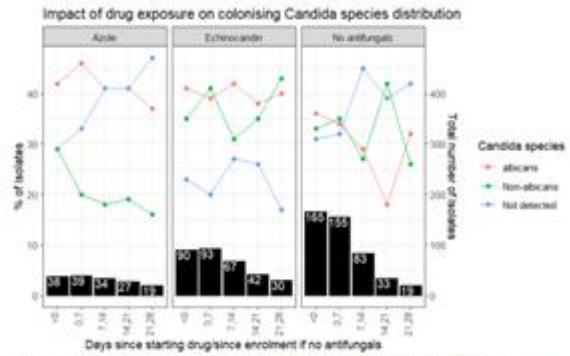

Figure 1 Impact of antifungal exposure on colonising Candida, by C.albicans, Non-C.albicans and Not Detected. Left-sided Y-axis= line graph species distribution, right-sided Y-axis corresponds to barchart of isolates number.

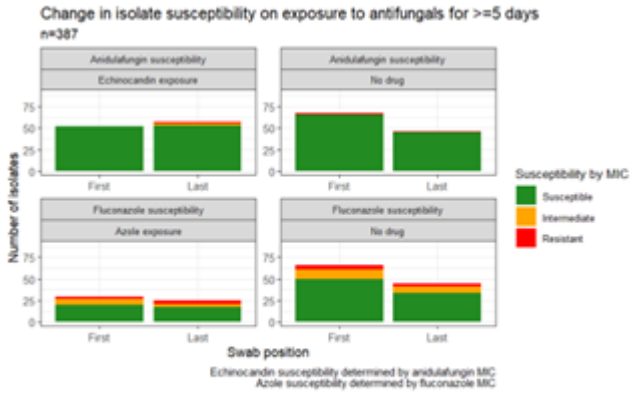

Figure 2 Change in Candida isolate susceptibility with ≥5d antifungals compared to unexposed isolates taken ≥5 days apart

P285

Candida Antigen and anti-Candida Antibody Assays for the Diagnosis of Invasive Candidiasis in ICU Patients: An Analysis of the CandiSep-Trial

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Introduction: Invasive Candidiasis (IC) is the most common fungal infection on ICUs and is associated with high mortality. Besides culture, the detection of fungal antigens and antibodies against those antigens are important methods to support the diagnosis of IC.

Objectives: We aimed to analyse the performance of 3 *Candida* antigen and 5 anti-*Candida* antibody assays in patients of the CandiSep study.

Methods: The CandiSep study was a randomized, multicentre trial comparing BDG-guided antifungal therapy versus standard of care in patients with sepsis and high risk for IC conducted in 18 German intensive care units (ICUs). 342 ICU patients were included. IC and candidemia were diagnosed in 48 (14.2%) and 14 (4.1%) patients, respectively. Sera from two consecutive days at the onset of sepsis were frozen at -20 °C. In the present study, those sera were tested for β-(1→3)-D-glucan with the Fungitell assay (BDG), for mannan with the Platelia *Candida* Ag Plus assay (Platelia-Ag) and the Serion ELISA antigen *Candida* (Serion-Ag), and for anti-*Candida* antibodies with the Platelia *Candida* Ab Plus assay (Platelia-Ag), the Serion ELISA *Candida albicans* IgA/IgM/IgG assays, and the Virclia *Candida albicans* germ tube antibody IgG Monotest (CAGTA-Monotest). All assays were performed according to the recommendations of the manufacturers.

Results: The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of a single or two consecutive positive test results for the diagnosis of IC and candidemia are shown in Table 1. The sensitivity for the diagnosis of IC was lower than that for candidemia, with BDG being the most sensitive antigen test (60.4% and 71.4%, respectively) and Platelia-Ab the most sensitive antibody test (29.2% and 42.9%, respectively). The diagnostic performance could be improved by using optimized cutoff values, e.g. >277 pg/ml for BDG in IC (sensitivity 45.8%, specificity 80.1%) or >356 for BDG in candidemia (sensitivity 64.3%, specificity 83.4%). The area under the ROC-curve (AUC) for the diagnosis of IC and candidemia was generally higher for the antigen assays compared to the antibody assays. Combining an antigen assay with an antibody assay increased the sensitivity with a loss of specificity (see Table 1). However, the combination did not significantly change the AUC.

Conclusion: The diagnostic performance of various *Candida* antigen and anti-Candida antibody assays in prospectively collected samples from ICU patients with high risk for IC varies greatly. BDG is the only assay with a moderate sensitivity. All other assays showed poor to unusable sensitivity. The combination of antigen and antibody assays did not improve the diagnostic performance significantly.

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P286

Clinical-Economic Evaluation of Pediatric Patients managed with Isavuconazonium Sulfate for Invasive Fungal Disease: A Retrospective Cohort Study in Real-World Settings

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Objectives: Invasive fungal diseases including invasive aspergillosis (IA) and invasive mucormycosis (IM), are life-threatening infections that occur primarily in immunocompromised and/or hospitalized pediatric patients. Isavuconazonium sulfate (ISAV) is approved for the treatment of adults with IA/IM, but its use in pediatric patients has yet to be explored fully. This real-world study aimed to describe the characteristics and outcomes of children prescribed ISAV in clinical practice.
Materials & Methods: This was a non-interventional, retrospective cohort study investigating the clinical outcomes and healthcare resource use in pediatric patients receiving ISAV. Patients aged <18 years at first administration of ISAV from March 2015 to June 2022 were identified from the TriNetX Electronic Medical Records (EMR) database. Endpoints included ISAV utilization, adverse events (AEs) of interest, length of stay, mortality rates (overall and at Days 42 and 84), and daily inpatient and outpatient ISAV dose. Data were summarized descriptively, and no statistical analyses were conducted.
Results: The patient cohort included 76 patients with a median (range) age of 14.0 (9.5–16.0) years (Table 1). Overall, median (Q1–Q3) treatment duration was 3.0 (1.0–11.0) days and most (81.6%) patients received ISAV in the inpatient setting: prior antifungal use was 43.4% and concomitant antifungal use was 34.2%. Additionally, 31.6% of patients experienced at least one pre-defined AE, the most frequent being electrolyte abnormalities, and hepatobiliary disorders (including elevated liver enzyme levels, Table 2). The median (Q1–Q3) length of stay was 31.0 (14.0–69.0) days and time from ISAV initiation to discharge was 12.0 (1.0–44.0) days. All-cause mortality rates were 6.6% at Day 42 and 10.5% at Day 84.
Conclusions: Findings from this large real-world evidence study showed that the age range of patients receiving ISAV was broad and prior/concomitant antifungal use was relatively common. Treatment duration was short, however this may be impacted by inherent limitations of the EMR database (such as inclusion of discontinued orders or cancellations). The AE profile in pediatric patients suggested relatively good tolerability for ISAV.

Table 1. Patient characteristics

Characteristic	Total (N=76)	1–11 years (n=29)	12–17 years (n=47)
Median age, years (Q1–Q3)	14.0 (9.5–16.0)	8.0 (5.0–11.0)	16.0 (14.0–16.0)
Male, %	43 (56.6)	15 (51.7)	28 (59.6)
Race, %			
White	48 (63.2)	21 (72.4)	27 (57.4)
Black	8 (10.5)	2 (6.9)	6 (12.8)
Other/Unknown	20 (26.3)	6 (20.7)	14 (29.8)
IA or IM, %			
with	10 (13.2)	N/A	N/A
without	66 (86.8)	N/A	N/A
Prior antifungal use[a], %	33 (43.4)	N/A	N/A
Concomitant antifungal use[b], %	26 (34.2)	N/A	N/A

IA, invasive aspergillosis; IM, invasive mucormycosis; N/A, not available.
[a]Most commonly used antifungal was Amphotericin B followed by voriconazole, micafungin and posaconazole
[b]Most commonly used antifungal was Amphotericin B followed by micafungin, fluconazole and voriconazole

Table 2. Adverse events (reported in >5% of patients, within any age category)

AE[a], %	Total (N=76)	1–11 years (n=29)	12–17 years (n=47)
Patients with any AE[b], n (%)	24 (31.6)	12 (41.4)	12 (25.5)
Hyperglycemia[c]	4 (5.3)	3 (10.3)	1 (2.1)
Hypoglycemia[d]	6 (7.9)	4 (13.8)	2 (4.3)
Hypokalemia[e]	6 (7.9)	3 (10.3)	3 (6.4)
Hypomagnesemia[f]	9 (11.8)	4 (13.8)	5 (10.6)
Hypophosphatemia[g]	6 (7.9)	3 (10.3)	3 (6.4)
Hepatobiliary disorders	5 (6.6)	3 (10.3)	2 (4.3)
Elevated[h] ALT	3 (3.9)	2 (6.9)	1 (2.1)
Elevated[h] AST	4 (5.3)	1 (3.4)	3 (10.3)

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase
[a]AEs were counted from ISAV initiation date to end of ISAV.
[b]Each patient can have multiple AEs.
[c]>115 mg/dL if fasting and >125 mg/dL for those with history of diabetes.
[d]<75 mg/dL
[e]<3.5 mEq/L
[f]<1.8 mg/dL
[g]<3 mg/dL
[h]>2x upper limit of normal.

P287

Rhinofacial Conidiobolomycosis – RARE FUNGAL INFECTION PRESENTING AS A NASAL MASS

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Objectives:
Conidiobolomycosis is caused by a fungus in the genus of *Conidiobolus.* Typical symptoms include nasal discharge, epistaxis, unilateral nasal obstruction, sinus tenderness, extensive and persistent facial swelling, which can lead to disfigurement. In this case report, we describe an immunocompromised young gentleman who develops an indolent presentation, complicating the diagnosis and leading to delay in treatment.

Case Presentation:
29 years old Malay gentleman with underlying hearing disabilities and smear-positive pulmonary tuberculosis, presented with a history of being unwell for five months and one-week history of poor oral intake. On clinical examination, he was alert and not tachypnoeic. There was painless swelling of the left nasal cavity with loss of nasolabial folds extending onto the bilateral maxilla, which was noticed in the last five months. He was diagnosed with facial cellulitis via computed tomography (CT) scan and nasal biopsy showing epidermal hyperplasia without granulomas. Oral fluconazole 200mg BD and anti-tuberculous treatment were started. Unfortunately, he declined further treatment and defaulted on his follow-up. Four months later, he was readmitted again due to worsening facial swelling extending to the lip associated with loss of weight. Repeated CT neck, paranasal sinus and thorax reveal worsening soft tissue mass causing upper airway narrowing with cervical lymphadenopathy. An open tracheostomy with direct laryngoscopy, nasoendoscopy and biopsy was successfully done. Intravenous amphotericin B 40mg OD was immediately started covering for invasive fungal infection. His infective and autoimmune screening were negative. Tissue biopsy shows inflamed tissue with features of fungal infection. Tissue for fungal culture reveals macroscopic and microscopic features, of *Conidiobolus sp.* which further confirmed as *Conidiobolus megalotocus* by fungal PCR. The MALDI-ToF identification failed since this isolate isn't in their database. Antifungal susceptibility testing was also performed on the isolate at a reference laboratory. His treatment regime for conidiobolomycosis started with additional Lugol's iodine 2ml OD, T. Fluconazole 200mg BD, and IV Amphotericin B 40mg OD. After 2 months of treatment, his facial swelling has improved and he continues to receive regular follow-up appointments.

Discussion:
Rhinofacial entomophthoromycosis or Conidiobolomycosis is an uncommon disease and the fungi that caused the disease are mainly found in tropical Africa, South and Central America and South East Asia including Malaysia. A definitive diagnosis for the infections requires histopathologic evidence of the fungal and its isolation in culture. However, difficulty in obtaining a good sample will delay the diagnosis. The combination of medical therapy (antifungal and potassium iodide) with surgical debridement of the affected paranasal sinus is the treatment of choice.Various drugs had been used as a single agent or in combination with different outcomes.

Conclusions:
Conidiobolomycosis is one of the differential diagnoses to be considered in a patient presented with a mass in the facial region. A high index of suspicion and relevant investigation can help in early diagnosis as the disease is usually chronic and indolent. In this case report, we highlight the importance of good samples to guide diagnosis for early treatment.

P289

"Double trouble: Pulmonary coinfection with Scedosporium apiospermum and Mycobacterium chelonae in an immunocompromised host."

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Objectives:
To describe an unusual case of pulmonary *Scedosporium apiopsermum* infection with *Mycobacterium chelonae* coinfection in an immunocompromised host.

Materials & Methods:

An 18-year-old male patient recently diagnosed with B Cell-Acute lymphoblastic leukemia (ALL) was admitted for chemotherapy. He was in the consolidation phase but developed fever and cough during admission. He had no history of any notable pulmonary infections in the past. In view of neutropenic fever and dry cough with blood culture showing no growth, a high-resolution computed tomography scan (HRCT) of the chest was taken. The scan revealed a thick-walled fibro cavity measuring about 16x22x21 mm involving the left lower lobe with perifocal ground glass opacity and air-space opacity and few intracavitary soft tissue density strands. The BAL sample was sent to rule out infective etiology. Aspergillus Galactomannan antigen done from serum and bronchoalveolar lavage (BAL) were negative. *Mycobacterium tuberculosis* infection was ruled out by CBNAAT.

Results:

The BAL sample received was negative for KOH microscopy but grew a mould after 3 days of incubation. The mould grew as white cottony colonies with aerial mycelium on Sabourauds dextrose agar and was identified as *Scedosporium* species microscopically. MALDI-tof helped to identify the species as *Scedosporium apiospermum*. However, the sample inoculated in automated mycobacterial growth indicator tube flagged positive and on subculturing yielded Mycobacterium other than tuberculosis (MOTT).MOTT was identified as *Mycobacterium chelonae* by MALDI-tof.

The culture findings were discussed with the treating team. In view of the immunocompromised state, supportive radiological findings, and isolation of the organisms from BAL, both were considered significant. The patient was started on oral Voriconazole 200mg twice daily for 2 weeks for the fungal etiology and intravenous Amikacin, Imipenem and Levofloxacin combination for 4 weeks duration for MOTT. The patient improved symptomatically. However, the patient is currently on follow up to look for resolution of radiological and microbiological parameters.

Conclusions:
Scedosporium apiospermum,an ubiquitous fungi is an emerging opportunistic pathogen that can cause infections, particularly in immunocompromised individuals. Infections caused by *Scedosporium* species can manifest in various forms, including localized skin and soft tissue infections, pulmonary infections, bone infections, and disseminated infections. Prompt

diagnosis allows for the initiation of targeted antifungal therapy, which can help prevent further dissemination of the infection. This approach can help prevent the spread of the infection and improve patient outcomes.

We present a case of *Scedosporium apiospermum* pulmonary infection with MOTT coinfection in an immunocompromised host. In our setting, invasive Aspergillosis is the most common fungal infection among ALL patients. However, this scenario presenting with mild symptoms yet showing a combination of pathogens was a rare finding. The management of such coinfections often requires a multidisciplinary approach. Radiography and timely culture have helped in prompt identification and has led to providing appropriate treatment to the patient and helped with better patient outcome. This case report emphasizes the need for a high index of suspicion and knowledge of atypical organisms of fungal and mycobacterial aetiology in causing lung infections in an immunocompromised host.

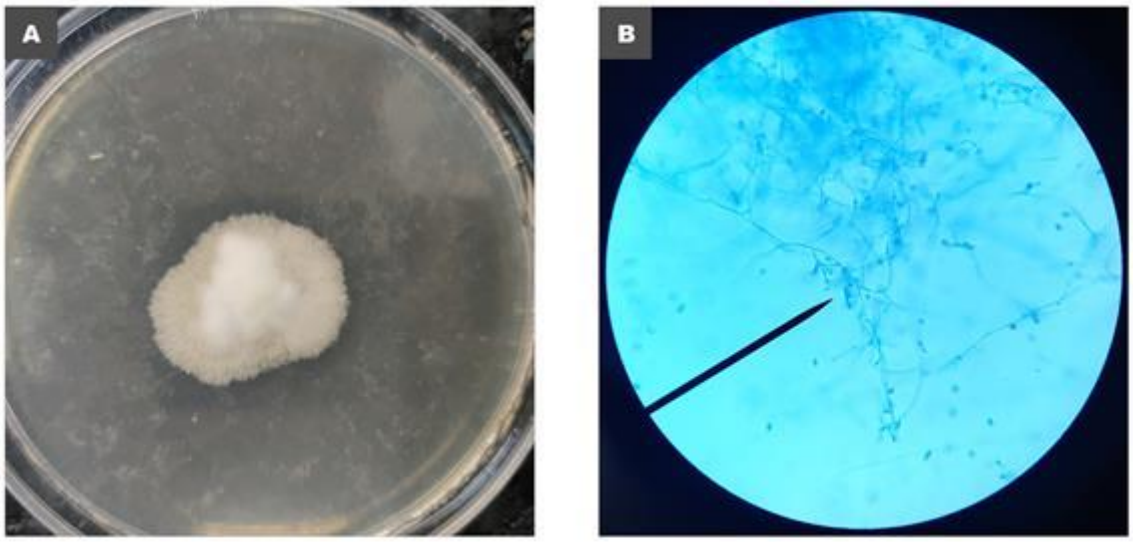

Figure2: (A) Colony morphology of *Scedosporium apiospermum* on Sabouraud dextrose agar shows white cottony aerial mycelium.

(B) Microscopic morphology of *Scedosporium apiospermum* on lactophenol cotton blue preparation shows hyaline branching septate hyphae with short or long slender conidiophores, bearing single conidia. The conidia were oval and unicellular, with the larger end toward the apex(400x).

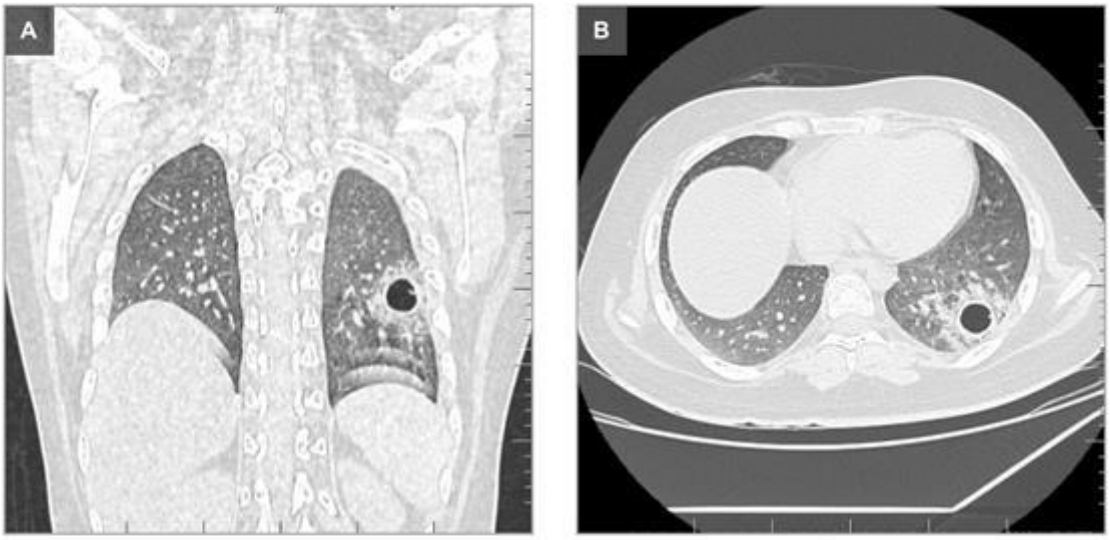

Figure1: CT chest- (A) Coronal and (B) Axial sections shows a thick-walled fibro cavitary lesion involving the left lower lobe with surrounding ground glass and air-space opacity and few intra-cavitary soft tissue density strands.

P291

COVID-19 associated cryptococcaemia

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Objectives:
The aim of this abstract is to report a case of COVID-19 related cryptococcaemia in an elder patient with multiple comorbidities treated in intensive care unit (ICU).

Materials & Methods:
A 78-year-old patient with *Cryptococcus neoformans* isolated from blood culture in February 2023 at the University Hospital Centre Zagreb is presented. Clinical information was obtained from medical records.

Results:
A 78-year-old female patient with a previous history of oxygen dependent chronic obstructive pulmonary disease and arterial hypertension was admitted to University Hospital Centre Zagreb presenting with symptoms of dyspnea, tachycardia and difficulty to maintain verbal contact. According to the emergency medical team, the patient collapsed the night before. Prior to admission, patient was tested positive for SARS-COV-2 virus. Initial diagnostic tests showed atrial fibrillation with rapid ventricular response (HR 170/min), respiratory acidosis with low oxygen saturation (SO2 66%), leukocytosis and high C-reactive protein (120 mg/L) and impaired kidney function (creatinine clearance 25 ml/min calculated with Cockcroft-Gault equation), leading to admission to the ICU with administration of high-flow nasal oxygen therapy (HFNO). Echocardiogram was done, showing heart failure with left ventricular ejection fraction about 20%. Blood tests showed extremely elevated liver enzymes and CT angiography of the abdomen was made to rule out thrombosis of hepatic vessels. After initial treatment with HFNO with maximum flow of 60L/40% FiO2, patient was sedated, intubated and provided with mechanical ventilation, hemodynamically supported with vasoactive drugs. Subsequently, haemorrhage was developed from deep femoral artery and red blood cell concentrate transfusions were given. Due to decrease in patients' alertness and further deterioration, tracheostomy was performed.
First three days the patient was treated with remdesivir, along with corticosteroids, antibiotic prophylaxis and antiaggregants which were given continuously during the stay. Several tests for SARS-COV-2 were performed, all with positive result. Blood tests showed daily decrease of CRP levels with progression of leukocytosis. Levels of β-D-glucan and galactomannan in serum were tested. Results were negative for galactomannan and indeterminate for β-D-glucan tenth and sixteenth day of inpatient care. *Cryptococcus neoformans* was isolated in blood culture obtained on the sixteenth day.

Conclusion:
This report suggests that cryptococcaemia as opportunistic invasive fungal infection may be considered in patients with severe clinical presentation of simultaneous COVID-19 infection. Weakened immune system, multiple comorbidities, intensive care unit treatment and extensive use of corticosteroids and antibiotics may contribute to development of secondary bloodstream opportunistic infection cause by *Cryptococcus spp.*

P292

Histoplasmosis : an emerging disease in Tunisia

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Objectives:

Histoplasmosis, caused by the dimorphic fungus Histoplasma capsulatum, is an exotic mycosis primarily found in tropical and subtropical regions.The aim of this study is to investigate the clinical presentation, management, and outcomes of patients diagnosed with imported histoplasmosis, focusing on factors contributing to the high mortality rate.

Materials & Methods:

In this retrospective study conducted at the department of infectious diseases at Rabta Hospital, we examined all the patients with confirmed diagnoses of histoplasmosis. We focused on describing the initial presentation, management, and progression of the disease.

Results:

Over a five-year period (2017-2022), we present a series of five cases of histoplasmosis, consisting of three women and two men. Four patients originated from Ivory Coast but were currently residing in Tunis and one tunisian patient whom residing in Congo. The average age was 33.5 years, ranging from 31 to 37 years. Initial clinical manifestations included a decline in general condition and fever in all patients, three patients exhibited an umbilical papulonodular rash and productive cough.

Histoplasmosis was the primary indicator of underlying retroviral infection in all cases. Two patients had positive serology for visceral leishmaniasis. Disseminated histoplasmosis was observed in four patients and lymph node involvement was noted in one patient..

The average time to diagnosis following admission was 9 days, ranging from 2 to 15 days. The diagnosis was confirmed through direct examination, revealing intracellular spherical yeasts identified as Histoplasma capsulatum var capsulatum in three cases and *Histoplasma duboisii*. Skin biopsies were used in two cases, a blood smear and nasal mucosa biopsy in another case, a myelogram in the fourth case and biopsy of lymph node in one case.

All patients received Amphotericin B followed by oral Itraconazole. Macrophage activation syndrome occured in three patients, leading to severe hemorrhagic symptoms that required multiple transfusions of red blood cells and platelets.

Two patients had a positive therapeutic outcome, with follow-ups of 15 and 12 months, respectively. The other two patients died despite initiation of antifungal therapy, with one passing away after 8 days due to a hemorrhagic syndrome and the other after 40 days due to CMV pneumonia.

Conclusions:

Histoplasmosis is an imported disease in our country. The high mortality rate is primarily associated with the disseminated form of the disease in immunocompromised individuals, which is enhanced by delayed diagnoses. Early detection and effective treatment are crucial for improving prognosis.

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Pulmonary pneumocystis in HIV infection: an epidemioclinical study

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Introduction :
Pneumocystis remains the most common opportunistic infection associated with HIV infection.It is characterized by a clinical polymorphism responsible for delayed diagnosis. It is still a public health problem.

Aim :
Our aim is to study the epidemiological and clinical criteria of pulmonary pneumocystis in HIV infection.

Methods :
We conducted a retrospective descriptive study including HIV-infected patients admitted to the infectious diseases department of la Rabta Hospital for pulmonary pneumocystis over a 30-year period from January 1991 to December 2020.

Results :
We collected 100 cases. The mean age was 38.5 years. The sex-ratio was 1.56. The mean time to diagnosis was 70 days. Pneumocystis was inaugural to retroviral infection in 60% of cases. CD4 count was 46.1 cells/mm when pneumocystis was diagnosed. The mean initial viral load was 756468.8 copies/ml. Of the patients who knew they were seropositive, 10 were on primary propbhylaxis. Compliance was good in 4 patients. Twenty patients were on antiretroviral therapy. Onset was abrupt in 35% of cases. Clinical signs were dominated by cough (94%), dyspnea (85%) and fever (83%). Pulmonary auscultation revealed crackling rales in 67 patients. It was normal in 22 patients. Oral candidiasis was detected in 49 patients. *P. jirovecii* cyst testing was positive in 36 cases. PCR was positive in 17 cases. Treatment was based on cotrimoxazole in 97 cases. Corticosteroid therapy was indicated in 81 patients. The average duration of treatment was 19.6 days. The mortality rate was 16%.

Conclusion :
Pneumocystis is responsible for significant mortality worldwide. It is thereforea clinical and therapeutic emergency.

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Co-infection of Aspergillosis and Nasal Demodicosis in an Aplastic Anemia Patient with COVID-19: a Case Report

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Objectives:
Demodex is a genus of mites that normally live in the pilosebaceous unit and gland. There are two types of demodicosis: primary and secondary demodicosis. Secondary demodicosis is usually related to local or systemic immuno-suppression like *Aplastic anemia* (AA). Patients with AA are the leading candidate for invasive fungal infections and invasive aspergillosis is among the most common infection in hematological malignancies. The predisposing factors of invasive aspergillosis are: *A. fumigatus*, *A. flavus*, *A. terreus*, and *A.niger* respectively. Although majority of invasive aspergillosis is caused by *A.fumigatus* overall in the united state, A.flavus is the predominant pathogen in tropical and sub-tropical area. In this study, we demonstrate an AA, COVID-19 patient who simultaneously had *Demodex folliculorum* and *Aspergillus flavus* in his paranasal sinuses.

Materials & Methods:
A 31 years old male was a known case of aplastic anemia on a treatment of cyclosporine, admitted with symptoms of high fever, dyspnea and severe thrombocytopenia at the Imam Khomeini Hospital Complex, in June 2022. Due to the COVID-19 pandemic, a chest CT scan was shown ground glass opacities. Real-time polymerase chain reaction from nasopharyngeal swab was positive for SARS-CoV-2. A routine laboratory examination revealed pancytopenia and infection. Para- nasal CT scan revealed pan sinusitis with bone destruction. A (10 %) potassium hydroxide of sinus debridement examination showed the presence of mite and fungal septate elements hyaline hyphae (Microscopic examination showed *Aspergillus* spp. For molecular identification DNA of *Aspergillus* spp. was extracted. The entire internal transcribed spacer (ITS) region (ITS1-5.8S rDNA-ITS2) was amplified by PCR with the universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR-amplicon was sent for Sanger sequencing and *Aspergillus flavus* was identified .

Results:

Even though the presence of *D. follicullorum* in debridement of the paranasal sinus increases suspicious rhino sinusitis due to the loss of patient, more investigation couldn't be done to find out its pathogenic implement. Some studies have suspended mites in transferring the fungal spores in sinuses.

Conclusions:
To the best of our knowledge, this is the first case of possible demodicosis associated with an aplastic anemia COVID-19 patient with a coinfection of invasive aspergillosis. In conclusion, this case shows more attention from clinicians and laboratory physicians about coinfection of Demodex and Aspergillus spp, especially in immunosuppressed patients. More studies are needed to investigate the role of aplastic anemia in the susceptibility of them.

P295

Recurrent Cutaneous Fusariosis in a Kidney Transplant Recipient – a Case Report and Review of the Literature

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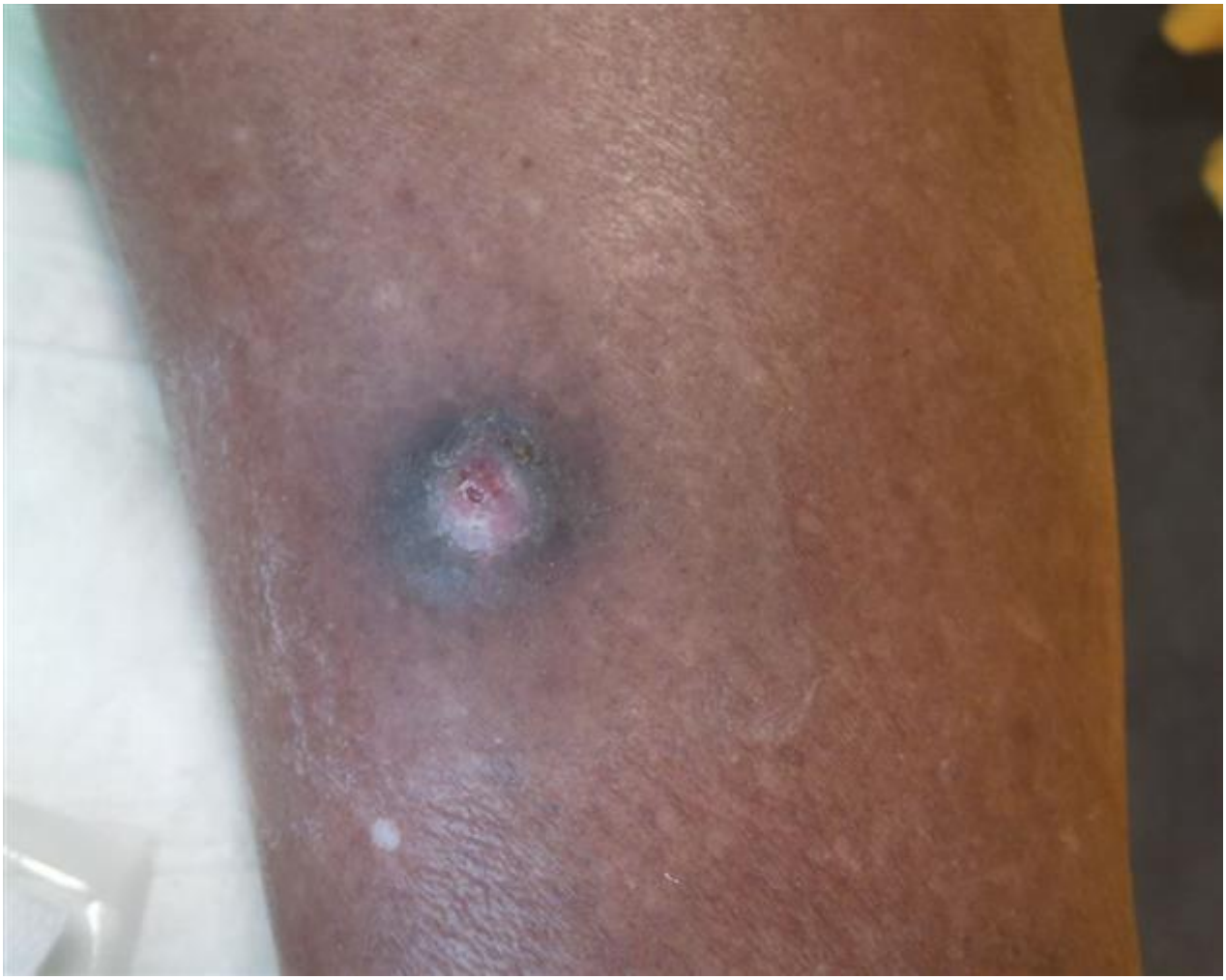
Recurrent Cutaneous Fusariosis in a Kidney Transplant Recipient – a Case Report and Review of the Literature *

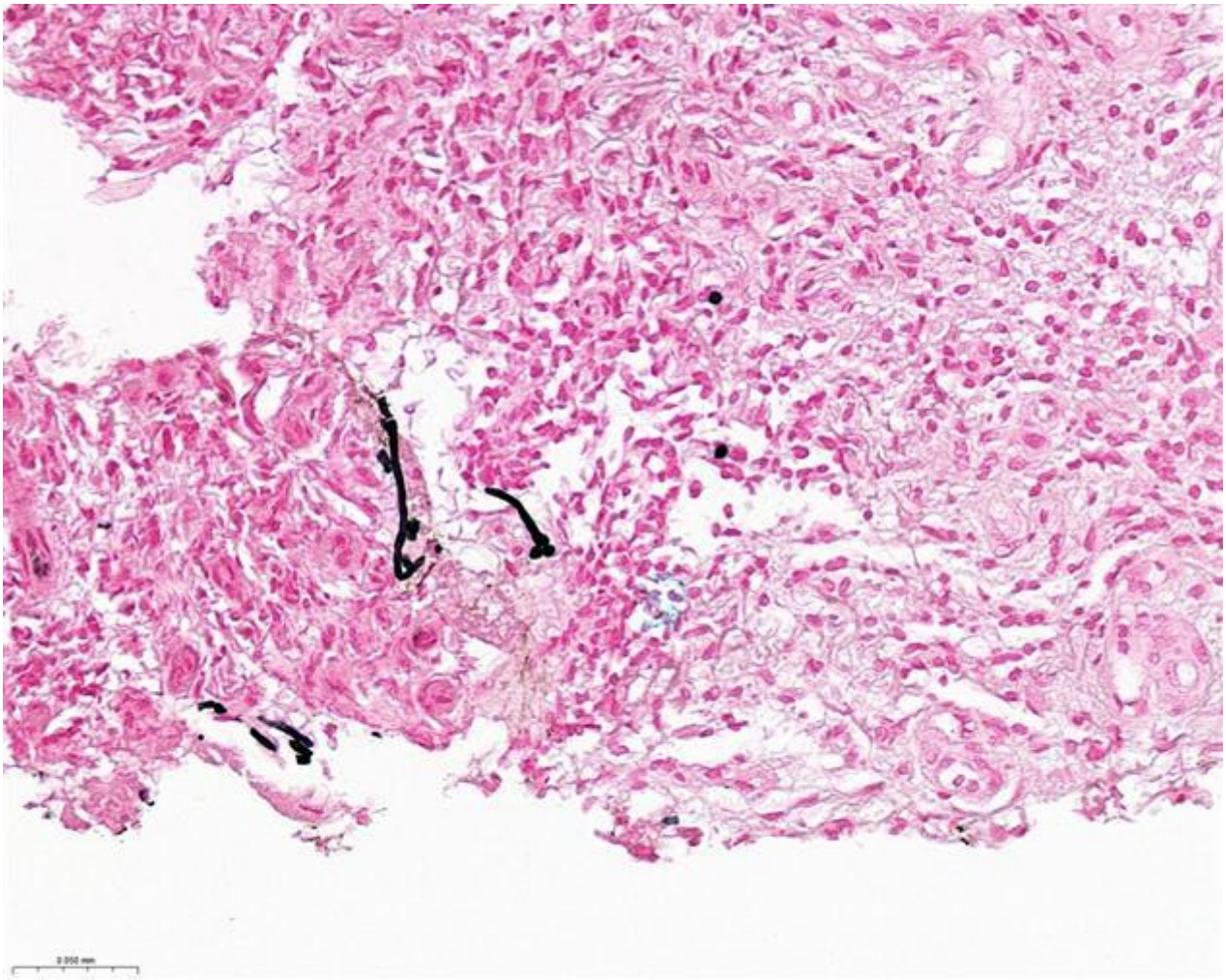
Introduction: We report an unusual case of cutaneous fusariosis in a kidney transplant recipient. *Fusarium* species are emerging fungal pathogens that pose diagnostic and therapeutic challenges. In severely immunocompromised patients, fusarial infections are associated with high mortality in the case of systemic dissemination.

Case presentation: A 69-year-old black male with a history of renal transplantation presented with recurrent purulent nodules and painful ulcers of the left lower leg. Based on repeated skin biopsies, focally invasive skin infection with *Fusarium solani* was proven histologically and microbiologically. After four months of treatment with oral voriconazole, lesions considerably improved. When the patient died one month later from Covid-19 pneumonia under continued antifungal therapy, there was no evidence of systemic fusariosis or fusarial superinfection.

Conclusion: Although rare, fusarial infections should be considered in immunocompromised individuals such as solid organ transplant recipients. Therefore, skin lesions in this patient population should be examined accurately. Histopathological and microbiological workups, including fungal cultures, are necessary for diagnosis and timely initiation of targeted therapy. Systemic antifungal therapy with voriconazole is the treatment of choice for focally invasive fusariosis.

* Case report accepted by *healthbook TIMES Oncology Hematology* for publication





P296

Fungal otitis : infectious diseases department experience

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Introduction :

Fungal otitis is an uncommon and potentially life-threatening invasive infection that affects the outer ear canal. If the infection spreads to the nearby tissues, it can cause severe inflammation of the bone and damage to the cranial nerves, which can have serious consequences.

Aim :

Our aim is to describe the epidemiological, clinical, microbiological and therapeutic features of fungal otitis.

Methods :

We conducted a retrospective descriptive study from January 2010 to December 2022. We included all cases of fungal otitis hospitalized in the Infectious Diseases Department of la Rabta Hospital.

Results :

We have collected 26 cases. The average age was 72 years. The sex-ratio was equal to 1.6. All patients had diabetes. No other immunosuppressive conditions were noted. The average time between consultation and onset of clinical signs was 75 days. The predominant clinical signs were otalgia in 25 cases, otorrhea in 16 cases, hypoacusis in 14 cases, headache in 7 cases and facial paralysis in 6 patients. Twenty-one patients had been treated with antibiotics before coming to our department. Candidiasis otitis was diagnosed in nine patients and aspergillosis otitis was diagnosed in 12 patients. In order of frequency, the yeasts isolated were dominated by : *Aspergillus flavus* (n=11), *Candida albicans* (n=4) and *Candida parapsilosis* (n=4). *Candida tropicalis* was isolated from one patient. Bacteria were present in 5 patients. Aspergillary antigenemia was performed in 15 patients, it was positive in seven cases. Locoregional complications were dominated by: tympanal bone lysis (n=22), temporomandibular arthritis (n=10), deep space damage (n=15), mastoid damage (n=15), sinus damage (n=16) and venous thrombosis (n=6). Antifungal treatment was based on voriconazole in 16 patients, followed by fluconazole in 6 and amphotericin B in 4 patients. A combination of two antifungal agents was prescribed in 4 cases. one patient developed renal failure on amphotericin B, a second patient developed hepatic cytolysis on voriconazole. Only one patient required surgical treatment. Recovery was observed in 18 patients. Total treatment time averaged 19.47 days, with extremes ranging from 2 days to 98 days.

Conclusion:

Fungal otitis is typically observed in older individuals, those with diabetes, or individuals with a weakened immune system. An increase in incidence of fungal infections of the ear are noted in the last few years due to the increase in cases of diabetes. Cases of fungal otitis caused by *Aspergillus fumigatus* are more common, which is not the case in our series. Early diagnosis and microbiological identification are essential to ensure optimal treatment.

P297

Population genomics of human pathogenic fungus Aspergillus fumigatus isolated from multi patient cohorts and possible link with environmental A. fumigatus

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Background *Aspergillus fumigatus* causes a range of diseases in humans, such as invasive aspergillosis (IA). The patients at risk for IA is broadening with cases emerging beyond neutropenia in critically ill patients, such as those with severe influenza (influenza associated pulmonary aspergillosis, IAPA, and COVID-19 associated pulmonary aspergillosis, CAPA). Increasingly host-related factors are identified that increase the risk in these patients, but fungal factor such as genetic relatedness and variation of these *A. fumigatus* isolates remain poorly understood. We set out to investigate genetic variation of *A. fumigatus* isolates collected from multi patient cohorts (IA in haematology, IAPA and CAPA) by whole genome sequencing of *A. fumigatus* isolates recovered from these patients.
Methods Genomes of 20 *A. fumigatus* isolates from patients with CAPA were compared with 20 isolates each from patients with IAPA and IA in patients with hematological malignancy. In addition, 20 isolates were included that were cultured from environmental sources. We investigated the genomic relatedness of these *A. fumigatus* cultured from specific patient cohorts and environmental niche using whole genome sequencing, population genomic analysis.
Results We found that CAPA isolate genomes do not exhibit significant differences from the genome of the isolates collected from other patient cohorts by examining the mutational spectrum of single nucleotide polymorphisms. Phylogenomic analysis of these isolates cultured from different patient cohorts and environment did not show strong genetic structuring into main genomic clades. However, majority of isolates cultured from patient cohorts and environment generally clustered together. Furthermore, we have shown the *A. fumigatus* cultured from CAPA, IA exhibit genetic relatedness with the isolates cultured from environmental ecology niche.
Conclusion These results will help us to understand the genomic relatedness/variation of *A. fumigatus* cultured from different cohorts. More importantly, this will provide the insight for understanding the spreading of *A. fumigatus* and it is essential for tracing the transmission route of *A. fumigatus*.

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Invasive Fungal Infections in Patients with Hematological Neoplasia: A Retrospective Multicenter Study in Greece – Preliminary Data

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Objectives: To retrospectively study invasive fungal infections (IFIs) in patients with hematological malignancy in seven tertiary-care centers in Greece.

Materials & Methods: We performed a retrospective multicenter analysis from January 2020 to March 2023. Chi-squared test, t-test and Mann-Whitney U test were used to compare categorical and continuous variables. P values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 28.0 SPSS Inc. Chicago IL USA.

Results: The present study included 45 cases of IFIs in 44 patients (females 45.4%); median age was 61 years. Among these patients, 50% had acute myeloid leukemia and 4 patients had undergone allogeneic stem cell transplantation. Upon the development of the fungal infection, 82.2% of the patients were neutropenic, and 68.9% of the individuals were on prophylactic antifungal agents. The commonest choices for antifungal prophylaxis were fluconazole and posaconazole. Regarding the fungal disease, candidiasis accounted for 43.2%, followed by aspergillosis (proven, probable, or possible) at 36.3%. The remaining cases included *Mucor*, *Rhodotorula mucilaginosa*, *Trichosporon asahii*, and *Fusarium* infections. Candidiasis was caused most frequently by *Candida parapsilosis* (36.8%). Based on the definitions from the most recent revision by EORTC, the majority of cases (66.6%) were proven, whereas the remaining ones were considered possible or probable fungal infection. Early mortality was estimated at 18% within the first 7 days from the IFI diagnosis; mortality rate was 27.3% within 28 days from IFI diagnosis; overall 31.8% of patients died from IFIs or from other related conditions. In this study, the highest percentage of IFIs involved lungs (40%), followed by bloodstream (33.3%), and less

commonly skin, CNS, liver, pharynx, and palate. Additionally, 62.2% of patients had central venous catheter (CVC). The retention or removal of CVC did not have a significant impact on the outcome of patients (mortality was 37.5% corresponding to 8 patients with removal of the CVC, and 33% in 15 patients with retention of the CVC). Concurrent bacterial infections were found in 42.2% of cases. Age (>60 years), presence of neutropenia, depth of neutropenia, history of diabetes, history of corticosteroid or broad-spectrum antibiotic use, concurrent bacterial infection, high ferritin levels, presence of chronic kidney disease or abnormal chest-computed tomography or X-ray findings were not associated with the outcome. Out of 14 cases with *de novo* mycosis one patient died, whereas among 31 patients with breakthrough mycosis 13 patients died. The median time of neutropenia was 21 days in patients who survived vs. 45 days in patients who died (*P*-value: 0.02). These findings indicate that there is a statistically significant correlation between the duration of neutropenia and an unfavorable outcome.

Conclusions: Candidiasis and aspergillosis were the most frequent IFIs, with *Candida parapsilosis* being the predominant species in patients with hematological malignancy. The duration of neutropenia was significantly related to an unfavorable outcome.

P299

Emergence of Pathogenic Aspergillus niger with Triazole Resistance: A Cause for Concern or a New Phenomenon?

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Abstract

Objectives
Aspergillus niger is an increasingly common fungal pathogen that can cause respiratory and non-respiratory infections in humans. Due to its potential for transmissible resistance to triazoles, treating *A. niger* infections can be challenging. This study aimed to assess the susceptibility patterns of *A. niger* isolates to commonly used antifungal agents and determine the prevalence of triazole resistance.

Material and Methods
Between 2019-2022, we prospectively collected *A. niger* isolates from clinical specimens. After subculturing, single isolates from growth plates were confirmed by DNA sequencing from the ITS1 to ITS4 region. Using the CLSI M38 standard, we determined the MICs of five antifungal agents, including itraconazole, voriconazole, posaconazole, isavuconazole, and amphotericin B, and calculated the range of MICs and resistance rates for each drug.

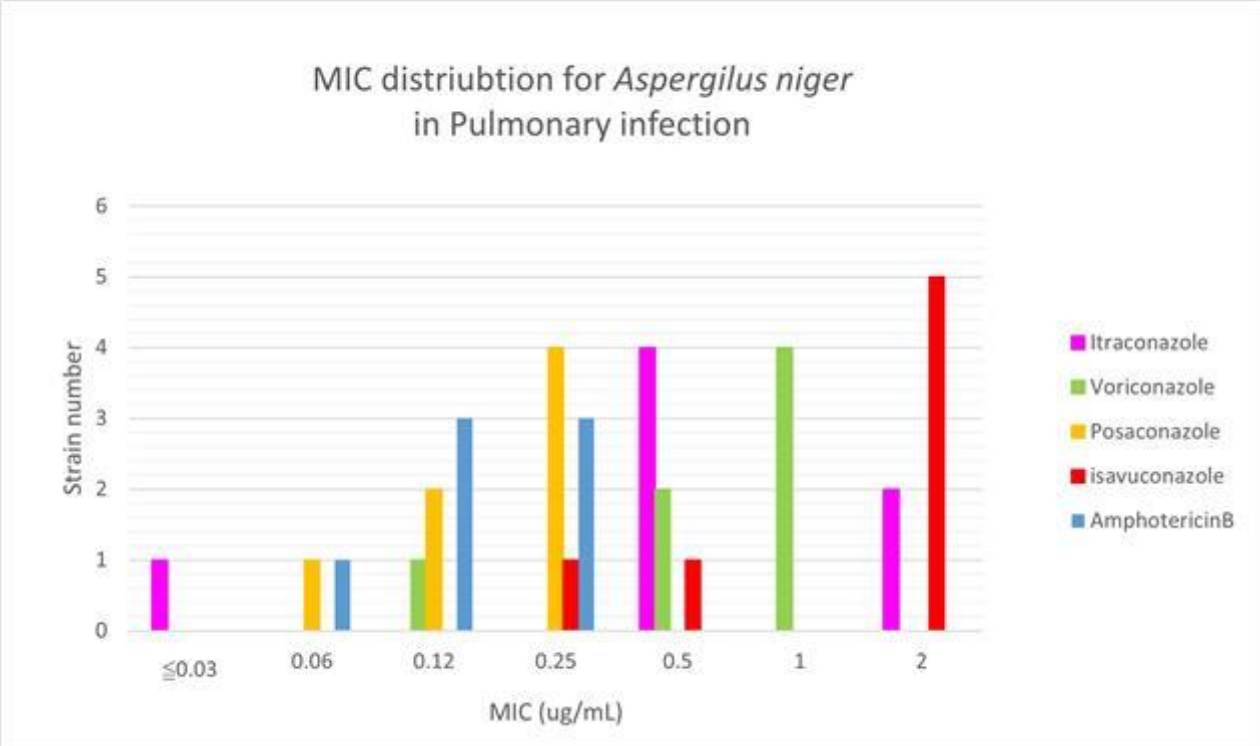
Results
Nigh *A. niger* isolates were included in this study, with 9 isolates obtained from 5 patients with pulmonary infection, and two from non-pulmonary infection. Among the 7 *A. niger* isolates from patients with pulmonary infections, five were from sputum in patients with COVID-19-associated pulmonary aspergillosis (CAPA). For non-pulmonary infections, one isolate was obtained from pus on the skin of a patient with chronic pulmonary aspergillosis (CPA) concurrent with skin and soft tissue infection (SSTI), and another one was obtained from a patient with sinusitis.

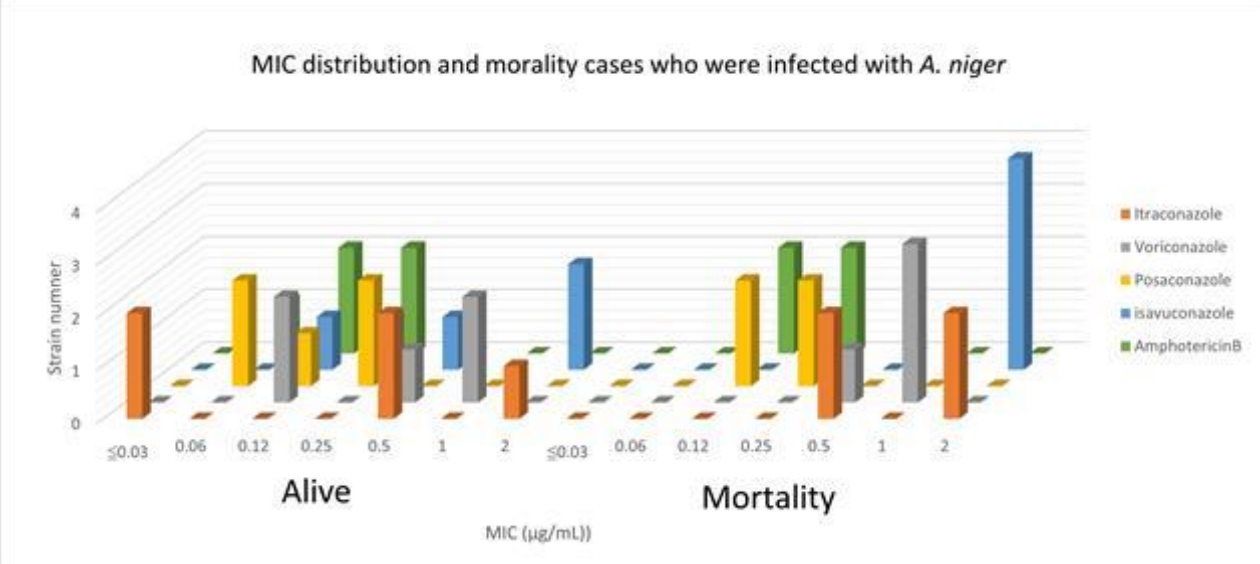
The results showed that the resistance rates of *A. niger* to antifungal agents varied depending on the drug and infection site. In pulmonary infections, the highest resistance rates were observed for isavuconazole (81.8%), followed by voriconazole (72.7%), itraconazole (54.5%), posaconazole (36.4%), and amphotericin B (0%) (Figure 1). From the five isolates from patients with CAPA, and the highest resistance rates were observed for isavuconazole and voriconazole. For non-pulmonary infections, all isolates were susceptible to itraconazole, posaconazole, isavuconazole, and amphotericin B. However, the number of isolates from non-pulmonary infections was small, and further studies are needed to confirm these findings.

During the study, two patients died due to Aspergillus infections, while five patients remained alive. Two patients with four mixed *A. niger* isolates with different MIC patterns were died.

Conclusion

Although with limited sample size, our study highlights the importance of carefully selecting antifungal agents for treating *A. niger* infections, especially in patients with CAPA. Isavuconazole and voriconazole showed high resistance rates, and patients with the triazole resistance *A. niger* also had a higher mortality (figure 2) in the current survey, especially in cases of IPA or CAPA. The prevalence of triazole resistance observed in this study emphasizes the need to monitor resistance patterns according to infection sites to ensure appropriate therapy. Further population based investigations is warranted.





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Trends in Medical Mycology

P300

What about fungal peritonitis in patients on continuous ambulatory peritoneal dialysis?: Results of a Tunisian study

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Introduction and Objectives:

Fungal peritonitis is a rare but potentially fatal complication of chronic peritoneal dialysis, associated with high morbidity and mortality ranging between 5% and 40%. Fungal peritonitis (FP) accounts for 1‑20% of all peritonitis episodes. The most important risk factor is a previous antibiotic therapy, particularly for bacterial peritonitis.

The purpose of this study was to describe clinical and microbiological features of fungal peritonitis in patients undergoing continuous ambulatory peritoneal dialysis in Tunis.

Materials & Methods:
It is a retrospective study conducted between January 2013 and December 2022 (09 ans). It included 122 Samples of the peritoneal dialysis fluid from 95 adult patients on continuous ambulatory peritoneal dialysis with peritonitis clinical signs (fever, abdominal pain, cloudy effluent, antibiotic resistance). Samples were submitted to mycological study. The processing of the peritoneal dialysis effluent sample included: centrifugation, direct examination, Sabouraud culture, identification of colonies and study of antifungal susceptibility for yeasts.

Results:
From 122 samples, FP was diagnosed in 12 cases (12,63%). The mean age of our patients was 21 years, with extremes ranging from 03 to 62 years. The sex ratio was 0,5. Clinico-biological signs suggestive of peritonitis were present in 9 patients: abdominal pain (7 patients), fever (9 patients). A biological inflammatory syndrome was present in six patients in addition to the clinical signs. Broad-spectrum antibiotic therapy had been previously prescribed in 7 patients and one patient has received also an antifungal treatment.
The mycological diagnosis isolated mainly yeasts of the *Candida* (C.) genus: *C. albicans* (5 patients), *C tropicalis* (2 patients), *C.glabrata* (1 patient). Other yeasts were isolated: *Trichosporon asahii* and *Saprochaete capitata* in one case each. While in two other cases a mold was incriminated: *Fusarium solani* species complex (FSSC) and *Penicilium sp* in one case each.
The study of antifungal susceptibility was performed for all strains of *Candida*. No resistance, particulary to Amphotericine B and caspofungine, was found for the eight yeast isolates. A single strain of *Candida albicans* was resistant to all azoles tested, including voriconazole.
Conclusions:
Fungal peritonitis is a recognized cause of morbidity and mortality in patients undergoing continuous ambulatory peritoneal dialysis. Prior antibiotic use was an important risk factor predisposing patients to the development of fungal peritonitis. Early detection of fungal peritonitis would lead to early institution of appropriate therapy and prevention of complications.

P301

Aspergillus flavus Necrotizing Fasciitis Following Doxorubicin Extravasation in A Lymphoma Patient: A Case Report

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Objectives: To demonstrate an unusual case of necrotizing fasciitis following doxorubicin extravasation caused by *Aspergillus flavus* in a lymphoma patient

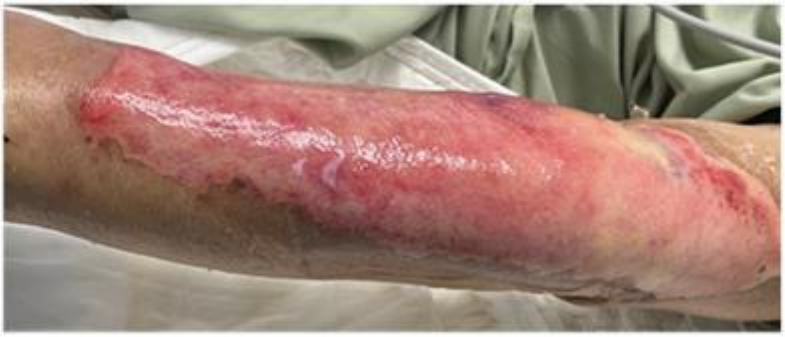
Materials & Methods: This is an interesting case report of a patient from a tertiary university hospital in Thailand.

Results: We report a case of a 90-year-old man who was diagnosed diffused lage B cell lymphoma (DLBCL) in December 2022, presented with left nasal mass. Nasal telescopy was performed and tissue biopsy from the mass demonstrated DLBCL. He received chemotherapy with rituximab, cyclophosphamide, doxorubicin and prednisolone (R-CHOP) regimen, started on 25 January 2023. In march 2023, while he was receivig the third course of chemotherapy, doxorubicin accidentally leaked from the vein at his left arm. Doxorubicin was stopped immediately. He developed erythematous and exfoliated skin on his left arm (Figure 1a), and turned to be a black eschar 2 weeks later (Figure 1b). The lesion was progressed and therefore skin biopsy was performed. Histopathology from skin biopsy revealed branching septate hyphae and the fungal culture grew *Aspergillus flavus*. *Aspergillus* necrotizing fasciitis was diagnosed and voriconazole was commenced as initial antifungal therapy. The surgeon preformed wide debridement of his left arm and the chemotherapy was halted. His clinical manifestation is improve and awiating for skin graft.

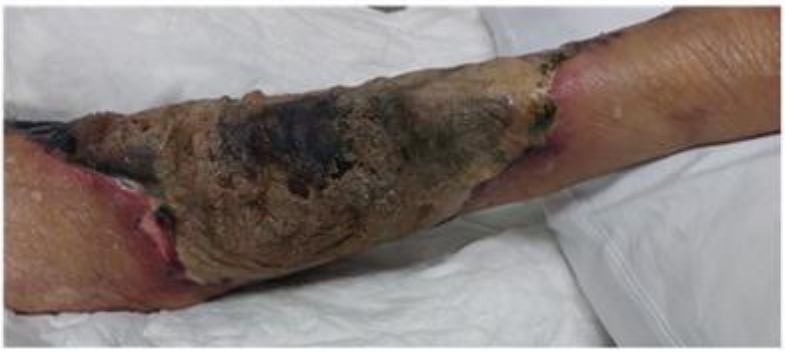
Conclusions: *Aspergillus* necrotizing fasciitis is a rare invasive fungal infection of the skin and it can be a serious complication from chemotherapy extravasation.

Figure 1. The skin lesion of the patient's left arm after extravasation of doxorubicin (1a) and it became black escahar 2 weeks later (1b)

1a



1b



P302

Rare presentation of disseminated cryptococcosis due to Cryptococcus deneoformans in an immunosuppressed patient – Look deep and think fungus!

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Objectives:

The prevalence of cryptococcosis in Haematopoietic Stem Cell Transplantation (HSCT) recipients is very low. However, these patients are at high risk of life-threatening complications as they need immunosuppressive therapy, and the coexistence of GvHD adds complexity to management regarding immunosuppressant drugs. Skin involvement in cryptococcosis is rare and can have different presentations, such as bacterial-like cellulitis, and is considered an early marker for disseminated disease.

The recently taxonomically redefined *Cryptococcus deneoformans* is more prevalent in Northern Europe and rarely causes disease.

We report a case of rare presentation of disseminated cryptococcosis due to *Cryptococcus deneoformans* in an immunosuppressed patient.

Materials & Methods:

A 71-year-old male patient was admitted in the Emergency Department with a 15-days evolution of inflammatory signs of the left forearm without improvement on oral flucloxacillin. Past history was relevant for Non-Hodgkin T-cell lymphoma in remission after Allo-HSCT in 2014, with coexistent chronic GvHD with pulmonary and cutaneous involvement, under prednisolone and ruxolitinib. Patient was started on piperacillin-tazobactam for possible left upper limb cellulitis and on the 4th day of hospitalization, he was transferred to the Pulmonology Department due to clinical deterioration with hypercapnic respiratory failure. In addition to the increase in acute phase reactants, soft tissue infection appeared to be worsening, with intense pain, functional impotence, paresthesia, distal pallor and skin detachment with drainage of purulent exudate that was promptly collected and processed for bacteriological and mycological studies. Urgent intervention by Plastic Surgery was requested, considering the probable diagnosis of necrotizing fasciitis and empirical antibiotic therapy was changed to meropenem, clindamycin and linezolid.

Results

At the laboratory, direct microscopic examination of the preoperative exudate revealed round small yeast forms with a circular empty space surrounding the cell and unstained by Gram stain, raising the suspicion of *Cryptococcus* sp. A preparation of the purulent exudate with India ink highlighted the encapsulated yeast consistent with the diagnosis of cryptococcosis and the clinician was immediately informed. Cultures revealed white smooth colonies at 18-24h of incubation that were identified as *Cryptococcus deneoformans* by MALDI-TOF mass spectrometry and confirmed by sequencing the IGS region. Later, blood cultures were also positive for *C. deneoformans*, confirming the diagnosis of disseminated cryptococcosis.

Susceptibility pattern showed low minimal inhibitory concentrations (MIC) to amphotericin B (0,125mg/L) and higher MIC values to fluconazole (16,0 mg/L).

The patient's clinical condition gradually improved after surgical drainage and debridement, followed by vacuum therapy and subsequent partial skin graft of the forearm. The patient underwent 45 days of liposomal amphotericin B followed by isavuconazole.

Conclusions:

The early recognition of cryptococcal disease in an immunosuppressed patient, in any clinical presentation, is essential for a timely treatment. The visualization of fungal elements in tissues collected from a normally sterile body site confirmed the diagnosis of invasive fungal infection. The recovery of viable fungus from the sample and the use of MALDI-TOF allowed for the identification of a less frequent species and enabled the antifungal susceptibility test leading to adequate treatment.

P303

Antifungal treatment considerations: treatment selection, sequence, and duration in patients with acute myelogenous leukemia and invasive mold infections.

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[1]Geneva University Hospital, Geneva, Switzerland, [2]Basel University Hospital, Basel, Switzerland, [3]Lausanne University Hospital, Lausanne, Switzerland

Objectives:
We have recently demonstrated that allogeneic hematopoeitc cell transplant recipients (allo-HCTR) with invasive mold infections (IMI) require on average 2 treatment changes during their treatment course (range 0, 8). We aim to describe antifungal treatment characteristics, focusing on changes and reasons of changing antifungal treatment in patients with acute myeloid leukemia (AML) diagnosed with IMI.
Materials & Methods: This was a retrospective observational multi-centre cohort study performed from 1.1. 2010 through 1.1.2020 including consecutive AML patients diagnosed with an IMI. For patients who underwent an allo-HCT, only IMI diagnosed prior to transplant were included. Patients from three Swiss University Hospitals (Basel, Geneva, Lausanne)) were included. Data on IMI diagnosis and treatement were collected, including number of treatment changes and reasons prompting those changes. The study was approved by relevant ethics committees and was endorsed by the Fungal Network of Switzerland (FUNGINOS).
Results: Overall 74 IMI in 72 AML patients were included.Antifungal prophylaxis was prescribed in 39/74 (52.7%) episodes within 30 days prior to IMI diagnosis: 23/74 (60.5%) fluconazole, 13/74 (34.2%) posaconazole, and 2/74 (5.3%) voriconazole. There were 47/74 (63.5%) probable and 27/74 (36.5%) proven IMI. The most frequent pathogen identified was *Aspergillus* spp. (50/74, 67.6%; *A. fumigatus* in 23/50, 46% cases), followed by Mucorales (11/74, 14.9%), and other molds (13/74, 17.6%). Two IMI (2/74, 2.7%) were mixed with *Aspergillus* spp. and Mucorales . Most frequently involved site of infection was the lung (69/74, 96.3%), followed by sinus (4/74, 5.4%), brain (4/74, 5.4%), and abdomen (6/74, 8.1%).
Median total duration of treatment was 200.5 days (IQR 113, 305). 25/72 (34.7%) patients did not have any change, while 43/72(59.7%) had at least 1 change of their antifungal treatment from initiation to completion (information not available in four patients) Hence, 147 treatment courses were administered with an average of 2.6 (0,9) different antifungal treatments *per* patient. Reasons for antifungal treatment changes included clinical efficacy (58/147, 39.5%), toxicity (38/147, 5.9%), logistical reasons (11/147, 7.5%) or other reasons (16/58, 10.9%); of note 1 treatment change could have been prompted by more than one reason. Clinical efficacy reasons included the following: clinical suspicion for invasive aspergillosis (IA) (5/58, 8.6%), clinical suspicion for non-IA IMI (5/58, 8.6%), targeted treatment based on microbiological diagnosis (18/58,31%), subtherapeutic azole therapeutic drug monitorig levels (4/58,6.9%), clinical/radiological improvement (6/58,10.3%), clinical/radiological progression (17/58,29.3%), other reasons (10/58, 17.2%). Toxicity reasons included liver dysfunction (16/38, 42%), renal dysfunction (8/38, 21%), skin rash (1/38, 2.6%), gastrointestinal intolerance (1/38, 2.6%), allergic reaction (1/38, 2.6%), drug-drug interactions (11/38, 28.9%) and other (4/38, 2.1%). Logistical reasons for treatment

changes included the following: switch to oral treatment (8/11, 72.7%), insurance problems (1/11, 9.1%), other (2/11, 18.2 %). Surgical therapeutic intervention was observed in 34/74 (45.9%) IMI cases, predominately a “wedge” resection (18/34, 52.9%).
Conclusions: Multiple changes in antifungal treatment in the majority of patients treated for an IMI were observed, prompted by a large variety of reasons. Our findings reflect the complexity of antifungal treatment in this high-risk population and call for additional, more effective and better tolerated antifungal treatment options.

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P304

Aspergillosis in patients with lymphoproliferative malignancy: new population at risk?

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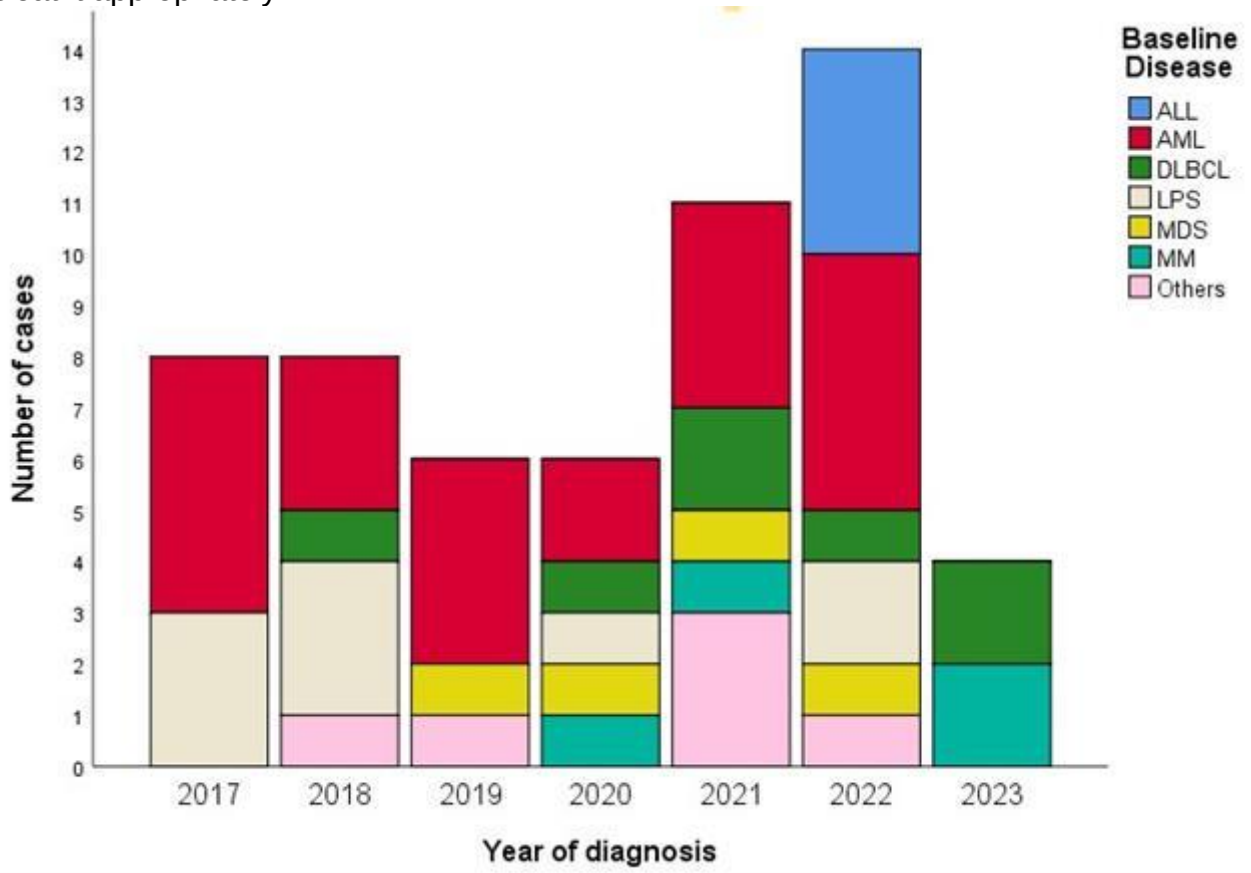
Objectives: Among patients with hematologic malignancy, patients with acute myeloid leukaemia and recipients of allogeneic haematopoietic stem cell transplants are at high risk of developing aspergillosis. New treatments or previous corticoid treatment have been related to an increased risk of developing aspergillosis. The aim of this study is to describe the clinical, radiological, and microbiological characteristics of patients with lymphoproliferative syndromes diagnosed of proven or probable aspergillosis.

Materials & Methods: We conducted a retrospective study in a tertiary centre in Spain. Data were collected from patients with haematological malignancies from January 2017 to April 2023. Patients fulfilling the following criteria were included: *Aspergillus*-positive culture (sputum, bronchoalveolar aspirate, bronchoalveolar lavage (BAL) or biopsy) or positive galactomanan in serum or BAL and diagnosis of lymphoproliferative syndrome prior to the determination. Proven and probable invasive aspergillosis were defined according to the Consensus Definitions of Invasive Fungal Disease from the EORTC/MSG. The isolation of *Aspergillus* spp. from a mucocutaneous surface in patients without evidence of invasive disease was considered colonization. A descriptive analysis was performed including demographic, clinical, analytical, radiological, and microbiological data.

Results: Out of 62 patients with hematologic malignancy and aspergillosis, 20 had a lymphoproliferative syndrome and met the inclusion criteria. Seven patients were diagnosed prior to 2020 and thirteen were diagnosed 2020 onwards. Twelve were male and median age was 60 years. Baseline disease was diffuse large cell B lymphoma in seven patients (35%); multiple myeloma in four patients (20%), follicular lymphoma in two patients (10%), T cell lymphoma in two patients (10%), chronic lymphocytic leukaemia in two patients (10%), and Hodgkin lymphoma, mantle cell lymphoma and lymphocytic lymphoma in the remaining patients. Eight patients had received three or more lines of treatment and seven of them had progressive disease at aspergillosis diagnosis. Eight patients received a haematopoietic stem cell transplant, six an allogeneic transplant and two an autologous transplant. One patient received CAR-T cell therapy and three patients bispecific antibodies. Most frequent risk factors were chronic obstructive pulmonary disease (3/20, 15%), bronchiectasis (12/20, 60%), previous treatment with corticoids (16/20, 80%), previous fungal infection (1/20, 5%), neutropenia (4/20, 20%) and graft-versus-host disease (5/6, 83.3%). Hospitalisation was required in 75% of the cases. CT-scan often showed several patterns mainly nodular lesions (7/18, 38.9%), consolidation (4/18, 22.2%) and ground-glass opacities (9/18, 50%). Mixed patterns were identified in five patients (27.8%) and bilateral affectation in fourteen (77.8%). Seven patients presented invasive pulmonary aspergillosis, four subacute aspergillosis and eight bronchial aspergillosis. Five patients had a breakthrough invasive fungal infection, three of them undergoing prophylaxis with fluconazole and nebulized amphotericin B liposomal. Species identified were *A. fumigatus* (12), *A. flavus* (3), *A. nidulans* (1), and *A. terreus* (1). Two

of the isolates were azol-resistant. All-cause 60-day mortality was 61.1% and aspergillosis-related mortality was 42.1%.

Conclusions: Aspergillosis is an opportunistic infection in patients diagnosed with lymphoproliferative syndromes. Correlated with particular risk factors and compatible findings in CT scan, the clinician must be aware to diagnose the infection as prompt as possible and treat it appropriately.



	Total (N=20)
Male	12 (60)
Age	60 ± 15
Baseline disease	
LDCGB	7 (35)
MM	4 (20)
FL	2 (10)
TL	2 (10)
CLL	2 (10)
Others[1]	3 (15)
Progressive disease at diagnosis	7 (35)
≥ 3 línies de tractament	8 (40)
AlloSCT	6 (30)
AutoSCT	2 (10)
CAR-T cell therapy	1 (5)
Bispecific antibodies[2]	3 (15)
Risk factors	
COPD	3 (15)
Bronchiectasis	12 (60)
Corticosteroids	16 (80)
Previous fungal infection	1 (5)
Neutropenia	4 (20)
GVHD	5 (83.3)
Hospitalization required	15 (75)
CT-scan	18 (90)
Nodular lesions	7 (38.9)
Consolidation	4 (22.2)
Ground glass opacities	9 (50)
Micronodules	2 (11.1)
Mixed patterm	5 (27.8)
Bilateral affectation	14 (77.8)
Invasive aspergillosis	7 (35)
Subacute aspergillosis	4 (20)
Bronchial aspergillosis	8 (40)
Proven aspergillosis	3 (15)
Probable aspergillosis	17 (85)
Breakthrough IFI	5 (25)
Fluconazole + amphotericine B	3 (33.3)
Isavuconazole	1 (5)
Voriconazole	1 (5)
Biomarkers	
GM BAL ≥ 1.0	7/12 (58.3)
GM serum ≥ 0.5	6/15 (40)
positive serum BDG	2/4 (50)
Outcome[3]	
30-day mortality	9 (45)
60-day mortality	11 (61.1)
Attributable mortality	8 (42.1)

P305

Mucormycosis Associated with COVID-19: Results Of Prospective Multicenter Study

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Objectives: to analyze risk factors, etiology, clinical signs and results of treatment **Mucormycosis Associated with COVID-19 (**CAM) in adult patients.

Materials and methods. In prospective multicenter study in 2021 y were included 100 COVID-19 adult patients with «proven» and «probable» mucormycosis (EORTC/MSGERC 2019\2020).

Results. The median age of CAM patients was 62 years (18 - 80), males – 57%. The main underlying conditions were diabetes mellitus (79%), obesity II-III (32%), hematological diseases (4%), and chronic sinusitis (2%).

The main risk factors were corticosteroids (98%), lymphocytopenia (47%), stay in the ICU (9%), ketoacidosis (32%), mechanical ventilation (7%) and agranulocytosis (2%). The risk of CAM developing was significantly increased with diabetes mellitus (OR=22,3 [5,2-96,5]), overweight (OR=3,6 [1.7-7,8]), and high dose glucocorticosteroids (prednisolone ≥100 mg per day) use (OR= 25,6 [7,5-86]), especially ≥10 days (OR= 28 [8,2-95,2]).

The main sites of CAM were paranasal sinuses (96%), the orbit (66%), CNS (35%), and ≥ 2 organs involvement (83%).

Diagnosis was established by histology and/or microscopy in all patients. The diagnosis was confirmed by culture in 15% of cases (*Rhizopus spp.* - 47%, unidentified mucormycetes - 40%, *Lichtheimia ramosa* - 7%, *Mucor* sp. - 7%).

Antifungal therapy: amphotericin B deoxycholate (63%), posaconazole (64%), amphotericin B lipid form (18%), echinocandins (2%) and isavuconazole (8%). Surgery was performed in 97% of patients. The 12 weeks overall survival rate was 90%.

Conclusions:

1. The main underlying conditions in adult CAM patients were diabetes mellitus (79%).
2. The risk of CAM developing was significantly increased with diabetes mellitus (OR=22.3) and high dose glucocorticosteroids (OR=25.6)
3. The main aetiology agents were *Rhizopus* spp. (47%).
4. The main sites of CAM were the paranasal sinuses (96%), the orbit (66%), CNS (35%), and ≥ 2 organs involvement (83%).
5. The 12 weeks overall survival rate was 90%.

P307

Impact of COVID-19 on the epidemiology and outcomes of candidemia: A Retrospective Study from a tertiary care center in Lebanon

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Objectives: Invasive fungal infections, notably candidemia, have been associated with COVID-19. The epidemiology of candidemia has significantly changed during the COVID-19 pandemic. We aim to identify the microbiological profile, resistance rates, and outcomes of COVID-19 associated candidemia (CAC) compared to patients with candidemia not associated with COVID-19.

Materials & Methods: We retrospectively collected data on patients with candidemia admitted to the American University of Beirut Medical Center between 2004 and 2022. We compared the epidemiology of candidemia during and prior to the COVID-19 pandemic. Additionally, we compared the outcomes of critically ill patients with CAC to those with candidemia without COVID-19 from March 2020 till March 2022.

Results: A total of 245 episodes of candidemia were identified with 156 occurred prior to the pandemic and 89 during the pandemic. Of the latter, 39 (43.8%) were CAC, most of which (82%) were reported from intensive care units (ICU). Non-albicans *Candida* (NAC) spp. were predominant throughout the study period (67.7%). *Candida auris* infection was the most common cause of NAC spp. in CAC (Figure 1). *C. glabrata* had decreased susceptibility rates to fluconazole and caspofungin during the pandemic period (46.1% and 38.4% respectively) (Table 1). Mortality rate in the overall ICU population during the pandemic was 76.6%, much higher than the previously reported mortality of candidemia from previous studies at our center. There was no significant difference in 30-day mortality between CAC and non-CAC (75.0% vs 78.1%; P =0.76). Performing ophthalmic examination (P = 0.002), CVC removal during the 48 hours following the candidemia (P = 0.008) and identifying the *Candida* spp. (P = 0.028) were significantly associated with a lower case-fatality rate.

Conclusions: The epidemiology of candidemia has been significantly affected by the COVID-19 pandemic at our center. Rigorous infection control measures and proper antifungal stewardship are essential to combat highly resistant species like *C. auris*.

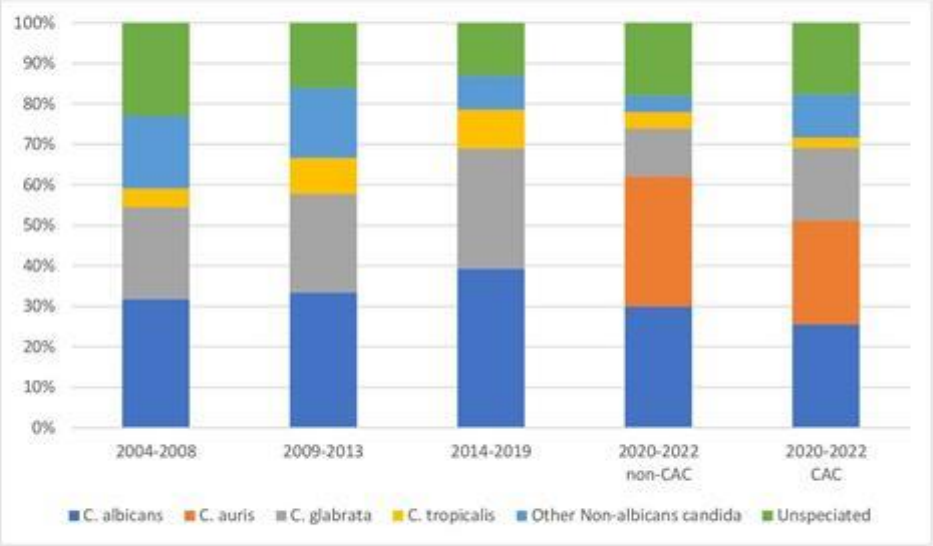

Figure 1. Predominance of *Candida* spp. over 18 years at AUBMC.

	2004-2008	2009-2013	2014-2019	2020-2022 (non-CAC)	2020-2022 (CAC)	Total
C. albicans						
Fluconazole	1/1	6/6	13/16	5/5	8/8	33/36
Voriconazole	-	3/4	14/17	5/5	8/8	30/34
Amphotericin B	-	3/3	17/17	5/5	8/8	33/33
Caspofungin	-	1/1	6/6	5/5	8/8	20/20
C. tropicalis						
Fluconazole	2/2	-	3/3	-	1/1	6/6
Voriconazole	-	4/5	7/7	1/1	2/2	14/15
Amphotericin B	-	1/1	7/7	1/1	2/2	11/11
Caspofungin	-	1/1	1/1	1/1	2/2	5/5
C. glabrata						
Fluconazole	3/4	3/10	17/23	2/6	4/7	29/50
Voriconazole	0/1	7/8	18/23	6/6	7/7	38/45
Amphotericin B	-	1/1	22/23	6/6	6/6	35/36
Caspofungin	-	-	9/9	3/6	2/7	14/22
C. parapsilosis						
Fluconazole	2/2	-	3/3	-	1/1	6/6
Voriconazole	-	-	3/3	-	1/1	4/4
Amphotericin B	-	-	3/3	-	1/1	4/4
Caspofungin	-	-	-	-	1/1	1/1
C. auris						
Fluconazole	-	-	-	0/3	2/9	2/12
Voriconazole	-	-	-	1/3	5/9	6/12
Amphotericin B	-	-	-	0/3	0/7	0/10
Caspofungin	-	-	-	8/8	3/3	11/11

P308

Candidalysin activates IL-1β-producing activity in vulvovaginal candidiasis

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Objectives:
Vulvovaginal candidiasis (VVC) is mainly caused by *Candida albicans* (*C. albicans*). Candidalysin encoded by *C. albicans ECE1* gene is involved in the IL-1β related inflammatory process. And a variant allele of *ECE1* appears correlated with a reduced pathogenicity of *C. albicans* in VVC. However, the host responsive immune mechanism of candidalysin in VVC has not been fully understood. Here we evaluated the proinflammatory mechanism of candidalysin in VVC.

Materials & Methods:
To rule out the pathogenic effects of candidalysin in IL-1β-producing activity, epithelial cell line A431was used in vitro to measure the activation of NLRP3/IL-1β in co-cultures with candidalysin, wild type *C. albicans* train (M1477), *ECE1*-deficient strain (M2057), *ECE1*-revertant strain (M2059) or candidalysin-deficient strain (M2174).

Results:
The results showed that M1477, M2059 and candidalysin significantly promoted IL-1β-producing activity and increased mRNA expressions of NLRP3 and IL-1β, when compared with M2057 and M2174 (P<0.05). In the mouse VVC model, we also found that IL-1β secretion and the expression of NLRP3/IL-1β signaling pathway were significantly higher in the mouse groups infected by M1477 and M2059 than those groups infected by either M2057 or M2074 (P<0.05).

Conclusions:
Our results indicate that Candidalysin could promoting IL-1β-producing activity and further promote the host inflammatory response against this fungus. The candidalysin could be an ideal target for antifungal strategies.

P309

A case of a deep mycosis due to a terbinafine resistant Diaporthe

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Background

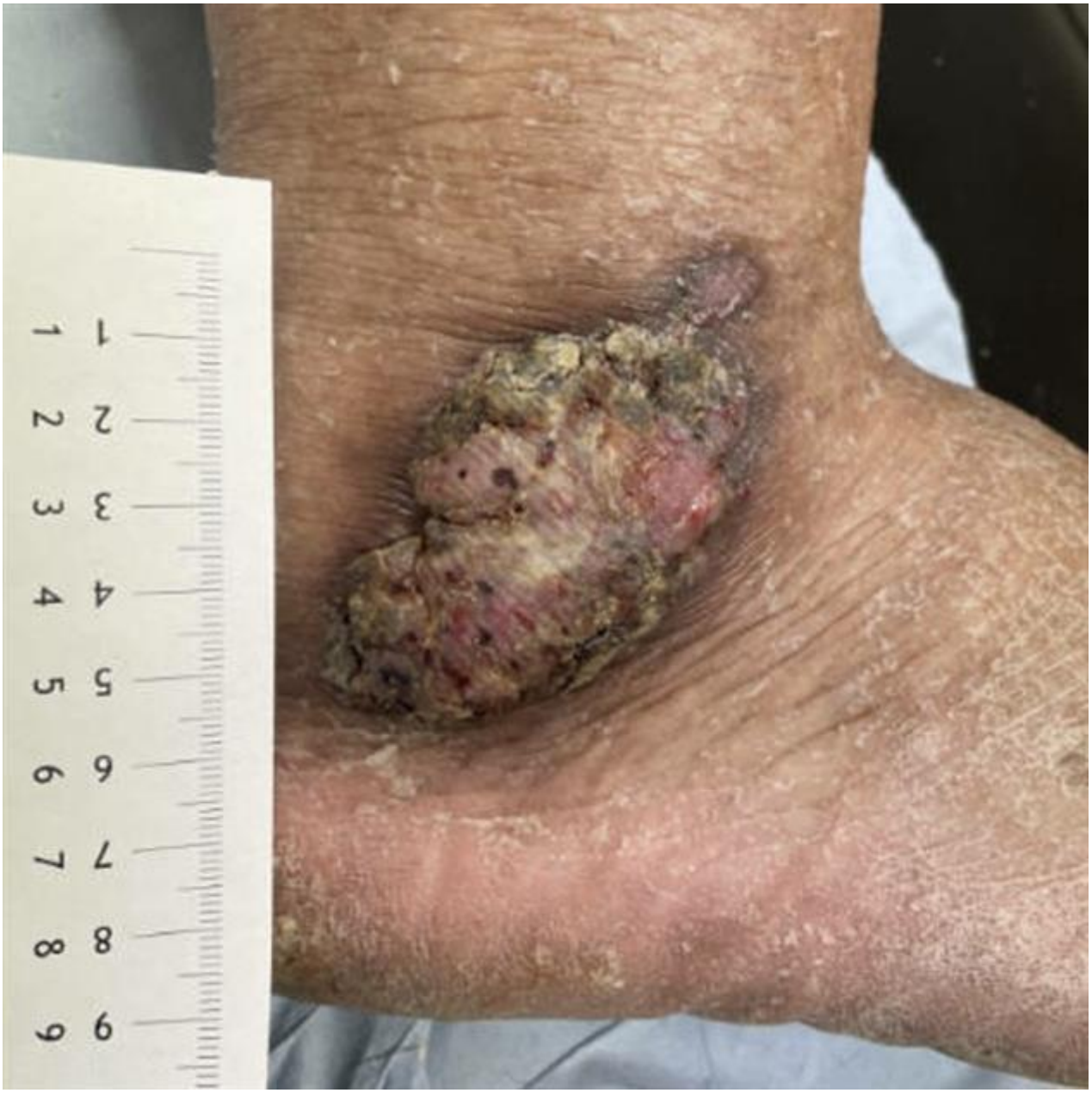
Phaeohyphomycosis is a caused by dematiaceous fungi from different genera. These fungi, also called "black fungi" are generally found in soil and are increasingly recognized in a variety of infections in both immunocompetent and immunosuppressed patients. Diaporthe belongs to the dematiaceous fungi and is mostly susceptible to terbinafine. Here we present a case of a terbinafine resistant Diaporthe, causing a cutaneous infection.

Case

A 76-year old patient presented in our out-patient clinic with a persisting wound on his right lateral malleolus since two years. Eight years before presentation he had undergone a prostatectomy and radiotherapy because of prostatic cancer and four years before presentation he had undergone a kidney transplantation because of diabetic renal insufficiency. He used prednisolon 7.5 mg two times daily and tacrolimus 18 mg once daily. The wound started as a small crust and slowly grew to a wound of 6 cm. At start the wound was a little painful and itchy, however at presentation it was painful. An exophytic, erythematous, tumor of 6 cm was seen, with outflow of pus at compression. At first, 1.5 years before presentation in our center, a malignancy was suspected and biopsies for histopathology were taken. These showed no malignant cells and possibly a ruptured folliculitis or epidermal cyst with reactive changes. Nine months later, the wound had persisted and a new biopsy was performed showing a granulomatous inflammation with spores and hyphae, no malignant cells. First he was treated with itraconazole, which was complicated by an acute kidney insufficiency for which he had to be admitted to the ICU. After that he was treated with terbinafine 250 mg once daily for three months, without any improvement and currently he was on ciclopirox crème. Since there was no improvement with this therapy, again a malignancy was suspected and he was referred to our center. New biopsies were taken and sent to the microbiology department. The blancophor stain was positive, showing hyphae and atypical spores and the biopsy was inoculated on Sabouraud agar and incubated at 25 ^{o}C and 37 ^{o}C. Seven days later, a white powdery fungus grew on the Sabouraud agar, which was incubated at 25 ^{o}C. Since the fungus did not sporulate, we were not able to determine it by microscopy. Therefore it was sent to the molecular diagnostics unit, where ITS sequencing revealed a Diaporthe spp, which was sent to the reference center for susceptibility testing. Since this fungus did not easily sporulate, susceptibility testing was difficult to perform. Since we found in literature, that Diaporhe is mostly susceptible to terbinafine, we advised the restart terbinafine. However, finally the results of the susceptibility testing were available and showed that the Diaporthe was resistant to terbinafine and itraconazole and susceptible to voriconazole. Therefore, treatment was changed to voriconazole.

Key learning points:

- Culture is essential in diagnosis of deep mycoses
- Susceptibility testing of fungi causing persistent mycoses is important
- Terbinafine resistant Diaporthe spp exist



P310

Hepatosplenic candidiasis in patients with hematological malignancies - a retrospective multicenter register study

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Objectives
Hepatosplenic candidiasis (HC) is a form of chronic disseminated candidiasis typically observed in patients with hematological disease experiencing prolonged neutropenia. This rare disease can be difficult to diagnose, as isolation of the causative agent is challenging. The treatment can be equally complex and tedious. Here we present cases of HC from 2010-2023, which have been documented at four German university hospitals from North Rhine-Westphalia, cooperating in the medical network “Centrum für Integrierte Onkologie (CIO) Aachen Bonn Köln Düsseldorf”.
Methods
Data were collected by trained medical students, as part of an integrated research and teaching concept at the University of Cologne, and recorded in CandiReg, a web-based registry developed by the European Confederation of Medical Mycology (ECMM).
Results
A total of twelve patients with hematological malignancies, mostly acute leukemia, were identified to have had probable or possible HC. In most cases disease was associated with chemotherapy and prolonged neutropenia. Patients presented with unspecific fever and tachycardia. A causative agent was only isolated in a few from a blood culture, including *Candida albicans* and *dubliniensis*. Hepatosplenic manifestation was effectively detected and monitored with the use of sonography and computed tomography. Treatment mostly consisted of caspofungin and a subsequent switch to an azole. Described are also successful treatments with the use of ibrexafungerp and cases in which an escalation to amphotericin B was needed.
Conclusions
This is the first German and one of few worldwide studies regarding this rare but serious fungal infection. Overcoming difficulties in diagnostics and minimizing the number of risk factors are still unmet goals. Clinicians need to be aware of the typical presentation and treatment strategies. Aside from that, novel antifungals may play an important role in treating chronic disseminated candidiasis effectively.

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The light and dark side of IFN-g in the immune challenge against fungi

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Objectives

Fungi can cause diseases in humans, from mucocutaneous (*Candida* spp.) to life-threatening systemic infections (*Aspergillus* spp.). Interferon-gamma (IFN-γ) has emerged as an extremely versatile cytokine mediating protection against a wide array of pathogens. Undoubtedly, such an important immune mediator is under stringent regulatory control. However, IFN-γ is double-edged sword: it can have therapeutic as well as aberrant effects depending on the type of disease. Two clear examples are: (1) chronic granulomatous disease (CGD), a genetic disorder of the NADPH oxidase characterized by increased susceptibility to *Aspergillus* infections and hyperinflammation associated with defective autophagy and increased inflammasome activation; and (2) autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), an autoimmune disease of impaired central immune tolerance characterized by selective susceptibility to mucosal but not systemic fungal infection.

Methods

First, we monitored DAPK1 expression and its modulation by IFN-γ in vivo *p47$^{phox-/-}$* in infected mice and in vitro in both *p47$^{phox-/-}$* lung macrophages and monocytes from CGD patients. Second, we broadly investigated oral mucosal immune responses both in a model of oropharyngeal candidiasis in *Aire$^{-/-}$* mice and in a large cohort of APECED patients.

Results

In mouse models and patient with CGD, we have described an IFN-γ/DAPK1 signaling that mediates LC3-associated phagocytosis (LAP), critical for dampening *Aspergillus*-triggered immunopathology. Of interest, DAPK1 activity was defective in murine and human CGD, a finding suggesting that the LAP/DAPK1 axis may represent a druggable pathway in CGD. Indeed, IFN-γ, the only FDA-approved treatment option in CGD, restored reduced DAPK1 activity and dampens fungal growth. Activated by IFN-γ, DAPK1 not only mediated *A. fumigatus*–LAP but also concomitantly inhibited nod-like receptor protein 3 (NLRP3) activation, thus restraining pathogenic inflammation. In contrast, in AIRE deficiency, we have shown that excessive IFN-γ–dependent responses at the mucosal level lead to susceptibility to oral mucosal infection by *Candida albicans*. Concordantly, genetic and pharmacologic inhibition of IFN-γ or JAK-STAT signaling ameliorated mucosal fungal disease and multi-organ autoimmunity. Thus, aberrant T cell-dependent, type 1 mucosal inflammation is a critical tissue-specific pathogenic mechanism that promotes mucosal fungal infection susceptibility.

Conclusions

Together, our data suggest that the bright side of IFN-γ in CGD in which it is beneficial is counteracted by the exaggerated IFN-γ/STAT1 response leading to tissue-specific autoimmune manifestations in APECED. Thus,

IFN-γ-mediated host-pathogen interactions are critical for both understanding disease pathogenesis and IFN-γ optimization by disease-oriented therapy.

P312

Clinical Characteristics and Prognosis of Hepatosplenic Candidiasis in Patients with Hematological Malignancies: A 15-Year Single Center Experience Study

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Objectives: Hepatosplenic Candidiasis(HSC) is an invasive fungal disease occurs in the period of neutrophil recovery in patients with hematological malignancies, which is rare and serious. The purpose of this study is to describe the characteristics and prognosis of HSC in patients with hematological malignancies in China.

Materials & Methods: All patients with hematological malignancies disgnosed with HSC from 2008 to 2022 were retrospectively reviewed in this study. The classification and assessment of HSC depending on the definitions of 2020 Eorepean Orgnisation for Research and Treatment of Cancer/Mycoses Study Group.

Results: Ultimately, Eighteen HSC patients were included, with 5.6%(1/18) proven, 55.6%(10/18) probable and 38.9%(7/18) possible HSC according to the 2020 European Organization for Research and Treatment of Cancer/Mycoses Study Group classification. HSC occured in 16.7%(3/18) patients after haplotype hematopoietic stem cell transplantation while 83.3%(15/18) after chemotherapy. At HSC diagnosis, 83.3%(15/18) patients received antifungal prophylaxis and voriconazole was the most commonly used. Mucosal damage were found in 22.2%(4/18) patients. Central vein catheterization were used in 80.0%(12/15) patients, with candidiemia in 50%(6/12) of cases. Blood cultures were positive in 33.3%(6/18) of cases, with 33.3%(2/6) positive for *Candida albicans* and 66.7%(4/6) for *Candida tropicalis*. β-D-glucans were positive in 43.8%(7/16). 33.3%(6/18) patients had liver biopsies, and 16.7%(1/6) was positive for *Candida tropicalis.* The first-line antifungal therapy was voriconazole and caspofungin. Efficacy evaluation showed that 58.8%(10/17) patients were partial response after 4 weeks of HSC; 6.3%(1/16) was complete response and 62.5%(10/16) were partial response after 8 weeks of HSC, respectively. Follow-up was conducted 6 months after diagnosis of HSC, 16.7%(3/18) patients died of hematologic recurrence and none died of HSC.

Conclusions: In this study, HSC patients with hematological malignancies of Chinese were characterized by difficult diagnosis, long period of treatment and slight clinical remission. Early identification of high-risk patients, specific diagnosis tools, and highly effective antifungal therapy will contribute to accurate diagnosis and treatment of HSC patients.

Keywords: hepatosplenic Candidiasis; hematological malignancies.

Table.1 Characteristics of HSC patients

Baseline Characteristics	Overall Poplulation（n=18） No.（%）或 Median（IQR）
Male gender	13（72.2）
Age,y	36（25,40）
Hematologic malignancy	
Acute myeloid leukemia	10（55.6）
Acute lymphocyte leukemia	6（33.3）
Acute promyelocytic leukemia	1（5.6）
Acute biphenotypic leukemia	1（5.6）
Stem cell transplantation before HSC	3（16.7）
Stem cell transplantation after HSC	7（38.9）
Characteristics at HSC diagnosis	
Mucosal damage	4（22.2）
Central venous catheterization	12（80）
ICU history	1（5.6）
Parenteral nutrition	5（27.8）
EORTC/MSG classification	
Proven HSC	1（5.6）
Probable HSC	10（55.6）
Possible HSC	7（38.9）
Antifungal prophylaxis	15（83.3）
Antifungal therapy	
Monotherapy	5（27.8）
Combination therapy	13（72.2）
Efficacy evaluation	
4 weeks at HSC diagnosis	
Partial remission	10（58.9）
Stable disease	4（23.5）
Progression disease	3（17.6）
8 weeks at HSC diagnosis	
Complete remission	1（6.3）
Partial remission	10（62.5）
Stable disease	3（18.8）
Progression disease	2（12.5）
Follow-up at 6 months after diagnosis of HSC	
Survival	15（83.3）
HSC-related deaths	0（0）
HSC-unrelated deaths	3（16.7）

P313

Clinico-epidemiological and microbiological parameters of mucormycoses outbreak during COVID-19 pandemic in and around New Delhi, India

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Objectives: There was a fulminant course of mucormycosis associated with high morbidity and mortality and enigmatic reason of its remergence with COVID-19 pandemic. Objective of this study was to know the recent change in epidemiology, new risk factors, causative agent, treatment and followup during pandemic in patients with or without COVID-19 infection in and around Delhi.

Materials & Methods: An observational study of mucormycosis was conducted in tertiary care and referral hospital in Delhi, north India. The demographic, clinical, mycological, radiological, host factor, management and follow up details of patients of mucormycosis was collected. All specimen (either biopsy from paranasal sinus or nasal discharge or bronchoalveolar lavage or sputum) were processed as per standard mycological techniques.

Results: Fifty-five consequetive patients with mucormycosis were diagnosed during the study period from April 2021 to December 2021.The age of the patients ranged from 3 months to 72 year (median 47 years). The patients were from Delhi 26 (47.27%) and its neighbouring states (53.73%).

Predisposing factors for mucormycosis included diabetes 41 (74.54%), steroid 20 (36.3%), oxygen support 13 (23.6%), hypertension 6 (10.9%), B cell acute lymphoblastic leukemia 4 (7.2%), chronic kidney disease 1 (1.8%), cardiac disease 3 (5.4%), hypothyroidism1 (1.8%), obesity 1 (1.8%), rheumatoid arthritis 1 (1.8%), tuberculosis 1 (1.8%). Seven (12.7%) had active covid infection, 25 (45.4%) patients had a history of recently treated covid infection (<6 weeks) and 23 patients had recovered from COVID 19 infection. In our patient cohort, most common presentation was rhino-orbito-cerebral mucormycosis 43 (78.2%), followed by disseminated 9 (16.4%), nasal sinus 4 (7.2%), pulmonary 2 (3.6%), cutaneous 1 (1.8%) etc. Intracranial extension was observed in 29 (52.7%) on imaging and 7 patient (12.7%) had cranial nerve palsies. The most frequent group of symptoms reported were oro-facial symptoms (toothache, facial swelling, pain and ophthalmological symptoms (swelling, pain, redness in eye, blurry and double vision) 43 (81.8%) followed by headache, weakness and fever 38 (69.09%), cough & breathlessness in 4 (7.2%), gastrointestinal symptoms or neurological symptoms. The most common species isolated was *Rhizopus arryhizus* 35 (63.6%), followed by *Rhizopus microsporus* 8 (14.5%), *Rhizomucor* spp. 4 (7.2%), *Mucor* spp. 4 (7.2%), *Lichthemia* spp. 2 (3.6%), *Apophysomyces variabilis* 2 (3.6%). Six (10.9%) patients had mixed infection with *Aspergillus* spp. 2 of whom had pulmonary disease. Treatment included intravenous amphotericin B and posaconazole in all patients. Orbital exenteration was done in 8 patients. Adjuvant retrobulbar amphotericin B injection was administered in 12 patients. Overall, 32 responded to therapy and mortality rate was 13 (23.6%) at a mean follow up period of 3 months.

Conclusions: In individuals with poorly controlled diabetes, COVID-19-associated mucormycosis can be a major consequence of COVID-19 infection. Despite having a reputation for being a difficult-to-treat fungal infection and having a high patient mortality rate, restricted use of corticosteroids, good glycaemic control, high suspicion by clinicians and microbiologists, advise on discharge about suspected mucormycoses symptoms, wearing of masks by susceptible populations to reduce exposure to Mucorales and avoiding of construction areas, can help in ameliorating mortality rate and increases successful management.

P314

A case of pulmonary cocidioidomycosis in the Netherlands

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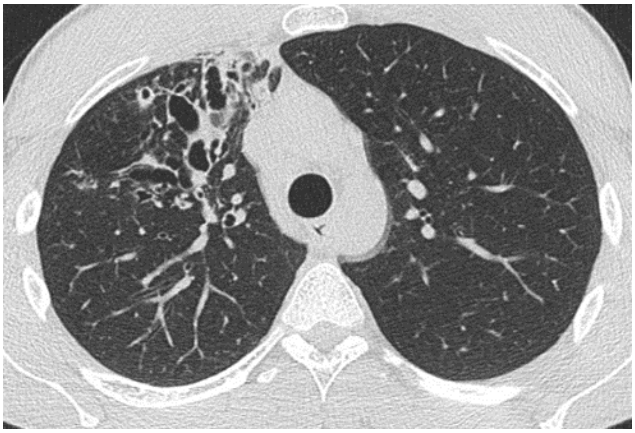
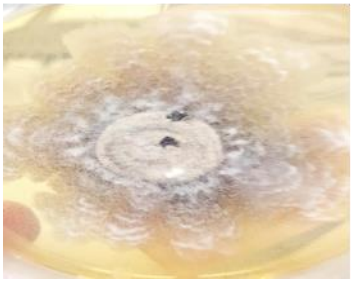
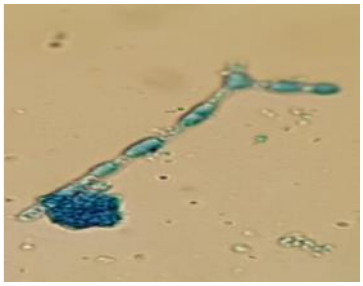
A case of pulmonary cocidioidomycosis in the Netherlands

Background
Coccidioidomycoses is caused by the dimorphic fungus *C. immitis*. In Europe this infection is extremely rare. Here we describe a case of pulmonary coccidioidomycosis in a 26-year-old man.
Case
A 26-year-old patient was referred to our center for diagnosis of cough. His medical history was positive for asthma, which had been stable for years without medication. Since April 2020 he was coughing for which the general practitioner started salbutamol per inhalation. In April 2021 his complaints increased and he reported coughing and thoracic pain. On the chest X-ray abnormalities were seen, and he was referred to the hospital. Laboratory results showed a high total IgE (4376 kIU/L), a high IgE (3,76 KIU/L) and IgG (30 mg/L) for *Aspergillus* and a mild eosinophilia ($0.4*10^9$/L). CT thorax showed an abnormal aspect of the right upper lobe with extensive bullous and bronchiectastic abnormalities, interspersed with areas of consolidations and nodular densities. Bronchoscopy showed no endobronchial abnormalities. Culture of the BAL showed growth of *Actinomyces odontolyticus* and a fungus, which had to be determined. Galactomannan in BAL was negative. He was treated with prednisone under the suspicion of a ABPA and doxycycline. The fungus was determined as a *Coccidioides immitis.* Therefore, itraconazole was given from the end of October 2021 until the end of December 2021. During additional anamnesis he reported that he had travelled in South America in 2017 for 3.5 weeks. After the start of prednisone, doxycycline and itraconazole his coughing and thoracic pain disappeared. However in spring 2022 his complaints returned and he was referred to our hospital. The CT scan again showed the same abnormalities in the right upper lobe with a new ground glass opacity in the right lower lobe. and *Coccidioides immitis* was cultured from the sputum. Fluconazol was started and will be continued for 6 to 12 months.
Key learning points:

- infection with *C. immitis* can occur in apparently healthy patients in Europe several years after visiting an endemic area
- Treatment with itraconazole for two months is not sufficient
- When complaints return, think of a continuing infection







P315

Utility and Pitfalls of β-D-Glucan for Diagnosis and Monitoring of Chronic Disseminated Candidiasis in Pediatric Cancer Patients

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Background:
Invasive fungal diseases (IFDs) are important complications in immunocompromised pediatric patients with cancer and associated with increased morbidity and mortality. β-D-Glucan (BDG) is a useful but nonspecific biomarker in patients with suspected IFDs and *Pneumocystis* pneumonitis. Little is known, however, about its utility for diagnosis and response monitoring of chronic disseminated candidiasis (CDC), a rare but difficult to treat condition.
Patients and methods:
We describe the utility and pitfalls of serum BDG in five pediatric cancer patients with suspected CDC for diagnosis and monitoring of treatment responses. BDG in serum was measured by a commercially available assay (Fungitell®; Associates of Cape Cod, MA, USA) and values were correlated to clinical, radiologic, and microbiological patient- and disease related variables. Antifungal treatment and treatment duration were analysed. Treatment response was determined by resolution of imaging findings and time to normalization of BDG levels.
Results:
Five pediatric patients (4f/1m; 4-18 years) with acute lymphoblastic leukemia (4) and Ewing sarcoma (1) and a diagnosis of probable CDC (2019 EORTC/MSG criteria) were identified between 2013 and 2021. All had a history of granulocytopenia and mucositis, and four had received prolonged courses of corticosteroids. CDC was located in spleen (5), liver (4), lungs (4), CNS (2), kidney (1), myocardium (1) and skin (1); and diagnosed on the basis of imaging, a positive blood culture (1), a positive BDG assay in serum (5), and absence of a bacterial, viral, and parasitic etiology. Patients received IV liposomal amphotericin B and/or caspofungin, followed by PO fluconazole for 184 to > 365 days, respectively. BDG concentrations in serum (35 time points) remained elevated for prolonged periods of time, were independent of clinical symptoms and returned to normal upon resolution of imaging findings in the four leukemia patients. In the remaining patient with Ewing sarcoma, a liver biopsy performed 5 months after diagnosis because of lack of improvement to antifungal therapy revealed disseminated aspergillosis. In this patient, repeated galactomannan testing in serum had been negative at presentation.
Conclusions:
The analysis demonstrates that BDG in serum is a useful adjunct to the diagnosis of probable CDC and may be useful for monitoring responses to antifungal treatment. However, as exemplified by one of the cases, it remains a non-specific fungal biomarker whose results need to be re-assessed in patients who do not respond to treatment as expected.

P317

Mucormycosis: A 14-year Retrospective Study from a Tertiary Care Center in Lebanon

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Objectives:

Mucormycosis (MCM) is a serious invasive fungal disease (IFD) caused by fungi of the class Mucoromycetes. Its manifestations vary widely depending on the site of infection. It is associated with high mortality, particularly in immunocompromised patients. A global surge in MCM cases was reported with the COVID-19 pandemic. Published data on MCM from the Middle East remains scarce. We analyzed all recorded cases of MCM at the American University of Beirut Medical Center (AUBMC), a tertiary care center in Lebanon, over 14 years. We aimed to identify the incidence, seasonal variation and predictors of mortality.

Materials & Methods:

We conducted a retrospective chart review between January 1, 2008, and January 1, 2022. All patients with proven or probable MCM were included in the study. Proven or probable MCM was defined by positive histopathology and/or positive cultures. Histopathology was consistent with MCM when broad, non-septate hyphae with irregular right-angle branching were visualized on tissue biopsy.

Results:

A total of 43 patients were identified to have MCM. Their median age was 53 years, and the majority were males (58.1%). Most of the cases were diagnosed in the Autumn season. 67.4% of the patients had hematological malignancies, and 34.9% had uncontrolled diabetes mellitus. The most common site of involvement was rhino-orbital-cerebral MCM (ROCM) (74%), and 5 cases of ROCM became disseminated (11.6%). The annual cases of MCM per 100,000 patient days increased markedly during the years of the COVID-19 pandemic (**Figure 1).** More than 50% of the cases occurred between 2018 and 2022, and 22% of them had COVID-19 infection. Liposomal Amphotericin B was used as a first line agent in most of the patients (86%). The median duration of total in-hospital antifungal therapy was 21 days and 51.2% of the patients received step-down therapy with azoles. Surgical debridement was done in 72 % of the cases, and it was significantly associated with survival (p-value: 0.02), (**Figure 2**). All-cause mortality was 46.7%, with renal failure and soft tissue involvement being significantly associated with mortality ($p < 0.05$).

Conclusion:

The incidence of MCM has been increasing at our institution particularly after the COVID-19 pandemic. Early diagnosis, treatment and surgical debridement improve patient outcomes and overall survival.

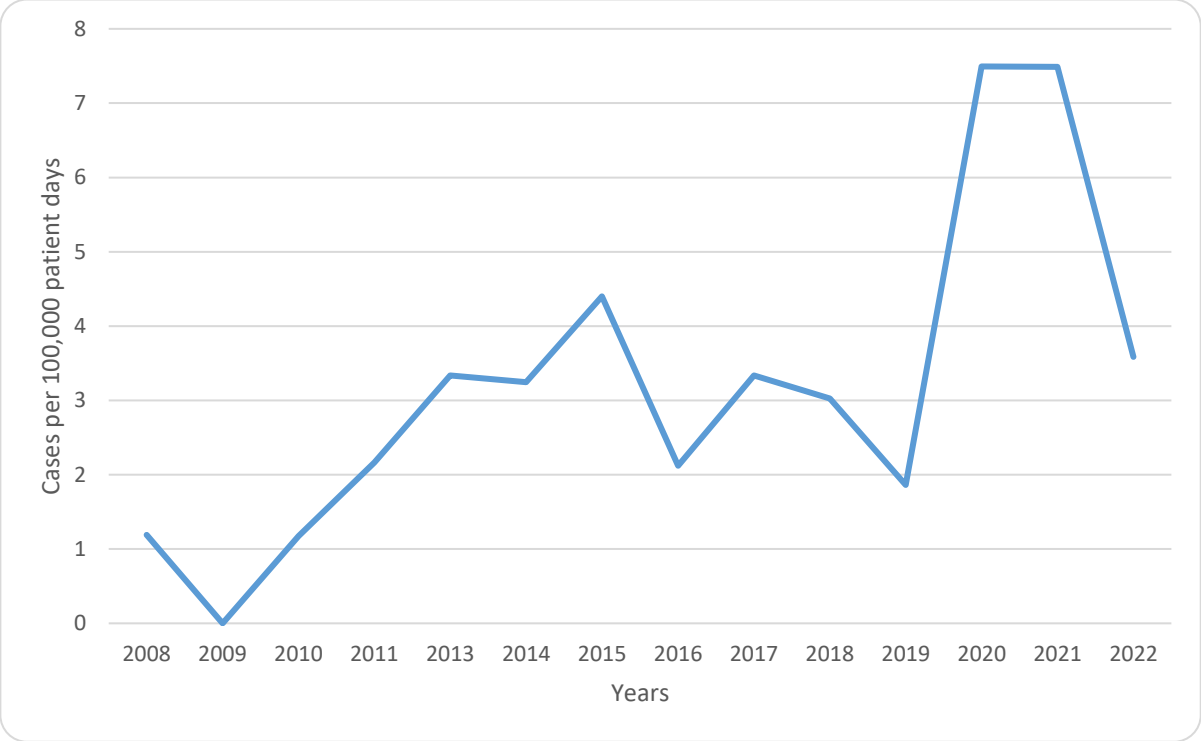

Figure 1: Annual cases per 100,000 patient days

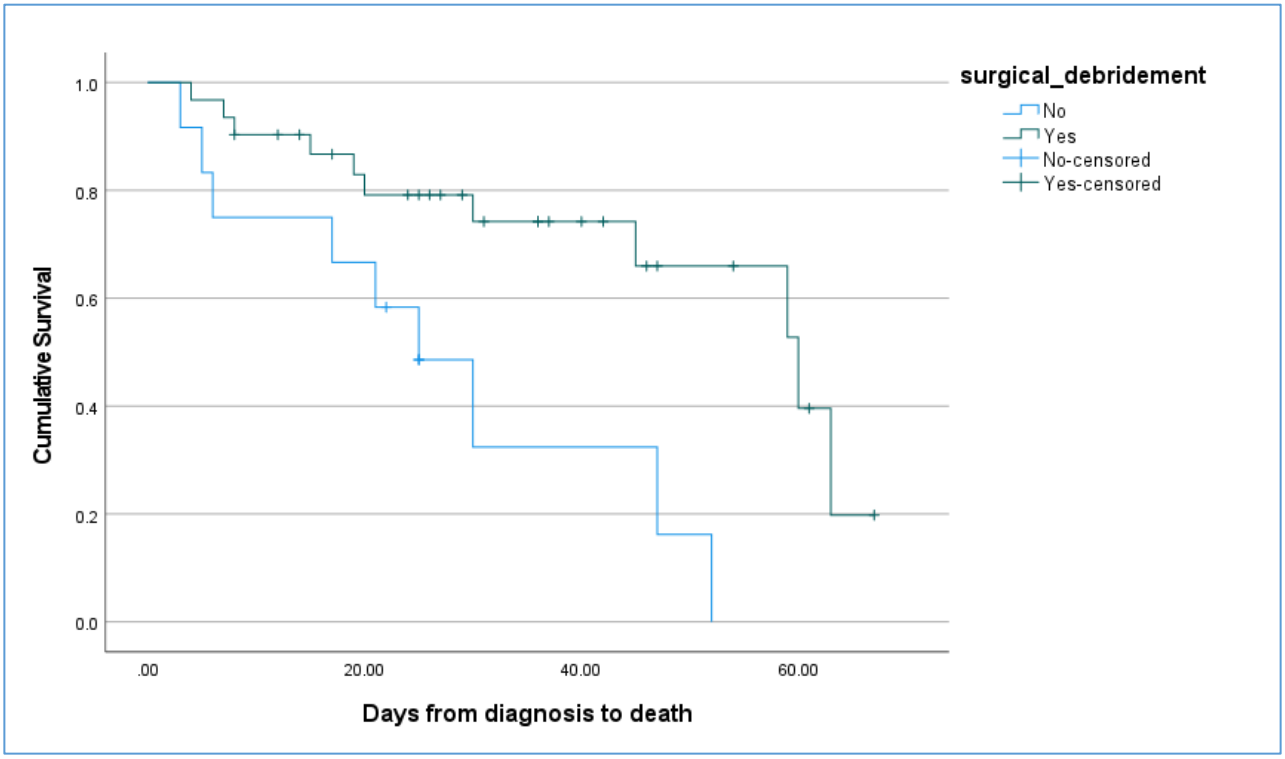

Figure 2: Kaplan-Meier survival analysis of patients with MCM who underwent surgical debridement

P318

Af-CAR-NK92 cells secreting IL-15 as potential off-the-shelf therapy for invasive pulmonary aspergillosis

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Introduction: Chimeric antigen receptors (CARs) are artificial fusion proteins consisting of an extracellular targeting domain, a spacer, a transmembrane domain, and an intracellular signaling domain, strengthened by a costimulatory domain. Originating from cancer treatment, CAR-engineered immune cells are a promising therapeutic option for treating infectious diseases. Immunocompromised patients often suffer from invasive aspergillosis, which leads to significant mortality, necessitating the exploration of alternative therapeutic options. Previously, we engineered T cells with an *Aspergillus fumigatus* (Af)-specific CAR and demonstrated their potent antifungal activity. CAR-NK92 cells offer certain advantages, such as intrinsic killing and minimal side effects after transplantation. Moreover, Af-CAR-NK92 cells could serve as off-the-shelf allogeneic therapeutics. Given the advantageous influence of IL-15 secretion on the viability, expansion, and cytotoxic potential of CAR-NK cells, we opted to integrate a gene cassette for continuous IL-15 expression into our CAR construct
Objectives: Engineering CAR-NK92 cells targeting *Aspergillus fumigatus* and enhancing their anti-fungal activity by incorporating an IL-15 gene cassette along with the CAR gene, resulting in constitutive IL-15 secretion.
Materials & Methods: We utilized the non-viral Sleeping Beauty transposon system to engineer NK92 cells and introduce the Af-CAR or the Af-CAR along with constitutive expression of IL-15. As a control, we generated cells expressing CARs that specifically target CD19. We selected and expanded the NK92 cells expressing the CAR and conducted *in vitro* functional assays to characterize their antifungal activity. To achieve this, we performed co-culture assays with Af hyphae and measured the secretion of cytokines, the degranulation of the cells, as well as assessed direct hyphal damage.
Results: Using the NK92 cell line, we achieved the generation of cultures containing more than 90% CAR-positive NK92 cells. The secretion of IL-15 proved to be sufficient for the expansion of NK92 cells, resulting in independence from IL-2. When exposed to *Aspergillus fumigatus*, Af-CAR-NK92 cells demonstrated specific activation, as evidenced by cytokine secretion and degranulation, whereas CD19-CAR-NK92 cells did not exhibit activation. We observed a substantial increase in cytokines such as IFN-γ, IL-10, and chemokines like CCL-3 and CCL-4. The constitutive secretion of IL-15 led to higher concentrations of these cytokines and an augmented percentage of CD107a-positive cells. Furthermore, Af-CAR-NK92 cells exhibited inhibition of fungal growth.
Conclusions: Af-CAR-NK92 cells have demonstrated functionality in *in vitro* assays, making them a promising therapeutic option. Their potential is further enhanced by the constitutive expression of IL-15, which augments their activation. Currently, the antifungal activity of Af-CAR-NK92 cells is being investigated *in vivo*.

P319

CARD9 deficiency promote immune-suppressive landscape in chronic fungal infections

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Objectives: We previously identified CARD9 deficiencies in ten Chinese patients with refractory, life-threatening phaeohyphomycosis, yet the specific molecular mechanism has not yet been adequately elucidated. To construct a detailed, high-resolution atlas of cell populations in the lesion of subcutaneous phaeohyphomycosis and study the cell-specific mechanisms involved in the pathobiology of CARD9-deficient individuals.

Materials & Methods: We performed single-cell RNA sequencing (scRNAseq) on skin lesions obtained from *Card9*-KO and wildtype (WT) mice with *Phialophora verrucosa* infection, as well as CARD9-deficient patient with phaeohyphomycosis. Cells were clustered Using RunUMAP function within the Seurat package, and each identified cell type underwent meticulous functional analysis to unravel its unique biological properties. Functional in vitro models, flow cytometry of human peripheral blood mononuclear cells (PBMCs), and immunostaining of murine and human lesions were used to validate the findings demonstrated by scRNAseq.

Results: Our data revealed remarkable T cell exhaustion in CARD9-deficient murine lesions, featured by the high expression of inhibitory immune receptors. Moreover, we found CARD9 deficiency promoted M2 polarization of macrophages, which showed a positive correlation with the expression of inhibitory immune ligands. Increased eosinophil cell abundance was found in CARD9-deficient murine lesions, and a subset of eosinophils, as well as neutrophils were also found to express inhibitory immune ligands during infection. Furthermore, prominent enhancement of inhibitory immune receptors mediated crosstalk between T cells and myeloid cells was observed in CARD9-deficient lesions. These findings were further supported in the scRNAseq data from a CARD9-deficient patient with subcutaneous phaeohyphomycosis, along with flow cytometry of PBMCs, and immunostaining of murine and human lesions.

Conclusions: Our study identified T cell exhaustion of lesions in CARD9-deficient phaeohyphomycosis and the underlying mechanisms, highlighting immune checkpoints as potential therapeutic targets for the intervention of persistent fungal infections with CARD9 deficiencies.

P320

Usefulness of broncho-alveolar lavage in classifying invasive fungal disease in paediatric malignancies

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Introduction

Invasive fungal diseases are an important cause of morbidity-mortality in children with haemato-oncological malignancies or who undergo a haematopoietic stem cell transplant (HSCT). The microbiological diagnosis of these infections is frequently challenging and often requires invasive procedures such as broncho-alveolar lavage (BAL) or a biopsy. These procedures might associate complications and morbidity. The diagnostic difficulties of IFD have an impact on the antifungal treatment of these patients, with prolonged use of empirical treatment, drug-to-drug interactions, side effects, cost a worse patient experience.

In this study we aimed to explore the usefulness of BAL in the diagnosis of IFD and the subsequent impact on antifungal therapy.

Methods

We collected data from children less than 18 years of age, with an underlying haemato-oncological malignancy or post-HSCT who were admitted with febrile neutropenia, underwent investigations for IFD and received empiric antifungal therapy at two different paediatric centres, between 2018 and 2021. All children underwent a BAL at different time points during their work-up for IFD. BAL samples were sent for microscopy, culture, Aspergillus PCR and/or BAL galactomannan (GM) following the respective local protocols. All children were classified as possible, probable, or proven IFD based on the EORTC definitions from 2020.

Results

A total of 35 children were included. Eighty per cent (28/35) of these children were under treatment for a haematological malignancy while 20% had solid tumours. The clinical suspicion of a possible IFD was based on High Resolution Computed Tomography (HRCT) chest findings in 71.4% (25/35) cases the remaining (28.6%) underwent BAL based on chest x-ray findings along with clinical features.

After investigations, 48% (17/35 patients) were reassigned to a category of probable IFD. Among these, 70% (12/17) were categorised based on the results of the BAL, the remaining had positive serum galactomannan.

The impact of the time of BAL form onset of symptoms was investigated in the subset of the patients in whom this data was available (n=24). The median days after the onset of symptoms to the performance of the BAL was 12 days (min 1day, max 48). Among children who had the procedure in the first 2 week of illness (n=16) 62.5% (10/16) had a positive finding on the BAL. Although we had small numbers, we did not find differences between performance during the first or the second week. After day 14, the chance of having a positive BAL result fell to 50%.

Conclusion

BAL was helpful classifying patients into probable IFD and this can impact the treatment decisions. The sooner the BAL is performed after onset of symptoms suggestive of an IFI the greater the yield with the optimal time being with in the first 2 weeks of illness.

P321

Use of Galactomannan from Bronchoalveolar Lavage to Detect Invasive Aspergillosis Early After Lung Transplantation

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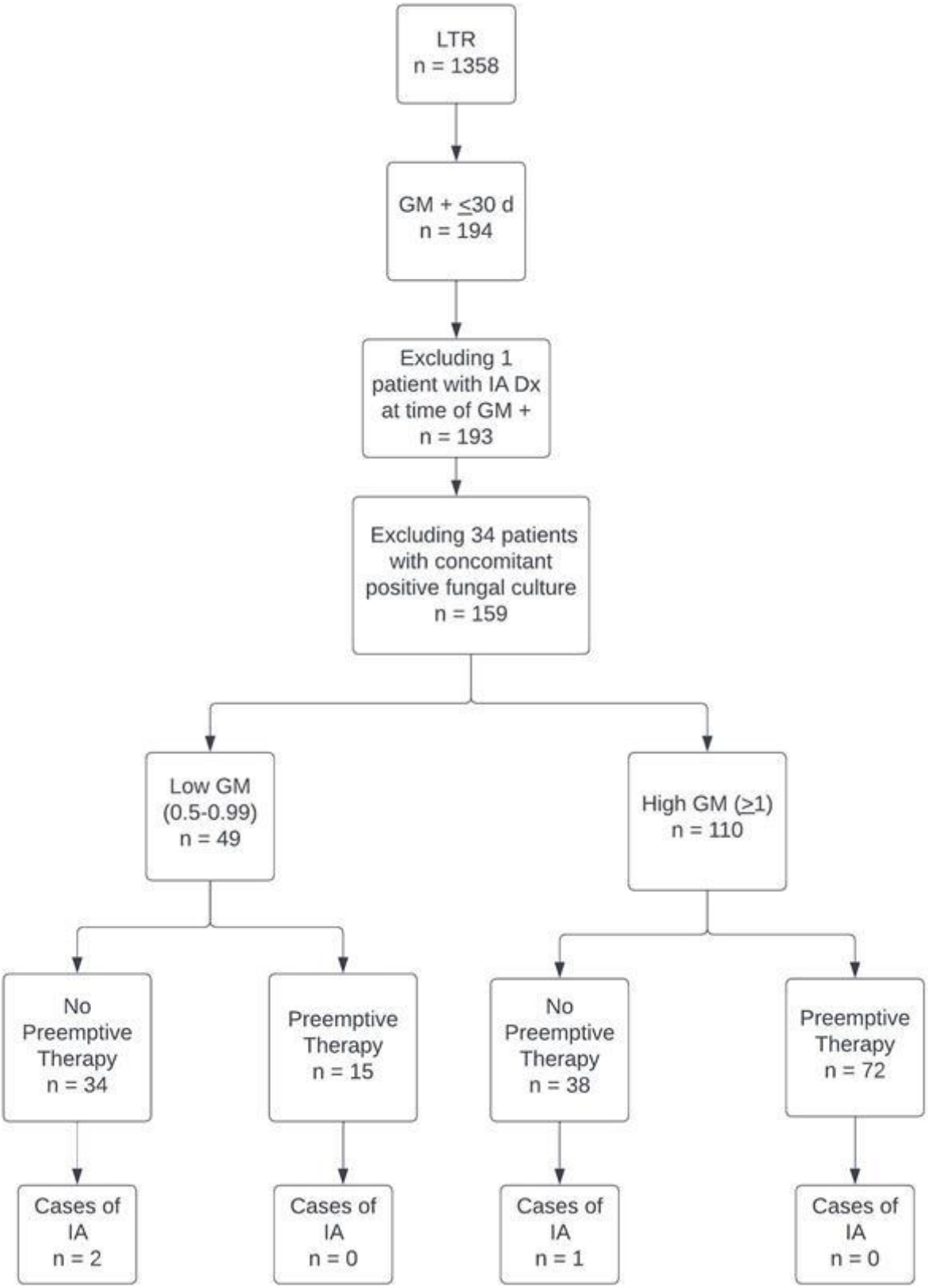
Objectives: Bronchoalveolar lavage (BAL) galactomannan (GM) testing has shown higher sensitivity than traditional culture methods for diagnosing invasive aspergillosis (IA). However, smaller studies have reported higher false positivity rates in the early post-transplant period. We aimed to evaluate the rate of IA among lung transplant recipients (LTRs) with positive GM from BAL but negative fungal culture within the first 30 days after lung transplantation. We also aimed to assess the preventive value of pre-emptive antifungal therapy in these cases.
Methods & Materials: LTRs who underwent lung transplantation at Toronto General Hospital between January 2010 and December 2019 were considered for the study. We included all LTRs who had a BAL GM ≥0.5 but a negative BAL fungal culture within the first 30 days after transplant. GM testing was performed by the microbiology laboratory at our hospital using the Platelia™ assay. All assays were run in triplicates and index value was determined by mean of the three values. Pre-emptive antifungal treatment was defined as mold-active antifungal drugs administered within 4 weeks of the positive GM result for a minimal duration of 2 weeks. Participants were followed for one year after transplantation, and the diagnosis of IA was established based on the International Society for Heart and Lung Transplantation criteria. The primary outcome measured was the occurrence of IA within 180 days of positive GM result.
Results: Among 1358 LTRs, 194 had positive GM results (≥0.5). Thirty-five patients were excluded; 34 because of concomitant growth of *Aspergillus* in fungal culture from BAL and an additional case because IA was diagnosed concurrently with the positive BAL GM. Out of 159 patients, 49 had GM of 0.5-0.99, while 110 had GM of ≥1 within 30 days post-lung transplant. Among patients with GM of 0.5-0.99, 15 received pre-emptive antifungal treatment, compared to 34 who did not. Two cases of IA (2/34, 5.9%) occurred within 6-months among those with BAL GM of 0.5-0.99 who did not receive pre-emptive therapy (after 96 and 178 days from positive GM test), while no cases were observed in patients who received pre-emptive therapy. In those with GM ≥1, out of 38 patients who did not receive pre-emptive antifungal treatment, one patient (2.6%) developed IA within the 6-month period (129 days after positive GM). Among the 72 patients with GM ≥1 who received pre-emptive therapy, no cases of IA occurred.
Conclusion: In our cohort, the likelihood of developing IA among those with low GM levels (0.5-0.99) was similar to those who had high GM levels (≥1). Nevertheless, the overall risk of developing IA within 6 months among LTRs with early GM-positivity was low (3/159, 1.89%, overall and 3/72, 4.2%, without pre-emptive treatment). The low absolute risk difference, coupled with the potential side effects of current antifungal treatments, underscores the importance of considering the benefits and cost-effectiveness of current treatment options.


LTR
n = 1358
GM + ≤30 d
n = 194
Excluding 1 patient with IA Dx at time of GM +
n = 193
Excluding 34 patients with concomitant positive fungal culture
n = 159
Low GM (0.5-0.99)
n = 49
High GM (≥1)
n = 110
No Preemptive Therapy
n = 34
Preemptive Therapy
n = 15
No Preemptive Therapy
n = 38
Preemptive Therapy
n = 72
Cases of IA
n = 2
Cases of IA
n = 0
Cases of IA
n = 1
Cases of IA
n = 0

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Histopathology of Cutaneous Invasive Fungal Infections in a Tertiary Cancer Center: Causes, Discordance with Culture, and Histopathologic Determinants of Outcome

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Background: Cutaneous invasive fungal infections CIFIs (primary or secondary to hematogenous seeding) are frequent and often fatal in immunocompromised cancer patients. There is a paucity of studies on the prognostic significance and concordance of histopathologic features with cultures.

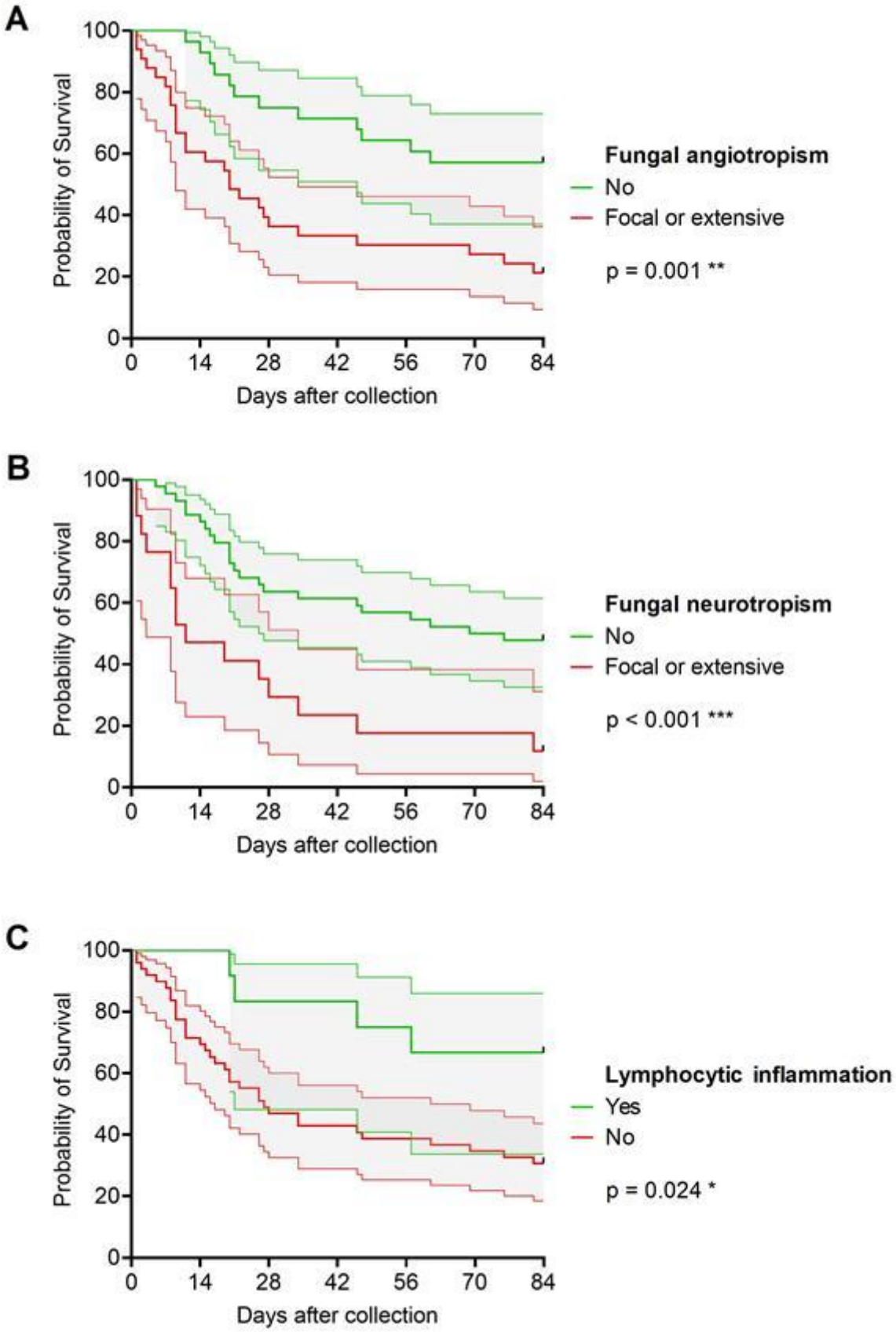
Methods & Materials: We reviewed all patients with histologically diagnosed CIFIs at the University of Texas MD Anderson Cancer Center, Houston, Texas, between June 2016 and June 2020. Demographic data, clinical characteristics, histopathologic features (organism, distribution [dermis/subcutis, blood vessels/nerves/epidermis], density of fungi, inflammation, fibrosis), culture findings, and outcome data were recorded. Independent predictors of 84-day all-cause mortality were determined using a multi-variable Cox' regression model.

Results: We identified 61 patients (median age 60 years, range 8-81); 37 (61%) were male. Most patients had hematologic malignancy (n=58, 95%), especially acute leukemia (n=40, 66%). CIFI was primary in 53 patients (87%), with acute onset (≤1 week) in 66% of patients; 37 patients (61%) had multiple skin lesions, most of which were painful or tender (n=35, 58%). The lesions were commonly ulcers (n=22, 37%) or papulonodules (n=19, 31%) and frequently localized to one anatomic region (n=35, 58%); lower extremity was the most common site (n=17, 28%). Fungal organisms were seen on hematoxylin & eosin-stained sections in 47 cases (77%), whereas ancillary studies (Grocott's methenamine silver stain/Periodic acid-Schiff) were required in 14 cases (23%). Of the 59 concurrent microbiology cultures, only 43 (73%) were positive. In 16 cases, fungal order/genus was identified by both histopathology and culture; 13/16 (81%) were concordant (Fleiss' kappa 0.67). The causative fungal order/genus was determined in 55 patients (90%), most commonly *Fusarium* (n=22, 36%) or Mucorales (n=12, 20%). Angiotropism was most frequently associated with *Fusarium* (19/22, 88%), and neurotropism with Mucorales (8/12, 67%, Figure 1). Eighty-four-day all-cause mortality was 62% (66% and 50% in CIFIs caused by molds and yeasts, respectively). Univariate analysis of the association of histopathological features and mortality outcomes (Mantel-Cox log-rank test) revealed that fungal angiotropism (p=0.001, Figure 2A) and neurotropism (p<0.001, Figure 2B) were associated with significantly increased mortality, whereas lymphocytic inflammation, seen only in 20% of patients, was associated with reduced mortality (p=0.024, Figure 2C). On multivariate analysis, female sex (adjusted hazard ratio [aHR] 2.68, 95% confidence interval [CI] 1.32–5.45, p=0.006), clinical presentation as non-ulcerated cutaneous lesions (papules/plaques/patches, aHR 2.60, 95% CI 1.31–5.15, p=0.006), fungal angiotropism (aHR 3.21, 95% CI 1.51–6.80, p=0.002), and culture positive for Mucorales (aHR 5.26, 95% CI 1.62–17.05, p=0.006) were independent predictors of 84-day all-cause mortality.

Conclusions: CIFIs have poor prognosis, especially when caused by Mucorales and if fungal angiotropism is identified. Lymphocytic inflammation may be associated with better prognosis. Since

cultures are frequently false negative (27%) or discordant (19%), more efforts are needed for culture-independent molecular detection of fungi. Incorporation of histopathologic features might inform prognostic risk stratification.

Characteristics		Fusarium (n=22)	Mucorales (n=12)	Other mold (n=7)	Yeast (n=14)	Total (n=55)	*P*-value
Pseudoepitheliomatous hyperplasia		2 (9%)	1 (8%)	0 (0%)	1 (7%)	4 (7%)	> 0.99
Necrosis		3 (14%)	2 (17%)	5 (71%)	2 (14%)	12 (22%)	0.019
Granuloma		0 (0%)	0 (0%)	0 (0%)	1 (7%)	1 (2%)	0.60
Angiotropism	No	3 (14%)	4 (33%)	5 (71%)	12 (86%)	24 (44%)	< 0.0001
	Focal	4 (18%)	1 (8%)	0 (0%)	2 (14%)	7 (13%)	
	Extensive	15 (68%)	7 (58%)	2 (29%)	0 (0%)	24 (44%)	
Neurotropism	No	13 (59%)	5 (42%)	7 (100%)	14 (100%)	39 (71%)	0.001
	Focal	6 (27%)	7 (58%)	0 (0%)	0 (0%)	13 (24%)	
	Extensive	3 (14%)	0 (0%)	0 (0%)	0 (0%)	3 (5%)	
Epidermotropism		9 (41%)	5 (42%)	5 (71%)	0 (0%)	19 (35%)	0.002


A
Probability of Survival
100
80
60
40
20
0
0
14
28
42
56
70
84
Days after collection
Fungal angiotropism
No
Focal or extensive
p = 0.001 **
B
Probability of Survival
Days after collection
Fungal neurotropism
No
Focal or extensive
p < 0.001 ***
C
Probability of Survival
Days after collection
Lymphocytic inflammation
Yes
No
p = 0.024 *

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Candidemia in patients over 80 years old; for the upcoming aging society

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Objectives
Current life expectancy is approaching over eighty years old in EU and Japan. Candidemia remains a major cause of morbidity and mortality among geriatric patients. Our purpose is to show the clinical features in candidemia among patients over 80 years old and to determine factors associated with death in 30 days.

Methods
All episodes of positive blood culture for candida species in patients who admitted in Tokyo Medical University Hachioji Medical Center (700-bedded university hospital), 80 years of age or older, were evaluated from January 2016 to December 2022. Patients had no antifungal treatment were excluded. Information on the patient's age, gender, underlying disease, use of intravenous catheter, immunosuppressive agent use, type of candida species from blood culture, White Blood Cell count (WBC), and Serum 1,3-βD-Glucan assay (BDG), and crude mortality in 30 days were collected. To determine the independent risk factors associated with death in 30 days, multivariate analysis was performed using variables with $p < 0.1$ in the univariate analysis; the level of significance was set at $p<0.05$. Statistical analyses were performed using R version 4.3.0 for Microsoft Windows 11.

Results
Thirty-one patients were included in this study. The median age was 84 years (80 to 94 years). Eleven out of 31 patients (35.4%) were female. The study population had dementia (29.0%), diabetes (3.54%), hypertension (3.54%). Patients used central intravenous catheter (IVC) (93.5%), steroids (22.5%), cancer chemotherapy (16.1%), and total parenteral nutrition (70.9%). The most common antifungal treatments were micafungin (77.4%). The first and second most common isolates were *Candida albicans* (51.6%) following *C. parapsilosis* (19.4%) and C. *glabrata* (19.4%). All of isolates were susceptible to micafungin. WBC ranged from 975/μl to 46300/μl (median 10000/μl), BDG ranged from 95.7 to 7670 pg/μl (median 464 pg/μl) at positive blood culture. The median of eGFR was 66.9 (3.4-109.7) ml//min/1.73㎡at admission. The follow-up blood culture had taken and remove IVC for 71% of the study population. No fundoscopy, 35.5% of echocardiogram had done. The crude mortality in 30 days was 61.3%. In univariate analysis, the no significant differences were found between the survived and the died group in BDG at diagnosis (mean 767 vs 2246 pg/μl, $p<0.28$). The follow-up blood culture with confirmed negative blood culture results [Odds Ratio; 0.0727, 95% Confidence Interval; 0.00768-0.689, $p=0.0223$] was the factor associated with survival in multivariate analysis.

Conclusion
The mortality rate in 30 days was getting worse according to aging among geriatric population (>65 years old; 32.2%[1], >75 years old; 45%[2], >80 years old; 61.3% by our result). In addition, the mean EQUAL candida score in geriatrics were significant lower compared to the young age[2]. Severe underlying diseases with insufficient management quality of candidemia may cause worse mortality. However, an indication of evaluation, such as fundoscopy, or echocardiography for geriatric population with, or without dementia should be discussed.

1. Barchiesi F, et al. PLoS One. 2017;12(5):e0176576.
2. Bal AM, et al. Mycoses. 2020;63(9):892-899.

P324

Paediatric Allergic Bronchopulmonary Aspergillosis an experience from tertiary care centre in southern India emphasizing the need for developing diagnostic guidelines

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Objectives:

- To analyse the risk factors, microbiological and radiological findings among a small subset of paediatric population for development of Allergic bronchopulmonary aspergillosis (ABPA) from whom samples were received at our referral centre
- To stratify these cases based on ISHAM-ABPA working group major criteria for ABPA meant for adults and highlight the need for paediatric specific diagnostic guidelines

Methods and materials:

We received respiratory and serum samples from around 11 paediatric patients over a period 6 months (October 2022-March 2023) from a Paediatric clinic for a complete ABPA workup. High volume culture on BAL, Total IgE, Specific Aspergillus IgE, Serum and BAL Galactomannan, a detailed clinical history and radiological investigations were performed.

Results:

The mean age of presentation was 6.7 years among the total of 11 cases. Asthma which is listed as major criteria accounted for 36% of cases (n=4) and one had Obliterative bronchiolitis. Repeated lower respiratory tract infections(LRTI) was the sole complaint in 45% (n=5) of cases. None had the classical cystic fibrosis as the risk factor. Total IgE was >1000 IU/mL in 36%(n=4), 100-1000 in 45%(n=5) of cases. *Aspergillus fumigatus* specific IgE was >0.35kUA/l in only 9% (n=1) of cases. Microscopic examination on KOH showed septate fungal filaments in 54% (n=6) and high volume culture yielded mould in 27% (n=3) of cases. Serum Galactomannan assay was positive in 45% (n=5) and BAL Galactomannan in 81% (n=9) cases. Radiology showed segmental collapse and ground glass opacities in 27%(n=3) each, bronchiectasis in 18%(n=2), atelectasis and high attenuation mucus in one case each. All except one received antifungal therapy (itraconazole) and the outcome was well controlled status in 54% (n=6), partially controlled in 18% (n=2) cases. We were able to classify 18% (n=3) each of the total cases as a) Classical ABPA b) Probable Aspergillus colonisation or sensitization and c) Other infective aetiology.

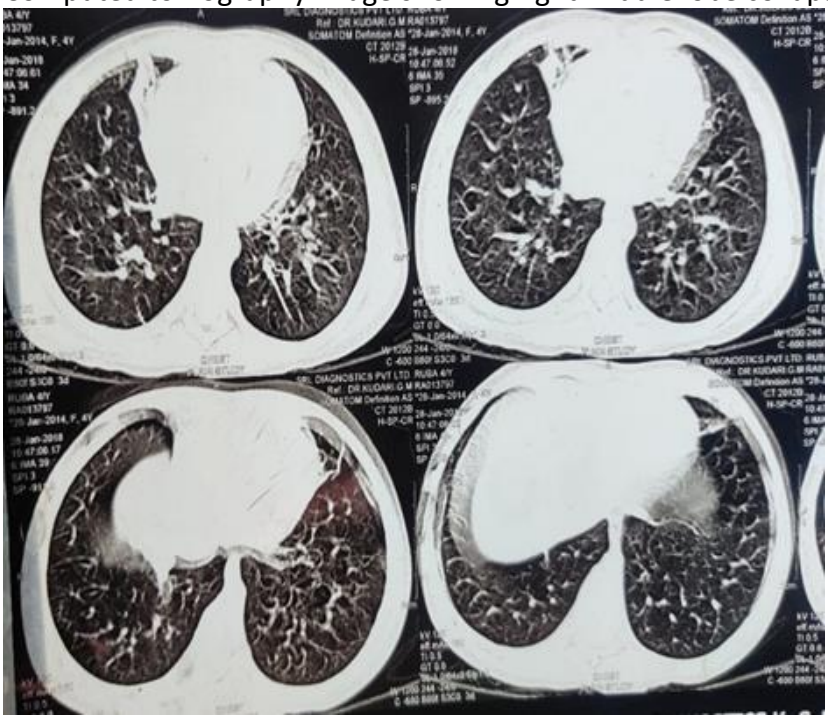
Conclusion:

ABPA is a condition characterised by hypersensitivity to *Aspergillus* species. Adults with ABPA have been the subject of extensive research. However not much data is available among the paediatric population. It has been described classically in cystic fibrosis and asthmatic children. The varied clinical presentation and laboratory tests posed a diagnostic dilemma in these children. Here we have analysed the data from this small subset of paediatric cases. We highlight the importance of

1. Analysing the predisposing host factors (factors such as repeated LRTI and not classically cystic fibrosis)
2. Evaluating the cut offs for Total IgE (we suggest considering values > 100 IU/mL for thorough evaluation)
3. *Aspergillus fumigatus* specific IgE (if <0.35 kUA/l, to repeat after 4 weeks and also perform Specific IgE for other moulds and allergens)

4. Correlating the radiological findings which might be different than in the adult population (GGO's and lung collapse)
5. Importance of high volume culture from respiratory samples for fungi and interpreting culture reports for other infective aetiology and galactomannan reports in these patients.
6. A high degree of suspicion and thorough screening is necessary to formulate diagnostic criteria for ABPA in children.

Computed tomography image showing right middle lobe collapse



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Aspergillus-Infection of Giant Neonatal Omphaloceles: Report of two Cases

Monika H. Tedy[1], Miriam A. Füller[1], Marc T. Hennies[2], Frieder Schaumburg[2], Volker Müller[1], Julia Sandkötter[1], Katja Masjosthusmann[1], Andreas Groll[1]
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Background
Invasive fungal diseases (IFDs) are important causes of infectious morbidity in critically ill neonates. Immature immune functions and disrupted protective barriers may lead to dismal outcomes. We present two cases of *Aspergillus* infections of giant omphalocele successfully managed with antifungal treatment.
Case Reports
Both infants were diagnosed prenatally and born at 36 and 37 weeks of gestation. Treatment started immediately after birth by sterile coverage. Microbiological smears were sampled weekly. Both received broad-spectrum antibiotics for suspected bacterial infection. Oral nutrition consisted exclusively of 5% dextrose and breast milk.
Pt. 1 had a positive superficial culture result (day 43) for *Aspergillus terreus.* Antigen-testing was first positive at day 46 (index: 7,2). All blood cultures remained negative for fungi. The pt. received liposomal amphotericin B (3 mg/kg/d) x 8 days and was then switched to voriconazole (18 mg/kg/d) x 99 days. Trough concentrations of VCZ were measured at 2,8 and 1,6 µg/ml.
Pt. 2 had a positive superficial culture (day 12) for *Aspergillus fumigatus.* Antigen-testing (day 26) was first positive at day 26 (index: 2,6); all blood cultures stayed negative for fungi. Voriconazole was given at 20 mg/kg/d for at least 13 days with a trough concentration of 2,9 µg/ml on one occasion. Pt. 1 was hospitalized for 83 days. Recovery was complete and the final surgical correction was planned within 3 years. Pt 2 was transferred on day 30 and died at 3,5 months due to non-fungal complications.
Conclusion
The cases demonstrate the risk of neonates with a giant omphalocele to develop potentially invasive mold disease. Diagnosis relies on the detection of the organism and may be supported by galactomannan antigen testing in blood. Treatment is longstanding, relies on *in vitro* susceptibility testing and appropriate antifungal therapy. Here, voriconazole coupled with TDM was well tolerated and associated with good outcomes.

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Candida Speciation and Antifungal Susceptibility in Pediatric Urine Samples at a Tertiary Care Hospital in Rajasthan, India

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Objectives: Candiduria refers to the presence of yeast cells in urine. While bacterial causes of urinary tract infections (UTIs) are more common in pediatric cases, the increasing use of antibiotics, the emergence of antifungal drug resistance, and the presence of relative immunodeficiency highlight the importance of regional surveillance for monitoring antifungal drug susceptibility profiles in pediatric candiduria.

Objectives : This study aimed to determine the speciation of Candida isolates and evaluate their antifungal susceptibility patterns in urine samples from pediatric patients attending a tertiary care hospital in Rajasthan, India.

Materials & Methods: This study was a laboratory-based observational study that enrolled 150 children (<14 years) who exhibited clinical suspicion of candiduria. Urine samples were collected from pediatric patients who presented at the hospital with clinical signs and symptoms of candiduria. To identify Candida isolates, standard laboratory techniques such as KOH mount and culture on Sabouraud dextrose agar were employed, and their speciation was determined using the germ tube test, Dalmau plate culture, and colour produced on HiChrome agar. Antifungal susceptibility testing was conducted using the Kirby-Bauer disk diffusion method following CLSI-2019 guidelines. Data pertaining to the prevalence and resistance patterns of Candida species were subsequently analyzed.

Results: The isolation rate was 21.33% (32) with maximum cases in infants (37.5%) and overall male to female ratio being 3.26:1. Majority of cases were reported from ICU (64%) with most prevalent risk factor being broad spectrum antibiotics (90.6%). We observed that NAC species (79.7%) had predominance over *Candida albicans* (20.3%) with *Candida tropicalis* being the predominant isolate (37.5%). We observed the maximum susceptibility to Amphotericin-B (92.19%) & maximum resistance to Voriconazole (10%).

Conclusions: The findings of this study provide valuable insights into the speciation and antifungal susceptibility patterns of Candida isolates in pediatric urine samples at a tertiary care hospital in Rajasthan, India. These results emphasize the need for effective surveillance and appropriate management strategies to combat Candida infections in pediatric patients.

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The first case of mucormycosis in a child with rheumatoid arthritis in Russia

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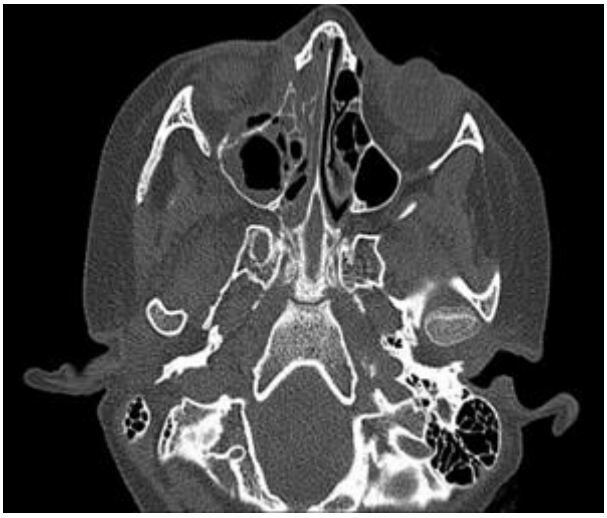
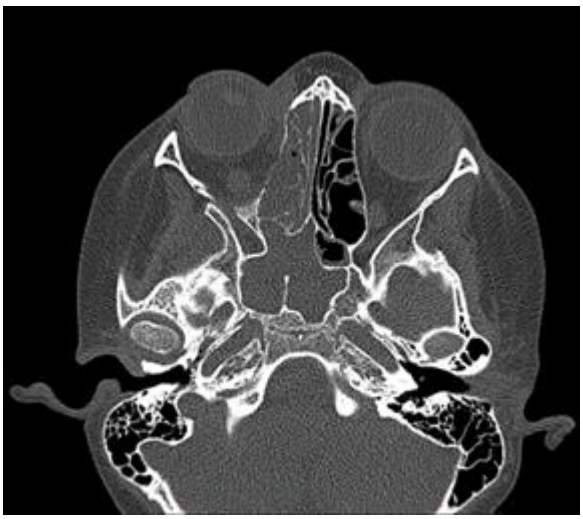
Objectives: The article presents the first case of mucormycosis caused by juvenile rheumatoid arthritis.

Materials & Methods: Diagnosis of IA was made according to EORTC/MSGERC criteria (2020).

Results: A 9-year-old girl with juvenile rheumatoid arthritis was hospitalized. This patient had treatment with high dose systemic glucocorticosterois and immunosuppressive drugs. Computed tomography of the paranasal sinuses revealed shading of the cells of the lattice labyrinth with destruction of the bone. The operation was performed – endoscopic right-sided ethmoidotomy (necrectomy), sphenotomy. Histological examination of the postoperative material revealed broad hyphae of the fungus, similar to mucormycetes. During PCR diagnostics, the genus of the fungus, Lichtheimia spp., was determined. The patient received combined antimycotic therapy with posaconazole and liposomal amphotericin B, followed by a transition to monotherapy with posaconazole. Girl was discharged in satisfactory condition.

Conclusions: High dose systemic glucocorticosterois and immunosuppressive drugs in severe juvenile chronic arthritis may develop mucormycosis. With prolonged fever, refractory to broad-spectrum antibacterial drugs and the presence of characteristic changes on computed tomography examination of the material from the lesion with mycological examination, if possible, it is necessary to perform a biopsy with histological and mycological examination. Upon confirmation of the diagnosis, it is necessary to immediately begin prescribe adequate antimycotic therapy with surgical removal of the affected tissues and elimination or reduction of the severity of risk factors.





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Liposomal amphotericin B als antifungal prophylaxis in children and adolescents undergoing allogeneic hematopoietic cell transplantation.

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Objectives:
Azole agents are recommended as antifungal prophylaxis in decreasing the incidence of invasive fungal disease (IFD) in high-risk patients in pediatric oncology, including high-risk patients receiving allogenic hematopoietic cell transplantation (HCT). However, azole related toxicity, pharmacological interactions with the conditioning regimen and growing incidence of azole resistance makes these antifungal agents not ideal in the transplant setting. Data on alternative antifungal prophylactic strategies in the pediatric allogeneic HCT are limited. We report on the contemporary incidence and outcome of IFD after allogeneic HCT in a cohort of high- risk pediatric patients who received prophylactic liposomal amphotericin B (L-AMB) twice weekly.

Materials & Methods:
This single-center retrospective study was executed at the Wilhelmina Children's Hospital and Princess Maxima Center for Pediatric Oncology in Utrecht and included all high-risk patients transplanted between 2010 and 2022. Pediatric patients with a hematologic malignancy (e.g., acute lymphatic, myeloid leukemias and lymphomas), myelodysplastic syndromes (MDS), bone marrow failure syndromes, immunodeficiencies and patients with prolonged neutropenia or long-standing immunosuppressive treatment were defined as high-risk. They received primary or secondary antifungal prophylaxis. We identified patients who developed proven, probable, or possible IFD using the definitions of the EORTC/MSG Consensus Group. Prophylactic antifungal treatment with L-AMB was given intravenously twice weekly (2.5mg/kg). Patients received antifungal prophylaxis from the start of conditioning until immune recovery (CD3 recovery >300cells/mL; CD4 > 200 cells/mL). Primary endpoint was the incidence of possible, probable and proven IFD. Secondary aims were the evaluation of toxicity of antifungal prophylactic treatment and survival assessed up to 180 days post-transplantion. Descriptive statistics were performed. Survival curves were measured using the Kaplan Meier method.

Results:
A total of 162 pediatric patients with transplant indications at high-risk for IFD received L-AMB. Incidence of breakthrough IFD within 180 days after allogeneic HSCT was 9.3% (15/162). The 15 cases comprised of four invasive yeast infections (2.5%) and 11 mold infections: three probable IFD (1.9%) and eight possible IFD (4.9%). All yeast infections were *candida* bloodstream infections. All probable and possible invasive fungal infections were localized in the lung. Most patients receiving L-AMB did not have any side-effects (125/162, 77.2%). Most common adverse effect was the need for potassium supplementation due to hypokalemia in 13% of patients. A hypersensitivity reaction to L-AMB occurred in ten patients (4.6%); seven patients (4.3%) experienced severe renal toxicity. Of the IFD patients, 11/15 (73%) survived. There was no IFD-related mortality in this cohort.

Conclusions:
High-risk children undergoing allogeneic HCT have a significant risk of breakthrough IFD under antifungal prophylaxis, but IFD-related mortality is low. L-AMB as antifungal

prophylaxis is well tolerated with manageable side effects. It should therefore be considered as an alternative option in high-risk pediatric allogeneic HCT recipients.

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Successful Treatment of Mucormycosis with Adjunctive Sargramostim and Hyperbaric Oxygen in Children and Adolescents with Acute Leukemia

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Objectives: Mucormycosis is a devastating invasive fungal disease associated with disfiguring morbidity and high mortality in patients with hematological malignancies. Current conventional antifungal therapy is limited in efficacy and associated with dose-limiting toxicity. Whether additional use of either immunomodulation and/or hyperbaric oxygen (HBO2) may improve outcome from mucormycosis in this population is uncertain.

Methods: Diagnosis was established by EORTC-MSGERC definitions. We implemented sargramostim (rhuGM-CSF) as adjunctive therapy in treatment of mucormycosis in 4 pediatric patients with acute leukemia and HBO2 in three. Standard antifungal therapy consisted of initial administration of liposomal amphotericin B at 5-7.5 mg/kg/d followed by isavuconazole or posaconazole. Surgical debridement was performed in the management of sino-orbital or cutaneous disease. rhuGM-CSF was administered at 250 mcg/m^2/dose daily while neutropenia and at 100 mcg/m^2/dose 3x weekly when not neutropenic. HBO2 was administered once or twice daily at 2.0ATA for 60 minutes.

Results: Patients ranged in age from 1-14 years and all were male. All patients had proven mucormycosis. Disease states included sino-orbital, pulmonary, and disseminated mucormycosis. GM-CSF was administered until completion of chemotherapy for acute leukemia. HBO2 was continued until pulmonary lesions decreased and sinus tissue demonstrated granulation. Treatment duration ranged from 3 to 12 months. All 4 patients are currently alive with median follow-up of 40 mos. (8-132 mos.) Two patients have achieved a complete response, while two other patients are responding with evidence of complete response. In a review of previously reported cases of pediatric mucormycosis, overall mortality was 36% (Zaoutis *et al*, 2007) and 32% (Otto *et a*l, 2019). Given the survival and favorable response of these 4 patients with acute leukemia, the potential role of rhuGM-CSF and HBO2 warrants further investigation alone or in combination.

Conclusion: The survival and favorable response of these 4 patients with acute leukemia and mucormycosis warrant further investigation of the potential role of rhuGM-CSF and HBO2 as adjunctive modalities to conventional antifungal therapy.

Case number	Age (Y)/ Sex	Stage of disease	Organism	Neutropenic	Treatment refractory prior to GM-CSF	Duration of GM-CSF (months)	Number of HBO2 treatments (2.0ATA, 60 mins each)	Total duration of therapy (mos.)	Follow-up (mos.)	Outcome
1	1/M	Disseminated	*Rhizopus* sp	+	+	1	26	3	132	Complete response
2	3/M	Fungal rhinosinusitis and pneumonia	*Rhizopus* sp and *Aspergillus* spp	-	+	3	40	12	69	Complete response
3	4/M	Sino-orbital-cerebral and pulmonary mucormycosis	*Lichtheimia corymbifera*	-	+	ongoing	86	8	8	Ongoing treatment with currently complete response
4	14/M	Pulmonary and hepatosplenic mucormycosis	*Rhizopus arrhizus*	+	+	ongoing	*	11	11	Ongoing treatment with currently complete response

*none

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Invasive aspergillosis in children with non-hematological diseases: results of a multicenter study

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Objectives: The article presents a multicenter study of invasive aspergillosis (IA) in children with non-hematological diseases

Materials & Methods: retrospective review of Saint-Petersburg register (1998-2023) of patients with IA. Diagnosis of IA was made according to EORTC/MSGERC criteria (2020).

Results: 42 children with IA with of non-hematological diseases were included, which accounted for 21% of all pediatric patients with IA (n=205). These fungal infections were diagnosed in children aged 1 to 17 years (median – 7.5 years), in boys – 52. The main background diseases were of primary immune deficits (38%) and oncological diseases (38%), but invasive aspergillosis developed with any other pathology (14%). Main risk factors were similar to risk factors in hematological pediatric patients: severe neutropenia (40%), lymphocytopenia (40%), the use of systemic glucocorticosteroids (38%) and immunosuppressants (38%). The main localization of invasive aspergillosis were the lungs. The predominant etiological agents were *A.fumigatus* (24%), *A.flavus* (12%), *A.niger* (12%). Based on the EORTC/MSG (2020) criteria, «proven» IA was diagnosed in 18% of patients «probable» - in 82%. Antifungal therapy received by 92% of patients, voriconazole – 80%. The overall 12-week survival rate of patients was 63%. The use of voriconazole significantly improved 12-week survival (p=0.02), the use of combined antimycotic therapy did not affect the survival rate (p=0.86). All children who did not receive antifungal therapy (8%) had a fatal outcome.

Conclusions: IA with of non-hematological diseases developed mostly in patients with prolonged neutropenia and lymphocytopenia. The predominant etiological agents were *A.fumigatus.* Antifungal treatment was used in 92% patients, voriconazole – 80%. The overall survival of patients in 12-weeks was 63%. The use of voriconazole significantly improved 12-week survival (p=0.02), the use of combined antimycotic therapy did not affect the survival rate (p=0.86).

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Fatal invasive aspergillosis in a child with chronic granulomatous disease

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Objectives: Patients with chronic granulomatous disease, a primary immunodeficiency, experience granulomatous complications and recurrent life-threatening opportunistic bacterial and fungal infections. We have reported a case of invasive aspergillosis in an 8-year-old boy with chronic granulomatous disease, who presented with pleural effusion and pneumonia, cerebral venous sinus thrombosis, and unusual skin lesions (Fig. 1) caused by Aspergillus fumigatus (Fig. 2).

Materials & Methods: It is noteworthy that treatment was immediately started according to the direct examination results. Due to the MRI findings suggestive of brain abscesses and brain edema, empirical amphotericin and voriconazole were started. The *in vitro* antifungal susceptibility tests (AFST) of *A. fumigatus* utilized the microbroth dilution method of the Clinical and Laboratory Standards Institute (CLSI) M38-A2 protocol for five antifungal agents including; voriconazole, itraconazole, fluconazole, caspofungin, and amphotericin B, that the minimum inhibitory concentrations (MIC) of antifungal agents were 0.25, 1, 16, 0.125, and 4 mg/L, respectively.

Result: Voriconazole and caspofungin were shown to be the most potent antifungal drugs against this A. fumigatus strain. On the other hand, the IDSA protocols recommend voriconazole as the initial therapy of invasive aspergillosis in most patients. So, in response to the results of antifungal susceptibility testing, treatment was switched to voriconazole at the dose of 100 mg twice a day (taken every 12 h), and interferon-γ subcutaneously (1-3 doses in even days) was introduced. Unfortunately, the patient passed away due to cerebral venous sinus thrombosis (CVST), and intracerebral hemorrhage (ICH) following increased intracranial pressure (ICP) after 1 month.

Conclusion: In conclusion, we have reported a case in which a patient who came with pleural effusion and pneumonia in the chest x-ray, CVST, and unusual skin lesion caused by *A. fumigatus* expired after one month. Our report suggests the importance of early diagnosis in children presenting with invasive fungal infections particularly, those involving the central nervous system.

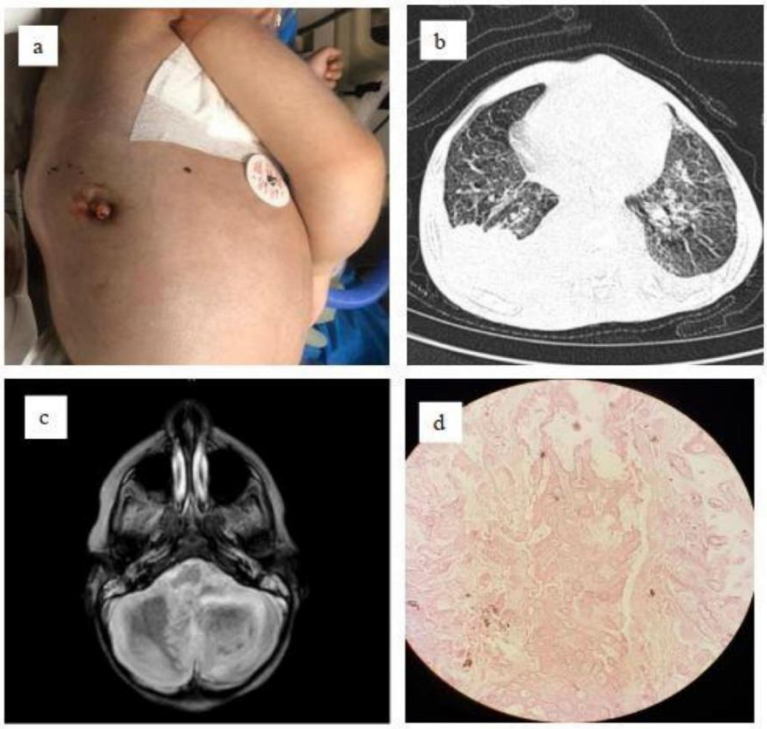

Figure 1. a: Cutaneous swelling and granuloma formation on the upper part of back consistent with abscess formation. **b:** Computerized tomography findings including pulmonary involvement with Pleural effusion. **c:** MRI of the brain showed lesion in left cerebellar hemisphere and a frontal brain white matter hemorrhagic lesion. **d:** Numerous fungal hyphae in the skin biopsies with hematoxylin and eosin staining. ×40.

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Unveiling the Emerging of Fluconazole-Resistant Candida Albicans in Preterm Neonate with Acute Kidney Injury: A Growing Challenge at Vajira Hospital

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Objectives: In order to raise awareness about the increasing resistance of Candida albicans to fluconazole, and its significant implications, particularly for children with renal diseases.
Materials & Methods: The patient's medical records were meticulously reviewed, thoroughly examining the clinical presentations, laboratory findings, culture results, and summarizing the data on drug susceptibility. A comprehensive report was then generated based on these findings.
Results: A case study was conducted on a preterm female Thai newborn with a gestational age of 25 weeks. The patient had a normal labor process with Apgar scores of 4 and 7 at 1 and 5 minutes, respectively. The birth weight was 650 grams. Immediately after birth, the newborn experienced dyspnea and desaturation. Chest X-ray examination revealed a ground glass appearance in both lungs, leading to a diagnosis of respiratory syndrome. The infant received treatment with surfactant therapy.
On the 30th day of life, the patient developed pneumonia with sepsis. Subsequent sputum analysis identified the presence of carbapenem-resistant Acinetobacter baumannii and Stenotrophomonas maltophilia. Treatment was initiated with meropenem, colistin, and levofloxacin. After one week, the patient's creatinine levels began to rise, accompanied by oliguria. Peritoneal dialysis was performed. However, the patient continued to experience fever and thrombocytopenia. A urine culture revealed a count of >105 Candida albicans, likely due to acute kidney injury and the nephrotoxic effects of multiple medications. Considering that C. albicans had previously shown full susceptibility to fluconazole, a risk-benefit analysis was conducted, and intravenous fluconazole was initiated as an antifungal treatment.
Despite receiving fluconazole treatment for one week, subsequent urine cultures continued to show the presence of C. albicans. The patient underwent abdominal, head ultrasonography, echocardiography, and retinal examinations, all of which showed no evidence of candida infection. Consequently, fluconazole-resistant C. albicans was considered the primary differential diagnosis, and the treatment was switched to intravenous amphotericin B. After three days, urine cultures no longer detected any organisms. Subsequently, the drug susceptibility test for C. albicans revealed a minimum inhibitory concentration (MIC) of 4 µg/mL for fluconazole, 0.5 µg/mL for amphotericin B, and 0.015 µg/mL for micafungin.
In this case, intravenous amphotericin B was continued for 14 days after the absence of any growth in the urine culture. Additionally, the patient underwent peritoneal dialysis for one month.
Conclusions: This case highlights the challenges faced in managing a preterm newborn with multiple complication and the emerging of drug-resistant Candida albicans. The case underscores the importance of accurate microbial susceptibility testing and a multidisciplinary approach to diagnosis.

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Co-infection Invasive Pulmonary Aspergillosis and Pneumocystis jiroveci pneumonia in a B-cell Acute Lymphoblastic Leukemia child

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Objectives: Coinfection between invasive pulmonary aspergillosis and *Pneumocystis jevorici* pneumonia (PJP) is a rare case in children. This case report showed coinfection in an immunocompromised child.
Materials & Methods: His clinical manifestations, laboratory, imaging, and treatment were reviewed. And demonstrated the timeline of diagnosis and treatment in this case.
Results: An 8-year-old Thai boy, presented with a two-week history of anemia and abnormal purpura in both legs. Subsequent medical examinations revealed bi-cytopenia with blast cells, and flow cytometry confirmed B-cell acute lymphoblastic leukemia (B-cell ALL). The patient received chemotherapy in accordance with Thai-POG guidelines. Following this, he developed febrile neutropenia and was initially treated with intravenous meropenem and amikacin, which yielded no improvement. After one week of febrile neutropenia, a computed tomography (CT) scan of the chest was performed, revealing multiple nodules in the right lung. Bronchoalveolar lavage (BAL) demonstrated a positive galactomannan level of 1.12 ng/mL. The patient was subsequently administered voriconazole, with therapeutic drug monitoring showing a level of 4.7 ug/mL. However, he developed progressive dyspnea with hypoxia, and a chest X-ray revealed a bilateral ground glass appearance in the lower lungs. Further analysis of sputum using Giemsa and silver staining detected PJP. Treatment for PJP consisted of trimethoprim-sulfonamides and prednisolone in combination with voriconazole. Within two days of commencing treatment, the patient's clinical symptoms improved. The total duration of trimethoprim-sulfonamides and prednisolone administration was 21 days, followed by continued trimethoprim-sulfonamides prophylaxis. Voriconazole was maintained until imaging studies confirmed resolution (approximately 6 months).
Conclusions: This case report highlights the coinfection between invasive pulmonary aspergillosis and *Pneumocystis jevorici* pneumonia (PJP) in a child, emphasizing the complexities involved in diagnosis and management. It underscores the significance of a multidisciplinary approach, timely identification, and appropriate therapeutic interventions in achieving successful outcomes in such cases.

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Invasive Aspergillosis in Hospitalized children in a TertiaryCare Hospital of New Delhi

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Background:

Diseases caused by *Aspergillus* species are numerous, ranging from allergic manifestations, to pulmonary saprophytic or locally destructive disease, to the almost uniformly fatal infection that disseminates from the lungs to distal organs in severely immunocompromised persons.

Material and methods:
Chest computed tomography as well as bronchoscopy with bronchoalveolar lavage (BAL) in patients with suspicion of pulmonary invasive aspergillosis (IA) were done.In most cases it was difficult to get deep samples due to high risk to the patients and hence microscopy and culture were not elicitable. Serum and BAL galactomannan and B, D-Glucan were the main stay for diagnosis. PCR was considered in conjunction with other diagnostic tests.
Results:
50 hospitalised children suspected of fungal infection were studied.Aspergillosis was diagnosed in 42% of our patients. There were 19 probable cases of Aspergillosis and 2 cases of mixed infection with *Aspergillus* with *Pneumocystis*. Twenty out of the 21 patients of Aspergillosis had chest X ray findings and CT-Chest showed ground glass opacity in 4 patients. Patients with aspergillosis associated risk factors were hospital stay >7 days, venous access device, prior use of antibiotics in all patients and catheterization in 90.5% patients (p=0.053). Presenting complaints were fever in 100% patients and cough 47.6% patients and chest infiltration was seen in 47.6% patients and abnormal USG present in 8patients (p=0.023). Most patients 76.2% were treated with Liposomal Amphotericin B but 33.3% patients expired.

Conclusion:
Early diagnosis of invasive fungal infection (IFI) is quite difficult leading to high morbidity and mortality. There is an urgent need for reliable screening methods facilitating timely diagnosis and treatment.

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Mucoral monitoring of the cases with SARS-CoV-2 in Northwest of Iran

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Mucoral monitoring of the cases with SARS-CoV-2 in Northwest of Iran

Objectives: During the surge of delta type Covid-19 in Iran, the rate of invasive mucormycosis considerably increased as a cluster in society. Covid-19 associated mucormycosis (CAM) immerged as rhino-sino-facial, rhino-sino-cerebral and acute sinusitis. The present report includes demographic, clinical and laboratory diagnostic information about new emerged CAM in Northwest of Iran.

Methods: During three months, from August to October 2021, about 65 cases with the clinical manifests suspected to mucormycosis and a history of recent severe Covid-19 and corticosteroid therapy with Dexamethasone were studied. Our subjects were the clinical specimens including 31 nasal biopsies, 24 paranasal sinus biopsies, 2 facial and palate biopsies, skin and sutures, one each. Also two samples of broncoalveolar lavage were used for investigating fungi in respiratory tract.

Results: Our findings of laboratory examinations showed 55 (84.6%) Mucoral elements. The suspected cases of CAM showed clinical manifests including acute sinusitis, rino-sino- cerebral 25(38.5%) , rino sino orbital 7(10.8%) , and sino facial 3(4.6%), involvements. The culture and identifications resulted *Rhizopus oryzae* as the most frequent isolate (44.6%) and *Candida* yeasts (albicans and non albicans *Candida* species) 6.2% and 7.7% respectively. *Aspergillus* species were detected 5 (7.7%) as well. A considerable number of cultures, 20 (30.8%) could resulted no growth for any fungi.

Conclusions: As a conclusion, delta type Corona virus causing a considerable increased invasive Mucormycosis in the recorded Covid-19 cases in the north west of Iran, Although, opportunistic candida and aspergillus were identified in lower frequencies as well.

Table: Clinical and Laboratorial data of northwest Iranian Black fungi infections

Code	Clinical Specimen	Reference	Predisposing Factor	Clinical Manifest	Direct Examination	Culture Identification
2441	Nasal	ICU	Diabetes Melitus	Sinusitis	Mucoral	*R. oryzae*
2152	Nasal	ICU	None	Sinusitis	Mucoral	*R. oryzae*
2152	Nasal	ENT	None	Necrosis	Filamentous	*A. flavus*
2162	BAL	Pulmonology	TB	Infiltration	Yeast	*C. albicans*
2163	Sinus	Nephrology	Kidney Graft	Sinusitis	Mucoral	*R. oryzae*
2166	Nasal	Nephrology	Diabetes Mellitus	RSCerebral*	Mucoral	*R. oryzae*
2167	Nasal	Neurology	None	RSCerebral	Mucoral	*R. oryzae*
2172	Nasal	Neurology	None	RSCerebral	Mucoral-Yst	*C. albicans*
2175	Nasal	Neurology	None	RSCerebral	Mucoral	*R. oryzae*
2176	Nasal	ENT	Diabetes Mellitus	RSOrbital**	Mucoral	*R. oryzae*
2177	Nasal	Neurology	None	RSCerebral	Mucoral	*R. oryzae*
2178	Nasal	Pulmonology	None	RSOrbital	Mucoral	*R. oryzae*
2183	Nasal	Pulmonology	None	RSCerebral	Mucoral-Yst	*Non alb Candida*
2184	Nasal	Neurosurgery	None	RSCerebral	Mucoral	*R. oryzae*
2185	Nasal	Neurosurgery	None	RSCerebral	Mucoral	*R. oryzae*
2189	Nasal	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2193	Nasal	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2198	Nasal	Neurosurgery	None	RSCerebral	Mucoral	*R. oryzae*
2203	Nasal	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2209	Nasal	ENT	None	Sinusitis	Filamentous	*A. flavus*
2213	Palate	Surgery	None	NOral	Yeast	*Non alb Candida*
2215	Sinus	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2228	Sinus	Neurosurgery	None	RSCerebral	Mucoral	*R. oryzae*
2229	Facial	ENT	Diabetes Mellitus	SFacial	Mucoral	*R. oryzae*
2242	Sinus	ICU	None	Sinusitis	Mucoral	*R. oryzae*
2243	BAL	Pulmonology	None	Dyspnea	Yeast	*C. albicans*
2244	Sinus	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2247	Sinus	Neurosurgery	Diabetes Mellitus	RSCerebral	Mucoral	*R. oryzae*
2251	Sinus	ICU	None	Sinusitis	Mucoral	*R. oryzae*
2253	Sinus	ENT	Diabetes	Sino-Orbit	Mucoral	*R. oryzae*
2264	Nasal	Pulmonology	None	Sinusitis	Mucoral	*R. oryzae*
2266	Sputum	ICU	None	Dyspnea	Yeast	*C. albicans*
2267	Sinus	Pulmonology	None	Sinusitis	Filamentous	*A. flavus*
2274	Nasal	Neurosurgery	None	Sinusitis	Mucoral	*A. niger*
2277	Nasal	ICU	Diabetes Mellitus	Sinusitis	Mucoral	*R. oryzae*
2283	Sinus	ENT	None	Sinusitis	Mucoral	*Non alb Candida*
2292	Sinusitis	Neurosurgery	None	SFacial	Mucoral	*R. oryzae*
2294	Sinus	ENT	None	Sinusitis	Yeast	*Non alb Candida*

2304	Nasal	Neurosurgery	None	RFacial***	Mucoral	*R. oryzae*
2306	Sinus	ICU	None	Sinusitis	Yeast	*Non alb Cand*
2319	Nasal	ENT	None	Sinusitis	Mucoral	*R. oryzae*
2320	Sinus	ENT	None	Sinusitis	Mucoral	*No groth*
2322	Skin	Infectious	None	Ulcer	Mucoral	*R. oryzae*

*Rhino-sino-cerebral, ** Rhino sino orbital, ***Rhino facial

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Clinical features and risk factors of invasive mold infections associated with COVID-19: a single center experience

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Objectives: Invasive mold infections (IMI) have become common in patients with COVID-19 pneumonia, which are difficult to diagnose and treat, have a high mortality rate. The objective of this study was to determine the causative frequency, clinical characteristics and risk factors of invasive infections associated with COVID-19.

Materials & Methods: In this prospective study, patients treated for COVID-19 pneumonia in intensive care units with invasive mold infection were included in the study group. A randomized control group was determined from patients with COVID-19 pneumonia and no secondary infection (bacterial or fungal). Demographics, treatments received and IMI outcomes were compared.

Results

Demographics: There were Twenty patients in the IMI group and 19 patients in the control group. Age, gender and comorbidities in the patients were similar in both groups (p>0.05). The severity of ARDS were not statistically different (p=0.333, p=0.751, p=0.661). While the need for standard O2 and high-flow O2 support did not differ between the groups (p=0.527, p=0.514), three patients (15.8%) in the control group had a history of intubation (p=0.106).

Risk factors for IMI: Steroids were used to treat COVID-19 in 18 (90.0%) patients in the IMI group and in three (15.8%) patients in the control group (p=<0.001). Dexamethasone in eight patients (40.0%) with IMI and one control patient (p=0.021); Methylprednisolone was used in 10 patients (50.0%) in the IMI group and in two (10.5%) patients in the control group (p=0.014). In multivariate analysis, steroid use was identified as the most important risk factor for the

development of IMI (90.0% vs. 15.8%, OR: 25.712, p=0.009). Invasive aspergillosis was observed in a total of 13 patients (65%). Seven patients (35.0%) were CAM (Table 2).

Isolates: A total of 21 agents were isolated in 20 IMI patients (Table 2). *Aspergillus fumigatus* was the causative agent in eight (38.1%) patients and *Rhizopus oryzae* in five (23.8%) patients (Figure 1). In case 12 with acute invasive fungal sinusitis.Antifungal susceptibility tests and galactomannan results are presented in Table 2.

Conclusions: Steroid use is the most important associated factor in the development of IMI. Its use in the treatment of COVID-19 should be minimized as much as possible.

Table 1. Demographic and Clinical Characteristics of the Patients

	Invasive mold infection (n=20)(%)	Control (n=19) (%)	Total (n=39)(%)	P	Multivariate Analysis OR (95% CI), P
Age-median (min-max)	73 (23-89)	62 (47-86)	67 (23-89)	0.050	
Male gender	15 (75.0)	12 (63.2)	27 (69.2)	0.501	
Symptoms of COVID-19					
Fever	9 (45.0)	10 (52.6)	19 (48.7)	0.752	
Cough	16 (80.0)	14 (73.7)	30 (76.9)	0.716	
Respiratory Distress	17 (85.0)	13 (68.4)	30 (76.9)	0.273	
Comorbidities					
At least a comorbidity	16 (80.0)	13 (68.4)	29 (74.4)	0.480	
Diabetes mellitus	11 (55.0)	8 (42.1)	19 (48.7)	0.527	
Hypertension	10 (50.0)	10 (52.6)	20 (51.3)	1.000	
COPD	2 (10.0)	3 (15.8)	5 (12.8)	0.661	
Coronary arter disease	2 (10.0)	3 (15.8)	5 (12.8)	0.661	
ARDS Severity (due to COVID-19)					
Mild	6 (30.0)	9 (47.4)	15 (38.5)	0.333	
Moderate	10 (50.0)	8 (42.1)	18 (46.2)	0.751	
Severe	4 (20.0)	2 (10.5)	6 (15.4)	0.661	
Respiratory support					
Standart O2	11 (55.0)	8 (42.1)	19 (48.7)	0.527	
High-flow O2	6 (30.0)	8 (42.1)	14 (35.9)	0.514	
Non- Invasive mechanical ventilation	3 (15.0)	-	3 (7.7)	0.231	
Invasive mechanical ventilation	-	3 (15.8)	3 (7.7)	0.106	
Immunosuppressive Therapy					
Steroid	18 (90.0)	3 (15.8)	21 (53.8)	<0.001	25.712 (2.257-292.909), 0.009
Dexamethasone	9 (45.0)	1 (5.3)	10 (25.6)	0.005	2.366(0.165-34.010), 0.527
Methylprednisolone	9 (45.0)	2 (10.5)	11 (28.2)	0.017	0.423 (0.029-6.075), 0.527
Immunomodulatory agent	8 (40.0)	1 (5.3)	9 (23.1)	0.010	1.984 (0.137-28.820), 0.616
Tocilizumab	5 (25.0)	1 (5.3)	6 (15.4)	0.182	
Anakinra	3 (15.0)	-	3 (7.7)	0.231	
Prognosis					
Mortality up to 28th day	12 (60.0)	3 (15.8)	15 (38.5)	0.008	

Table 2. Risk factors, clinics and agents of IMI patients

	Respiratory support	High-dose steroid (drug/dose)	Steroid (drug/dose)	Immunomodulator Agent (Drug/totally dose)	Invasive mold infection	Fungal Agent	Sequence analysis	Outcome	Susceptibility Minimum Inhibitor concentration (MIC) (μg/mL)			Galactomannan (ng/mL)
									Voriconazole	Amphotericin B	Caspofungin	
Case 1	NIMV		Dexamethasone 16 mg/day	Anakinra/300 mg	CAPA	Aspergillus flavugatus	JQ776545.1	Death				
Case 2	Standard O2		Dexamethasone 16 mg/day		CAPA	Aspergillus Flavus	MT645322.1	Death	1.00	4.00	2.00	
Case 3	Standard O2				CAPA	Aspergillus flavugatus	MT597427.1	Alive	1.00	2.00	1.50	
Case 4	NIMV		Dexamethasone 8 mg/day		CAPA	Aspergillus niger	MT620753.1	Death	0.38	2.00	0.25	25.0
Case 5	Standard O2		Dexamethasone 6 mg/day	Tocilizumab 100 mg	Rhino-orbital mucormycosis	Cladosporium sllicinum	MF472917.1	Alive	0.25	1.00	1.50	
Case 6	Standard O2		Dexamethasone 8 mg/day		Rhino-orbital mucormycosis	Rhizopus Oryzae	MT540020.1	Alive				
Case 7	Standard O2		Dexamethasone 16 mg/day		Rhino-orbital mucormycosis	Rhizopus Oryzae	MT316366.1	Death				
Case 8	Standard O2		Dexamethasone 16 mg/day		Rhino-orbital mucormycosis	Rhizopus Oryzae	MH715977.1	Death				
Case 9	Standard O2				CAPA	Aspergillus niger	MT597435.1	Alive				
Case 10	HFNO	Methylprednisolone 1000 mg			Rhino-orbital mucormycosis	Rhizopus Oryzae	MT603963.1	Death	0.25	1.00	0.19	
Case 11	Standard O2		Methylprednisolone 60 mg/day	Anakinra/300 mg	CAPA	Aspergillus flavugatus	MT635279.1	Death				
Case 12	Standard O2		Dexamethasone 16 mg/day	Tocilizumab 100 mg	Invasive fungal sinusitis	Lichtheimia Corymbifera/ Aspergillus terreus	MT316349.1/ MT358939.1	Alive	1.00	4.00	2.00	
Case 13	HFNO	Methylprednisolone 750 mg		Tocilizumab 200 mg	CAPA	Aspergillus flavugatus	MT635279.1	Death				
Case 14	HFNO		Methylprednisolone 40 mg/day	Tocilizumab 200 mg	CAPA	Aspergillus terreus	MT358939.1	Alive	1.00	4.00	2.00	2.0
Case 15	NIMV		Methylprednisolone 60 mg/day		CAPA	Aspergillus flavugatus	MT597427.1	Death	0.50	3.00	0.75	
Case 16	HFNO		Methylprednisolone 40 mg/day	Tocilizumab 200 mg	CAPA	Aspergillus flavugatus	MT597427.1	Alive	0.50	1.00	1.50	
Case 17	Standard O2		Methylprednisolone 40 mg/day	Anakinra/300 mg	CAPA	Aspergillus flavugatus	MN634608.1	Alive	0.38	1.00	1.50	4.0
Case 18	HFNO		Methylprednisolone 80 mg/day		CAPA	Aspergillus flavugatus	MH864623.1	Death	0.50	12.00	0.38	
Case 19	HFNO	Methylprednisolone 750 mg			Invasive fungal sinusitis	Aspergillus flavus	MT645322.1	Death	0.50	<32.00	0.50	
Case 20	Standard O2		Methylprednisolone 40		Rhino-orbital	Rhizopus oryzae	LC514313.1	Death	1.00	2.00	1.50	

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Is COVID-19-associated-pulmonary aspergillosis (CAPA) a myth? Frequency of Aspergillus detection from respiratory samples in ICU patients with and without COVID-19.

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Introduction: COVID-19 associated pulmonary aspergillosis (CAPA) is reported to be an emerging disease with a mean incidence of 13.5% (range 3%-35%). Interestingly, most studies on CAPA focused exclusively on COVID-19 patients. Therefore, we performed a study in order to compare the frequency of *Aspergillus* detection from respiratory samples and the rate of pulmonary aspergillosis in intensive care unit (ICU) patients with and without COVID-19.

Materials and Methods: We conducted a prospective observational study between November 2020 and May 2021 at the University Hospital Erlangen. All ICU patient with a microbiological examination of a respiratory specimen were included. All specimens were analyzed by mycological culture, Blancophor® staining, *Aspergillus* PCR (PathoNostics AsperGenius®) and by three galactomannan antigen assays (BioRad Platelia Aspergillus Ag EIA, Vircell Aspergillus Galactomannan Ag Virclia® Monotest, IMMY sona *Aspergillus* Galactomannan Lateral Flow Assay). Serum samples from patients with positive *Aspergillus* assays from respiratory samples were additionally tested for galactomannan and (1→3)-β-D-glucan.

Results: 650 respiratory specimens (BALF 39.5%, bronchial secretion 34.0%, tracheal secretion 26.5%) from 262 patients were included. 125 patients (47.7%) were treated for COVID-19. Surprisingly, the rate of patients with at least one positive *Aspergillus* assay was higher in the non-COVID-19 group (47.4% versus 40.0%, p=0.23). Correspondingly, the rate of positive *Aspergillus* assays was higher in the non-COVID-19 group for the Platelia Aspergillus Ag EIA (8.8% versus 6.5%, p=0.26), the Aspergillus Galactomannan Ag Virclia® Monotest (22.9% versus 14.2%, p=0.01), the sona Aspergillus Galactomannan Lateral Flow Assay (12.5% versus 9.3%, p=0.40) and for serum (1→3)-β-D-glucan (39.1 pg/ml versus 28.7 pg/ml, p=0.13). Only the rate of positive *Aspergillus* PCRs and serum Platelia *Aspergillus* Ag EIA was higher in the COVID-19 group (12.2% versus 8.4%, p=0.22 and 6.3% versus 0%, p=0.01). The mean levels of galactomannan and the mean ct-values of the *Aspergillus* PCR were comparable between COVID-19 and non-COVID-19 patients. These results remained consistent if only BALF samples were analysed.

Based on these results, the patients were classified for CAPA according to the 2020 ECMM/ISHAM consensus criteria. There was no significant difference in the mycological evidence for CAPA between COVID-19 and non-COVID-19 patients (no mycological evidence: 67.5% vs. 63.2%; possible mycological evidence: 23.6% vs. 28.7%; probable mycological evidence: 8.9% vs. 8.1%). Interestingly, in COVID-19 patients with possible or probable mycological evidence for CAPA, antifungal therapy had a negative effect on the outcome, i.e. more patients died if they received antifungal therapy.

Conclusions: The mycological evidence for invasive pulmonary aspergillosis is not more prevalent in COVID-19 patients compared to non-COVID-19 on ICU. Our results question the hypothesis that pulmonary aspergillosis is more common in COVID-19.

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Mucormycosis, COVID & Diabetes: Triad or a Dyad?

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Objectives: To compare the prevalence and to evaluate the risk factors and clinical presentation of patients with confirmed invasive mucormycosis during the preCOVID (Jan 2017-Dec 2019) and the COVID period (Jan 2020 – Dec 2022).

Materials & Methods: This descriptive retrospective study was carried out at the Pondicherry Institute of Medical Sciences, Pondicherry, India during the period of Jan 2017 –Dec 2019(PreCOVID) and Jan 2020 to Dec 2022(COVID era). All patients who were diagnosed as invasive mucormycosis by fungal culture were included and their data were retrospectively analysed by reviewing the medical records. All these patients were diagnosed with microscopy (KOH) and the isolates that grew on Saborauds dextrose agar (SDA) were morphologically confirmed by microscopy using lactophenol cotton blue (LPCB) stain.

Results: During the PreCOVID period out of the 180 samples received from patients, 6(3.3%) were confirmed as invasive mucormycosis, all the 6 isolates were *Rhizopus arrhizus*. In the COVID period, out of 95 samples, 9(9.4%) patients had culture confirmed invasive mucormycosis. The major risk factor during both the period was found to be diabetes mellitus (100%) and 33.3% (1/3rd) of them were ketoacidotic at initial presentation, 22.2% of patients in our study were newly diagnosed diabetics. Clinically, 50% of the patients in the preCOVID era presented with rhino-orbital mucormycosis followed by sino-nasal (33%) mucormycosis. During the COVID period 44.4% of patients presented with sino-nasal mucormycosis followed by pulmonary mucormycosis (33.3%). Treatment with Amphotericin B was initiated to all patients (100%) during the preCOVID period, however during the COVID period majority of the patients were referred to nodal centre for further management. During the COVID era only 4 (44.4%) patients were tested positive for the SARS CoV 2 virus by RTPCR, and these patients had mild COVID disease and were not on steroids.

Conclusions: The prevalence of culture confirmed mucormycosis was found to be high in the COVID era at our tertiary care centre. Pulmonary mucormycosis (33%) was noted only during the COVID era, however they were independent of COVID disease as all these patients were never tested positive to SARS CoV 2 virus neither at presentation nor in the past. Diabetes mellitus was the single most common risk factor among patients in both the period. In a country with a high prevalence of diabetes mellitus there should be a high index of suspicion of mucormycosis irrespective of COVID status in patients with rhino-orbital or pulmonary symptoms.

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COVID-19 associated invasive candidiasis: Results of a Multicenter Study

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Objectives: To study of CAIC in actual clinical practice in Russia.
Materials & Methods: Prospective, multi-center study included of 86 adult patients with CAIC from the 6 medical facilities of the St.Petersburg. (2020-2023 yy).. For diagnosis of invasive candidiasis we used criteria EORTS/MSGERC, 2020.
Results: The age of the patients was 29-96 (median – 61.0÷14.6) years, women – 51%. The median time from detection of Sarc-Cov-2 to diagnosis of CAIC was 16÷15 (0-52) days. The median time in the intensive care unit was 15.08÷13.08 (5-63) days. The risk factors were CVC for more than 10 days (OR = 32 [10-59]), abdominal surgical treatment performed in the previous 2 weeks (OR = 8,2 [1,6-30,3]), bacteremia (OR = 9 [4,0-19,6]), prolonged (median 11÷10 day) pulmonary ventilation (OR = 10,9 [5-22]), hemodialysis (OR = 10 [2,5-50,8]). Previous or concomitant bacteremia was detected in patients with COVID-IC in 83%. Signs and symptoms were non-specific: fever – 59%, hypothermia – 10%, renal failure –33%, and liver failure – 23%. The median SOFA score at the start of candidemia was 7. The main etiology agents were *C. albicans* (41%), *C. auris* (23%), *C. parapsilosis* (8%), *C. guilliermondi* (7%), *C. glabrata* (5%), *C. tropicalis* (3%). A combination of different *Candida* species was found in 3 patients: *C.tropicalis* + *C.parapsilosis* + *C. auris*; *C.tropicalis* + *C. auris*; *C. albicans* + *C.parapsilosis*. CAIC was an autopsy finding in 4 patients who did not receive antifungal therapy. Empirical therapy was used in 27% of CAIC patients: fluconazole - 93%, echinocandin - 7%. The majority (73%) of CAIC patients received antifungal therapy after laboratory confirmation of the diagnosis of IC (fluconazole – 47% and voriconazole – 25%, Echinocandin - 26%, AmB – 2%). The 30 days overall survival rate was 46%.
Conclusions: The main risk factors were long-term (10 days) CVC (OR = 32), long-term (11 days) mechanical ventilation (OR = 10,9), surgery in the last 2 weeks (OR = 8,2), bacteremia (OR =9), hemodialysis (OR = 10). The main isolated species were *C. albicans* (41%) followed by *C. auris* (23%). The 30 days overall survival rate was 46%.

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Invasive aspergillosis in adult patients with COPD

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Objectives: Analysis of risk factors, aetiology, clinical features, treatment and survival rates in patients with chronic obstructive pulmonary disease (COPD) and aspergillosis (IA).
Materials & Methods: In a prospective study 60 patients with COPD and «proven» or «probable» IA (EORTC / MSG, 2019) were included, median age - 62 (31 - 86), males - 73%. The control group included 60 patients with COPD without IA, 64 (36 - 86) years, males 60%. EORTC/MSGERC 2020 criteria were used for IA diagnosis.
Results: Our study showed that IA more often develops against the background of moderate and severe COPD (GOLD III-IV) – 67%. Other significant comorbid conditions were hematological diseases (53%), severe bacterial infection (38%), COVID-19 pneumonia (35%), and decompensated diabetes (22%). The main risk factors for the IA development in patients with COPD were prolonged (>10 days) lymphocytopenia (38% vs 5%), (OR=11.811 [3.309-42.154], p=0.00001), use of systemic corticosteroids (67% vs 15%), (OR=11.333 [4.659-27.570], p = 0.00001).
The main localization of IA was lungs - 98%, paranasal sinuses involvement noted in 6%, and dissemination of infection with ≥2 organs - 13%. Most frequent clinical symptoms were fever (87%), and cough (86%). Typical signs of IA severe were respiratory failure (75%), local pain syndrome (27%), and haemoptysis (25%). Typical CT features were areas of consolidation (80%), hydrothorax (46%), and foci of destruction (41%).
The main causative agents of IA were *A. fumigatus* (41%), *A. niger* (31%), and *A. flavus* (14%). The most commonly used drug was voriconazole (78%). The overall 12 weeks survival rate of patients with COPD and IA was 72%.

Conclusions: In patients COPD, the main risk factors for IA development were prolonged lymphocytopenia (38%), steroid use (67%). Causative agents are *A. fumigatus* (41%), *A. niger* (31%) and *A. flavus* (14%). The main sites of infection were lungs (98%). Respiratory failure (75%), haemoptysis (25%) are clinical characteristic. Typical radiological signs were areas of consolidation (80%), hydrothorax (46%), and foci of destruction (41%). The overall 12-week survival rate of COPD patients with IA was 72%.

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Does antiviral and immunomodulatory treatment of COVID-19 influence the outcome of patients with COVID-19-associated pulmonary aspergillosis?

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Objectives:

The incidence of pulmonary aspergillosis increased during COVID-19 pandemic along with increased risk of death, duration of hospitalization, ICU burden and costs. Risk factors identified for COVID-19 associated pulmonary aspergillosis (CAPA) are: age, mechanical ventilation and use of tocilizumab. If antiviral treatment (as remdesivir) and immunomodulatory treatments (as anakinra and tocilizumab) used for COVID-19 influence the outcome of CAPA is not established yet. The aim of our study was to asses if the outcome of CAPA is influenced by COVID-19 treatment.

Materials & Methods:

We prospectively screened for CAPA all patients with severe/critical forms of COVID-19 admitted in The University Hospital of Infectious Diseases Cluj-Napoca, Romania, (a COVID-19 dedicated hospital from March 2020 till Dec 2022) in case of respiratory failure despite standard of care treatment. It includes thoracic CT scan, sputum / tracheal aspirate / bronchoalveolar lavage culture and serum / BAL galactomannan (Platelia *Aspergillus* Ag Bio-Rad and *Aspergillus* galactomannan Ag Virclia Monotest -Vircell on VirClia Thunderbolt platform). CAPA was defined according with 2020 ECMM/ISHAM consensus criteria. All patients were treated for severe/critical COVID-19 (according to WHO criteria) with: oxygen supplementation / mechanical ventilation, dexamethasone 6mg/day, some with anakinra 100mg/day after a loading dose of 200mg, or with Tocilizumab 8mg/Kg/dose or with both. After the diagnosis of CAPA was established, all patients received Voriconazole treatment. Baseline characteristics between patients during follow up were compared with rank-sum tests, χ^2 tests and Fischer's exact test, as appropriate. Univariate was made targeting the risk factors of death.

Results:

From 2764 severe or critical COVID-19 patients admitted in our hospital from the beginning of pandemic till 31 Dec 2022, 45 patients (32 with critical COVID-19) were diagnosed with possible/probable CAPA (1.62%). No influenza co-infection was detected. 14 patients had positive cultures and galactomannan and 31 had positive cultures (3 with negative galactomannan and 28 without galactomannan performed). For patients with CAPA: the average age was 71.6 (min 36, max 88), 27 were men, 44 had comorbidities, the median duration of hospitalization was 22 days (min 4 max 87) and the time from admittance to diagnosis of CAPA was in average 13 days (min 3, max 51). The pulmonary involvement on CT scan was around 70%. Before the diagnosis of possible CAPA all patients were treated with dexamethasone (median 12 days), 18 were treated with remdesivir (median 8 days), 1 was treated with tocilizumab, 19 were treated with anakinra (median 13 days) and 7 with both tocilizumab and anakinra. 37 patients (82%) died. Age over 57 years old, IL6 >24.8 pg/ml, SOFA score>7, APACHE 2 >15 and use of remdesivir were significant associated with death ($p<0.05$). Treatment with tocilizumab, anakinra or both were not associated with risk of death ($p=0.57$, $p=0.92$).

Conclusions:

CAPA is a complication of severe and critical COVID-19 with high risk of mortality. The risk of death was not influenced by immunomodulatory treatment used for COVID-19 but it was found higher in patients treated with remdesivir. Further studies should address the question

if antiviral and immunomodulatory treatment for COVID-19 influence the outcome of patients with CAPA.

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Sino-nasal mycobiome characteristics of COVID-associated mucormycosis and severe COVID-19: a prospective comparative study

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Objectives:
COVID-19 associated mucormycosis (CAM) is a relatively recent entity, thought to result from a complex interaction between the viral disease, diabetic status as well as steroid induced immunosuppression. It is not known how local nasal micro-environmental changes in response to these factors and if it has a role in the devastating disease. We aimed to compare the upper respiratory tract mycobiome of healthy adults and severe COVID-19 patients and compare it with those suffering from CAM.
Materials & Methods:
This prospective observational study was conducted in the dedicated COVID-19 wards of JPNATC, AIIMS, New Delhi. Consecutive patients with CAM (group 1, n=10), and severe COVID-19 (group 2, n=) were included. Those patients who had undergone surgical procedure for mucormycosis or received anti-fungals for > 5 days were excluded. The control group (group 3, n=10) included healthy adults. Sampling was done at one time point in group 1 and 3. In the COVID-19 cohort sampling was done at baseline and at 7 days or discharge (whichever was earlier). The upper respiratory sample was obtained with a cotton tip by rubbing the midle meatus under direct vision. In patients with CAM sampling was done from the unaffected side. For the mycobiome analysis, fungal DNA was extracted by using the PowerSoil DNA isolation kit (MO BIO Laboratories, Solana Beach, CA) according to the manufacturer's protocol. PCR was performed with the primers specific to fungal ITS1 region. Sequencing was performed with the PacBio RS II/Oxford Nanopore technologies using commercially available DNA Sequencing Kit.
Results:
A total of 40 upper respiratory samples from 30 patients were taken for processing. The demographic features and outcomes are mentioned in Table 1. Mycobiome analysis was possible in 10 CAM patients, nine COVID-19 patients (at two time points) and seven healthy controls. The major phylum in the group 2 and group 3 patients was *Ascomycota*, while *Basidiomycota* was abundant in group 1. *Curvularia* sp was the most common fungi identified in the group 2 and 3. The other species sequenced included *Candida* sp, *Cladosporium* sp, *Metschnikowa* sp. While in group 1 *Malassezia* sp predominated followed by *Wallemia* sp. Figure 1 depicts the mycobiome as well as the alpha-diversity indices (Shannon and Chao1) of the various groups.
Conclusions:
The mycobiome of CAM patients is significantly ($p<0.05$) different than that of healthy subjects and severe COVID-19 patients with a lower alpha diversity index. The mycobiome of healthy adults did not differ from the severe COVID-19 patients at both time points. Further studies should explore the sequential changes in mycobiome of severe COVID patients developing CAM.

Table 1: Demographic profile, comorbidities, and outcome of the included patients

Parameter	Mucormycosis (n=10)	Severe COVID (n=10)	Healthy (n=10)
Age (mean (SD)) years	51.2 (12.9)	61.7 (13.9)	30.1 (5.9) years
Sex (male) %	90%	60%	90%
Comorbidities (n%): Diabetes mellitus Hypertension Malignancy Lung Disease	 7 (70%) 0 0 0	 2 (20%) 2(20%) 2(20%) 1(10%)	 0 0 0 0
Severe COVID (n%)	1 (10%)	10 (100%)	-
Areas involved (n%) Rhino Orbit Cerebral	 10(100%) 6(60%) 2 (20%)	-	-
Use of steroids (n%)	5 (50%) (before diagnosis of mucor)	10(100%)	0
Outcome (n%) Discharge Death	 5 (50%) 5 (50%)	 8 (80%) 2(20%)	-

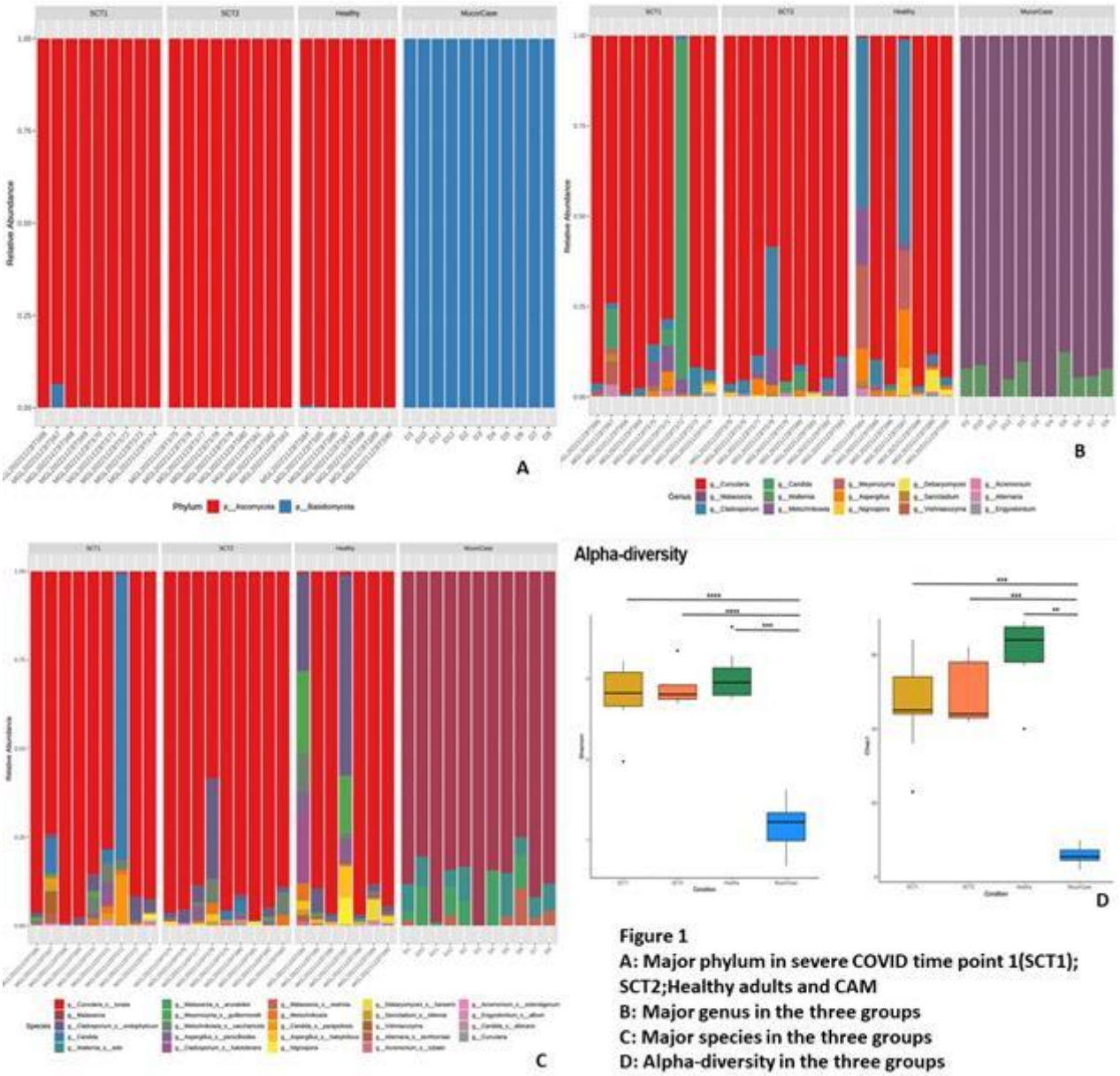

Figure 1
A: Major phylum in severe COVID time point 1(SCT1); SCT2;Healthy adults and CAM
B: Major genus in the three groups
C: Major species in the three groups
D: Alpha-diversity in the three groups

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IMMUNE RECONSTITUTION IN PLHIV AND HISTOPLASMOSIS

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Histoplasmosis is one of the most frequent opportunistic diseases in patients living with HIV (PLHIV). In those cases which CNS is not affected, antiretroviral treatment (ARV) is recommended to be iniciated as soon as possible. Paradoxical immune reconstitution (PIRD) is known as an exaggerated activation of the immune system that may occur after diagnosis and treatment of the opportunistic disease due to persistence of the antigen, or prior to diagnosis with the viable pathogen (unmasking -UIRD).

Objectives: To evaluate the effect of antiretroviral treatment in PLHIV and histoplasmosis (immune reconstitution syndrome - IRD).

Materials & Methods: A descriptive, retrospective and observational study was carried out. The medical records of 329 patients with histoplasmosis nursed within 2013 and 2022 were analyzed. These were divided into two periods: from 2013 to 2017 (A) and from 2018 to 2022 (B).

The Chi 2 test was used to evaluate statistical differences in relation to the frequency of immune reconstitution events in the two periods studied. A difference of $p < 0.05$ was considered significant.

Results:

A total of 329 cases of histoplasmosis were diagnosed and 302 of them were PLHIV. Thirty-two had IRD (10.6%), 23 were male, the median age was 38 years (24-68) and the median of the LTCD4+ count was 43 cells/µL (0-195). Seventeen were UIRD and 15 were PIRD. Signs, symptoms and evolution presented by the patients under ARVs are described in Table 1.

During period A there were 10/164 patients with IRD (6,1%), while in period B there were 22/138 cases (16%). The difference between both periods was significant (p: 0.0056).

The two most frequent signs and/or symptoms associated with IRD were cutaneous-mucosal lesions and intestinal obstruction. Out of the 12 patients who suffered intestinal compromise, 8 required emergency surgery (75%), and 3 died (25%).

Conclusions:

In the recent years, the number of cases of immune reconstitution syndrome has increased, which could be due to the extensive use of integrase inhibitors. Cases of intestinal involvement were severe enough to ruel out this involvement prior to initiation of ARVs.

Table.1

		UIRD N:17	**PIRD N:15**
	Skin and mucosal lesions	9	3

Symptons related IRD	Fever	1	0
	Polyarthralgias	0	1
	Pulmonary envolvement	3	0
	Lymph node involvement	2	1
	Bowel involvement	2	10
Period A 2013-2017 (N:164)	**Total: (%)**	**3**	**7**
	ARV: NNRTIs + NRTIs	0	6
	ARV: NRTIs + Pis	3	1
	ARV: NRTIs + INTi	0	0
Period B 2018-2022 (N:138)	**Total (%)**	**14**	**8**
	ARV: NNRTIs + NRTIs	2	0
	ARV: NRTIs + Pis	4	0
	ARV: NRTIs + INTi	8	8
death		0	3

ARVs: antiretrovirals; **NNRTIs**: non-nucleoside reverse transcriptase inhibitor; **NRTIs:** nucleoside reverse transcriptase inhibitor; **Pis**: protease inhibitor; **INTi**: integrase inhibitor.

		UIRD	**PIRD**
		N:17	**N:15**
Symptons related IRD	Skin and mucosal lesions	9	3
	Fever	1	0
	Polyarthralgias	0	1
	Pulmonary envolvement	3	0
	Lymph node involvement	2	1
	Bowel involvement	2	10
Period A 2013-2017	**Total: (%)**	**3**	**7**
	ARV: NNRTIs + NRTIs	0	6
	ARV: NRTIs + Pis	3	1
	ARV: NRTIs + INTi	0	0
Period B 2018-2022	**Total (%)**	**14**	**8**
	ARV: NNRTIs + NRTIs	2	0
	ARV: NRTIs + Pis	4	0
	ARV: NRTIs + INTi	8	8
death		0	3

ARVs: antiretrovirals; **NNRTIs:** non-nucleoside reverse transcriptase inhibitor; **NRTIs:** nucleoside reverse transcriptase inhibitor; **Pis:** protease inhibitor; **INTi:** integrase inhibitor.

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Derivation of an immunological biomarker model to predict invasive mould infection more than 10 days before diagnosis

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Objectives: As mortality of invasive mould infections (IMI) remains high, there is a need for improved biomarkers for timely diagnosis and patient stratification. There has been a shift from focus on the pathogen towards focusing on the host and its immune system. Various moulds have been shown to have an impact on host protein and induce T-helper cell (Th) 1, Th2, Th9 and Th17 subsets resulting in potent anti-Aspergillus T-cells effector mechanisms, and in elevated serum levels of cytokines such as IFN-γ and IL-6, IL-8, IL-15 and IL-17. The ECMM study “Immunologic Markers for Treatment Monitoring and Diagnosis in Invasive Mold Infection” aimed to identify circulating immunological markers that could be useful for an early diagnosis of IMI.

Materials & Methods: We collected longitudinal serum samples from 33 cases with probable/proven IMI and matched controls without IMI, and from an independent validation cohort with 20 cases and 20 matched controls. All patients were at-risk haematology patients of which none received mould active prophylaxis or empiric treatment. A panel of 92 circulating proteins involved in inflammation was measured using a targeted proteomics platform (Olink Proteomics AB (Uppsala, Sweden)) and protein concentrations were compared using a linear regression model. Correction for multiple testing was performed by Benjamini-Hochberg method. Based on the significant proteins, a random forest model was created.

Results: The differential abundance analysis on the derivation cohort identified multiple dysregulated proteins at the time of diagnosis and in samples 10 days before diagnosis of IMI, including IL-17C, which showed a consistent higher expression in cases with IMI compared to non-infected controls at both time points. This up-regulation was replicated in the validation cohort at the time point 10 days before diagnosis (p=0.024). We then created a machine learning algorithm including four proteins (TRANCE, TWEAK, EN-RAGE and CCL20) that was able to classify people who will develop IMI with 87% of accuracy based on the samples collected more than 10 days before diagnosis (Figure 1A). However, the model

could not be validated in the validation cohort. The validation cohort did significantly differ from the derivation cohort in some aspects (Figure 1B), including significantly higher rates of neutropenia (85% versus 35%), systemic corticosteroid use (43% vs 12%), and active GVHD (15% versus 2%), but lower rates of T-cell suppressive treatment (20% vs 38%) (Table 1). **Conclusions**: We identified a model to predict IMI more than 10 before diagnosis, however it could not be replicated in validation cohort, which might be due technical or biological differences in both cohorts. Future studies or collaborations are needed to validate our predictive model in at-risk patients that better match our derivation cohort.

Figure 1

Panel A: Random Forest model with the four most deviant proteins from the primary cohort: TRANCE, TWEAK, EN-RAGE and CCL20. Error rate was 12.73%.

Panel B: Illustration that there is batch effect between the two cohorts (batch 1 = primary cohort, batch 2 = validation cohort).

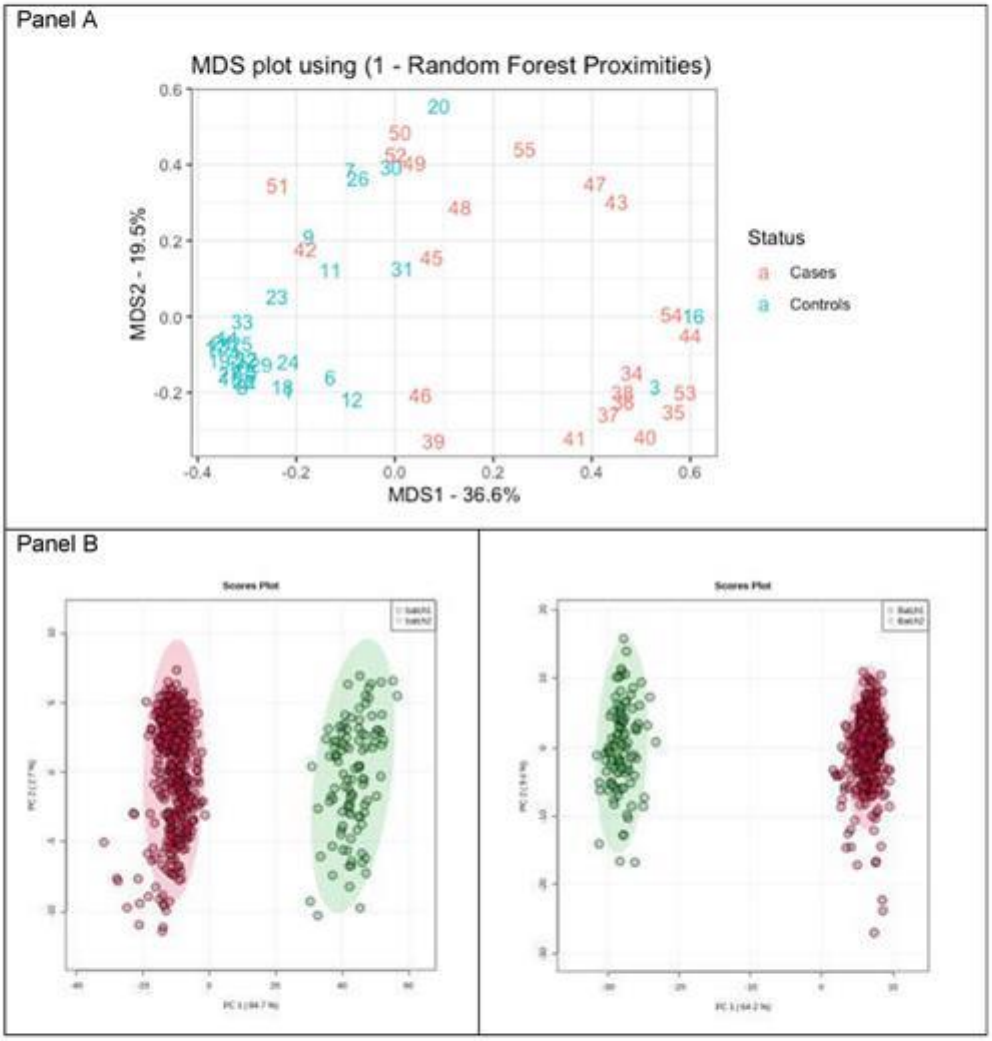

Table 1

An overview of the main characteristics of and differences between the derivation cohort and the validation cohort, including characteristics of all cases and all controls.

		Primary cohort n = 99	**Validation cohort** n = 40	
Characteristic		**Total(%)**	**Total(%)**	**p-value**
Median age		60,0 years	60,1 years	0,23
Female sex		42,4	42,5	0,99
Underlying disease				
	ALL	8,1	27,2	*0,0025*
	AML	57,6	52,5	0,59
	MDS	10,1	7,5	0,63
	Other	24,2	12,5	0,12
EORTC certainty				
	Proven IA	5,1	7,5	0,57
	Probable IA	28,3	42,5	0,1
	No IA	66,7	50,0	0,067
Previous allo-HCT		26,3	30,0	0,65
Neutropenia		35,4	85,0	*<0,001*
High dose steroids		12,1	42,5	*<0,001*
T-lymphocyte inhibitors		38,4	20,0	*0,037*
Active GvHD		2,0	15,0	*0,0029*

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Analysis of unconventional T-cell response in blood during Pneumocystis pneumonia

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Objectives

Pneumocystis pneumonia is a severe infection caused by an original ubiquitous fungus with opportunistic behavior, referred to as *Pneumocystis jirovecii.* The immune response of the host to *P. jirovecii*, particularly the innate one, plays a crucial role in managing and controlling the infection. While the involvement of various components of the innate immune response, such as macrophages, dendritic cells, neutrophils, and natural killer cells, is well-documented in *Pneumocystis* pneumonia, there is limited or no available data on unconventional T-cell populations, namely Mucosal Associated Invariant T (MAIT) cells, γδT cells, and invariant Natural Killer T (iNKT) cells. These cell populations have been more recently described, and their role during *Pneumocystis* pneumonia remains largely unexplored. Therefore, the objective of our study is to investigate whether unconventional T cells exhibit a distinct cellular pattern or profile associated with *Pneumocystis* pneumonia. By doing so, we aim to gain a deeper understanding of the intricate mechanisms involved in the host's inflammatory response to this infection.

Materials & Methods

Patients admitted at Regional University Hospital Centre of Tours (France) from January 1, 2022 to May 31, 2023 were prospectively included. The patients were divided into two groups: the Pj+ group (n=10), consisting of patients with positive *Pneumocystis jirovecii* testing; the Pj- group (n=10), composed of immunocompromised patients with negative *Pneumocystis jirovecii* testing. We aimed to characterize the cellular immune responses, specifically focusing on unconventional T cells, in the blood of hospitalized patients based on their *P. jirovecii* status. Flow cytometry was used to perform this characterization, and patients were selected as long as there were sufficient EDTA blood volumes for the flow cytometry analysis.

Results

Among the cell types examined, only iNKT cells exhibited significantly higher levels in the blood of Pj+ patients in comparison to Pj- patients. Conversely, no significant differences were observed between the two patient groups for MAIT and γδT cells. Additionally, there were no significant differences between Pj+ and Pj- patients regarding CD4 and CD8 T lymphocytes.

Conclusions

The available data, though limited, indicate the potential systemic involvement of unconventional T cells, especially iNKT cells, during *Pneumocystis* pneumonia. Ongoing research project aim to expand upon these findings and provide additional insights by comparison with a third group of patients composed of non-immunocompromised patients with negative *Pneumocystis jirovecii* testing. Furthermore, to address the pulmonary response more specifically, flow cytometry analyses are currently being conducted on bronchoalveolar lavage samples from patients in the thdifferent groups.

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Investigating invasive aspergillosis and neutrophils response against Aspergillus in patients treated with acalabrutinib

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Objectives:
Ibrutinib, first generation drug of Bruton Tyrosine Kinase (Btk) inhibitor, is known to be a risk factor for aspergillosis with increased cerebral damage. Several works report defect in neutrophils impregnated with ibrutinib, probably due to an off-target activity of the drug. Acalabrutinb is a second generation (Btk) inhibitor used since 2017, expected to have fewer side effects due to its greater selectivity on Btk. Nevertheless, similar defect in neutrophils mediated anti-*Aspergillus* response has been shown. So far, only one case of aspergillosis in patient with acalabrutinib has been reported. Here-in, we identified three cases of invasive aspergillosis in patients treated with acalabrutinib for chronic lymphocytic leukemia (CLL) and analysed neutrophil response to *Aspergillus* from patients treated with acalabrutinib.

Materials & Methods:
Cases were collected from three hospitals in Paris, France.
Blood samples from CLL patients were taken before initiation and after one month treatment with acalabrutinib (patients differ from reported cases). Whole blood stimulation with *Aspergillus fumigatus* germinating conidia was performed, and oxidative burst and surface expression of CD11b and CD62L were monitored by flow cytometry. Microbicidal activity of neutrophils was also evaluated. Neutrophils isolated from whole blood, were incubated with *A. fumigatus* germinating conidia overnight and video-microscopy was performed to quantify destruction of conidia.

Results:
First case concerns a 80 years old man who developped an invasive aspergillosis with cerebral lesion one month after introduction of acalabrutinib for CLL. Second case is a 68 years old woman who developped *Pneumocystis jirovecii* and *Aspergillus fumigatus* invasive pneumonia after one month of acalabrutinb for CLL. Last case is a 77 years old man who presented a proven invasive pulmonary aspergillosis after six weeks of acalabrutinb for CLL. All patients died within 90 days after acalabrutinib initiation.
Functionnal analysis indicates that neutrophils from patients treated during one month with acalabrutinib have a defective production of reactive oxygen species, as well as CD62L cleavage when stimulated with *A. fumigatus*. The CD11b integrin, which plays an important role in various neutrophil functions and which we had previously shown to be impacted by ibrutinib, does not appear to be impaired in this situation (Figure 1). Live video-microscopy approach indicates a striking loss of the

microbicidal activity of neutrophils with a germinating conidia killing rate dropping from 34.8% to 4.8%, before and after one month of acalabrutinib therapy (Figure 2).

Conclusions:

We report 3 cases of invasive aspergillosis occurring within the first month of acalabrutinib therapy in patients with CLL. While corticosteroids was administrated in this cases, these were short-term treatments (<5 days) for two patients. In addition, our functional analysis using flow cytometry coupled with video-microsocopy approach highlights an alteration in anti-*Aspergillus* responses in these patients. These preliminary data need to be confirmed by the inclusion of other patients, as well as *in vitro* studies using neutrophils from healthy donors impregnated with Btk inhibitors. In the meantime, it seems important to follow up patients treated with acalabrutinib for the risk of aspergillosis (in the same way as those treated with ibrutinib) and to encourage any concerned team to actively report every occuring cases.

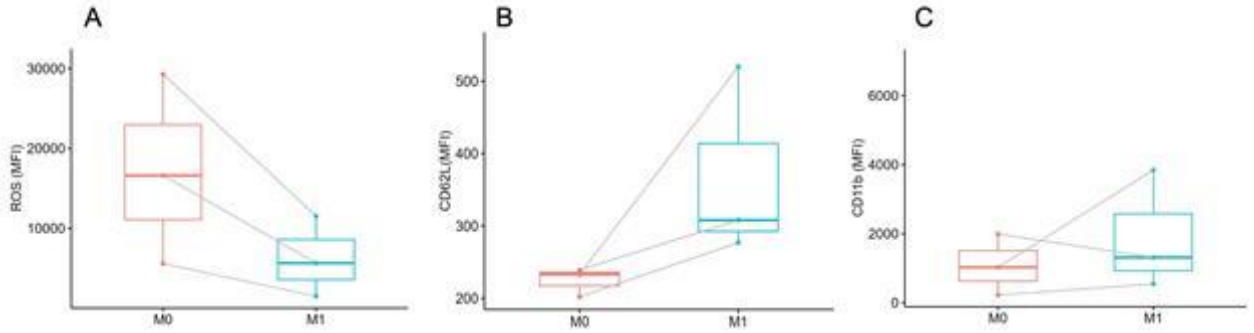

Figure 1. Neutrophils oxydative burst (A) and surface expression of CD62L (B) and CD11b (C) from patients (n=3) before (M0) and after one month (M1) treatment with acalabrutinib. Whole blood was stimulated with *Aspergillus fumigatus* germinating conidia.

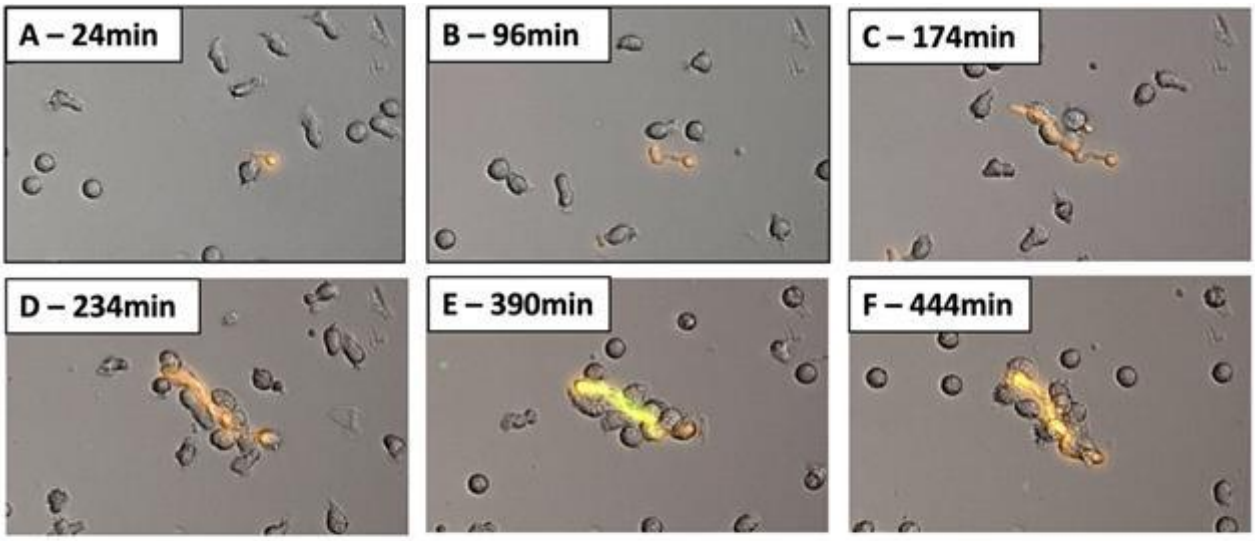

Figure 2. Videomicroscopy : neutrophils cultured with DsRed *Aspergillus fumigatus* germinating conidia (orange). (A-C) Hyphae growth. (D) Engulfment (deformation and tight attachment of neutrophils around conidia). (E-F) Killing (as indicated by staining of conidia with Sytox Green and stopping of conidial growth).

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C5a licenses phagocytes for sterilizing anti-fungal immunity during systemic candidiasis

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Objectives:

Systemic candidiasis, a life-threatening nosocomial infection, has recently emerged as a complication of anti-complement C5 or C5AR1-targeted therapeutics. We aimed to characterize the involvement of complement C5 and C5AR1 signaling in protective immunity during systemic candidiasis.

Materials & Methods:

For candidemic patients and healthy volunteers, we analyzed the whole blood transcriptome and complement C5a serum levels. We evaluated complement transcriptional induction for its utility as a diagnostic tool for candidemia while we assessed the potential of C5a serum levels and a *C5* cis eQTL (expression quantitative trait locus) as prognostic markers. For gaining mechanistic insights into the role of C5 in systemic antifungal defense, we utilized C5aR1 knockout, reporter, bone marrow chimera, and cell-type specific conditional knockout mice. We analyzed them using an established model of systemic candidiasis. To probe the role of phagocyte-intrinsic, extrahepatic C5 in protection during systemic candidiasis, we employed C5 reporter and phagocyte-specific C5 conditional knockout mice. We used flow cytometry, renal intravital microscopy, *Candida albicans* reporter strains, murine and human primary phagocytes, histological analyses, *in vivo* and *ex vivo* phagocyte effector function assays, and immunoblotting to assess the function of C5 in antifungal host defense comprehensively.

Results:

Our findings revealed that the complement pathway genes, including complement *C5*, were transcriptionally induced in candidemic patients, with the complement pathway being the topmost enriched biological pathway in candidemic patients. Furthermore, complement induction served as a predictive marker of candidemia. Using a mouse model of systemic candidiasis, we determined that C5a signaling through C5aR1, rather than C5aR2, was crucial for fungal clearance and host survival. Mechanistically, C5a signaling promoted myeloid phagocyte effector functions and ERK- and AKT-dependent survival in infected tissues. Without C5a signaling, dysregulated macrophage metabolism downstream of mTOR resulted in apoptosis and increased mortality through kidney injury. Apart from hepatocyte-derived C5, phagocytes contributed significantly to the local C5 pool, promoting antifungal protection. Lower levels of serum C5a or a *C5* eQTL leading to decreased leukocyte *C5* expression were independently associated with unfavorable patient outcomes during candidemia.

Conclusions:

During systemic candidiasis, C5a signaling through C5aR1 facilitates antifungal effector functions and the survival of myeloid phagocytes. Decreased complement C5a levels are associated with poor patient outcomes. Myeloid phagocytes act as a non-trivial source of complement C5 for the protective antifungal response.

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Vancomycin disrupts macrophage antifungal immunity

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Background and Objectives:
Prolonged antibiotic use is a controllable risk factor for systemic candidiasis. In recent years, antibiotics have been shown to have off-target detrimental effects on immune cells, but whether antibiotics disrupt antifungal immunity is unknown. We recently found that the antibiotic vancomycin enhanced mortality from invasive candidiasis in mice, whereas other antibiotics (e.g. metronidazole) did not affect susceptibility. To examine the mechanisms by which vancomycin impairs anti-*Candida* immunity, we explored how vancomycin pre-treatment of bone-marrow derived macrophages (BMDMs) impacted their responses to *C. albicans* challenge.
Materials & Methods:
Bone marrow was isolated from hind limbs of WT mice and differentiated in RPMI with M-CSF. BMDMs were differentiated in the presence or absence of 20 µg/ml of vancomycin for 5 days then harvested for the different experiments. The effect of vancomycin on macrophage anti-*C. albicans* responses were assessed using techniques including confocal microscopy, seahorse assay, RNA-Seq, western blotting, flow cytometry and ELISA.
Results:
Vancomycin-treated macrophages had reduced fungal killing, although phagocytosis of yeast cells was unaffected. Instead, vancomycin-treated macrophages exhibited impaired mitochondrial function, in which they failed to upregulate their respiratory capacity when stimulated with *Candida*. We used confocal microscopy to examine the mitochondria, which showed reduced mitochondrial mass and morphological changes in vancomycin-treated macrophages that have been previously linked to dysfunctional metabolic activity. Indeed, both an unbiased metabolite screen and bulk RNA-sequencing revealed several dysregulated pathways linked with mitochondrial dysfunction. For example, vancomycin-treated macrophages have an early upregulation of genes involved in inflammasome activation and pyroptosis, along with enhanced expression of mitochondrial-localised anti-inflammatory enzymes and lipids that have not been previously explored in the context of antifungal immunity.
Conclusions:
Our ongoing studies aim to determine which of these mediators prevents fungal killing by the vancomycin-treated macrophages. Taken together, our results improve our understanding of the pathways regulating antifungal immunity in macrophages and suggest that antibiotic-induced susceptibility to *C. albicans* may be partly driven by disrupted macrophage function.

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Host ecto-5'-nucleotidase (CD73) suppression impairs neutrophils NET formation response upon Candida albicans infection

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Objectives: We have previously shown that *Candida albicans* ecto-5'-3'-nucleotidase enzyme is capable of degrading the extracellular DNA of neutrophil extracellular traps (NETs), allowing the escape and proliferation of the pathogen (PMID: 34327150). Besides this direct escape, we propose an indirect relationship between the host ecto-5'-nucleotidase (CD73, the critical enzyme for adenosine formation) and the inhibition of NET production. In line with this, the present work aimed to further explore the host CD73 relevance in the interaction of the immune system, in particular of neutrophils, with *C. albicans*, especially in the development and regulation of NETs upon yeasts infection. In debilitated or immunocompromised individuals, the commensal *C. albicans* can switch to an opportunistic pathogen capable of causing life threatening infections. As part of the host first line of defense, neutrophil innate immune cells are responsible for the uptake and destruction of invaders, using several strategies to achieve it, namely by releasing NETs. However, different studies have revealed that the conversion of extracellular ATP into adenosine, through ectophosphatase and ectonucleotidase activities, can impact and determine the outcome of infection.

Materials & Methods: To accomplish our objectives, bone marrow derived neutrophils (BMDN) from wild type (WT) and CD73 knockout (CD73KO) groups of mice were isolated and studied. Neutrophils were infected with *C. albicans* SC5314 and YP0037, from the CYC-UC (Clinical Yeast Collection – University of Coimbra), at a MOI 1:1, and, through immunofluorescence/immunocytochemistry assays and microscopy analysis, the morphology of both cells types was scrutinized. BMDN incubation with phorbol myristate acetate (PMA, a strong NET inducing agent) was used as a NET formation positive control, and NETs were observed and quantified in all conditions. For the quantification of *C. albicans* filamentation gene expression we used RT-qPCR assays; RNA extraction was performed with a MagNa Pure Compact RNA Isolation Kit (Roche), transcribed into cDNA with a Transcriptor First Strand cDNA Synthesis Kit (Roche) and RT-qPCR assays, using a SsoFast EvaGreen Supermix (BioRad).

Results: The profile of NET production was significantly different in CD73KO neutrophils than the observed in WT neutrophils ($p<0.01$). Both PMA and *C. albicans* fairly induced NETs production in CD73KO neutrophils. Moreover, CD73KO NETs did not co-localize with yeasts, as observed in control WT neutrophils. Besides, *C. albicans* filamentation gene expression (*e.g.* EFG1) is modified, corroborating the microscopy observations showing early *C. albicans* yeast-to-hypha switch when exposed to CD73KO neutrophils.

Conclusions: The results demonstrate that host CD73 activity is essential for the control of *C. albicans* infection, by modulation of neutrophil-mediated response through NET efficacy. Clearly, the activity of host CD73 enzyme, by controlling the levels of adenosine in the extracellular infectious environment, modulates inflammation but also seem to compromise the effective response of innate immune cells upon a yeast infection.

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CD56-mediated activation of human natural killer cells is triggered by galactosaminogalactan of Aspergillus fumigatus

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Introduction & Objectives: Invasive *Aspergillus fumigatus* infections are a major cause of severe complications in immunocompromised patients. Delayed natural killer (NK) cell reconstitution in allogenic hematopoietic cell transplant recipients is associated with higher susceptibility to invasive pulmonary aspergillosis, indicating that NK cells are indispensable for fungal clearance. Previously, our group identified CD56 as a *pathogen recognition* NK cells *receptor* that recognizes *A. fumigatus* and is required for the antifungal activity. However, the underlying cellular mechanisms and the fungal ligand of CD56 are still unknown.
The objective of this project are to identify the fungal ligand interacting with CD56 on NK cells and to gain deeper insights into the cellular mechanisms of this interplay.
Materials & Methods: We used a combination of purified cell wall components, biochemical treatments, and *A. fumigatus* cell wall gene deletion mutants and investigated their relevance for the interaction of *A. fumigatus* with NK cells by flow cytometry, microscopy and ELISA. Their effects on CD56 surface expression, expression of activation and degranulation markers, as well as chemokine and cytotoxic effector molecule secretion were studied. Furthermore, PI3K and Pyk2 inhibitors were used to evaluate their involvement in the signalling pathway of *A. fumigatus*-induced NK cell activation. Their impact on CD56 surface expression, NK cell activation and chemokine release was investigated.

Results: CD56 on NK cells showed binding to the *A. fumigatus* cell wall polysaccharide galactosaminogalactan (GAG). Surface expression of CD56 on NK cells was significantly reduced after stimulation with purified GAG and the *A. fumigatus* wildtype strain but not with GAG-deficient mutant strains *Δagd3* and *Δuge3*. Likewise, purified GAG and *A. fumigatus* wildtype strain induced NK cell activation and elicited strong secretion of cytotoxic effector molecules (e.g., granzyme B) and chemokines (e.g., CCL3 and CCL4). Specifically, deacetylated galactosamine residues of GAG played a role in interaction with CD56 and triggered strong NK-cell activation, along with potent release of cytotoxic effectors and immune-enhancing chemokines. Inhibition of PI3K and Pyk2 decreased *A. fumigatus*/GAG-mediated activation of NK cells and secretion of chemokines.

Conclusion: Our data suggest that *A. fumigatus* GAG is a ligand of CD56 on human primary NK cells and stimulates potent antifungal effector responses under the involvement of PI3K and Pyk2.

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Analysis of mutations in ERG11 gene of Candida albicans

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Objectives:
Emergence of *Candida albicans* strains resistant to azoles has become a significant problem as fluconazole is widely used in clinical settings. The primary mechanism of azole resistance involves mutations in three specific regions of the *ERG11* gene. In Pakistan, fluconazole resistance in C. albicans emerged in 2014, however the resistance mechanisms have not been described for local strains. Therefore, the objective of this study was to investigate *ERG11* mutations in fluconazole-resistant *C. albicans* strains isolated at the Aga Khan University Hospital Laboratory in Karachi, Pakistan.

Method:
This study was conducted on 17 clinically significant isolates from 2020-2023, which either tested fluconazole resistant or were refractory to azole treatment. The antifungal susceptibility pattern was determined using YeastOne Sensititre according to the Clinical and Laboratory Standards Institute guidelines. The *ERG11* gene of *C. albicans* was amplified and sequenced. Sequences were analyzed using MEGA 11 tool.

Results:
In this study, out of 17 strains, nine were found to be resistant to fluconazole (MIC >8 µg/ml). Among these strains, three exhibited resistances to all azoles, whereas five showed intermediate resistance to voriconazole. Seven non-synonymous mutations were identified in these strains. Specifically, the mutations T123I, Y132H, A114V, and F145L were observed in fluconazole-resistant isolates, whereas sensitive strains harbored the K128T and D116E mutations. The single strain that did not show a clinical response to antifungal treatment but remained susceptible in vitro exhibited a unique A02G mutation.. Silent mutations F72, F105, K119, S137, and Y220 were also detected.

Conclusion:
In this study, we investigated the presence of azole-resistant *C. albicans* strains and mutations known to confer resistance to azoles. Synonymous mutations *ERG11* were observed, indicating potential genetic variations may influence drug susceptibility. However, the presence of these mutations alone does not guarantee resistance or sensitivity, suggesting the complex multifactorial nature of resistance. Further comprehensive investigations are required to fully understand the complete resistance profile and to identify additional contributing factors.

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Survey the effect of Licorice extract on Gene regulation of aflR and Aflatoxin production in Aspergillus Parasiticus by Real-time PCR

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Objectives Recent investigations revealed the effects of herbal extracts on both fungal growth as well as aflatoxins production. Methods & Materials

In the present study, we tried to evaluate antifungal activity as well as antitoxin activity of *Glycyrrhiza glabra* (licorice)extract. Strain American Type Culture Collection 15517 of *Aspergillus parasiticus* was used to perform antifungal susceptibility test according to Clinical andLaboratory Standards Institute document M27-A3, and the rate of aflatoxin production was determined using high-performance liquid chromatography technique after exposure to different concentrations of licorice extract. Quantitative changes in the expression of the *aflR* gene were analyzed by measuring the cognate *aflR* mRNA level with quantitative real-time reverse-transcription polymerase chain reaction assay. Results Our obtained results demonstrated the inhibitory effect of licorice extract on *Aspergillus parasiticus* growth at 500 mg/mL of licorice extract. In addition, a significant decrease in aflatoxin production was revealed at the same concentration. However, the production of aflatoxin B1 was entirely inhibited in 10 g/mL of licorice extract. The level of *aflR* gene expression was significantly decreased after the exposure of fungal cells to 500 g/mL of licorice extract. Conclusion: Evaluation of the antifungal and antitoxin activity of licorice extract on *Aspergillus parasiticus* revealed its antifungal properties as well as its effective ability to decrease aflatoxin production.

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Analysis of Microsatellite Length Polymorphism for Clinical Isolates of Candida albicans from Animals

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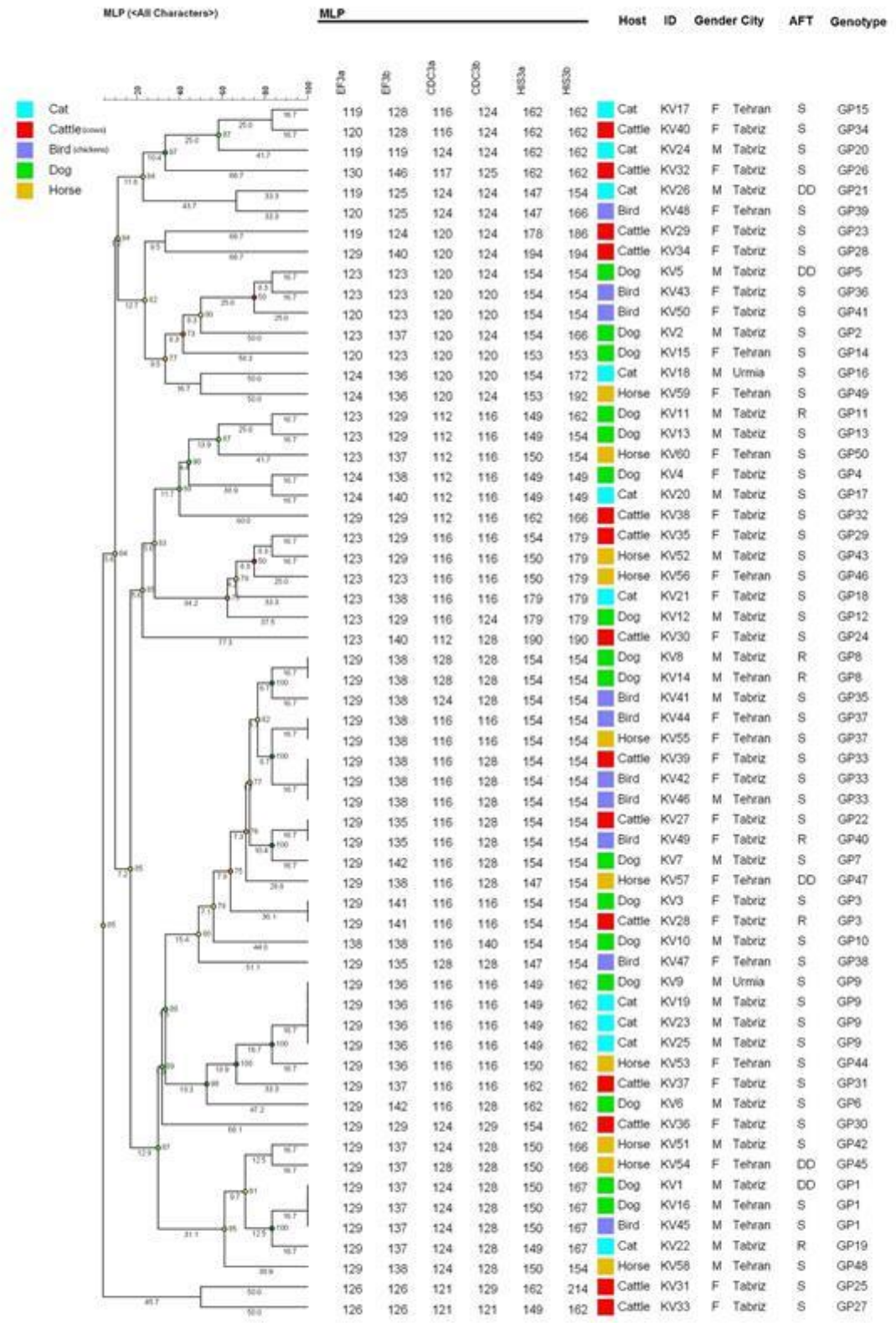
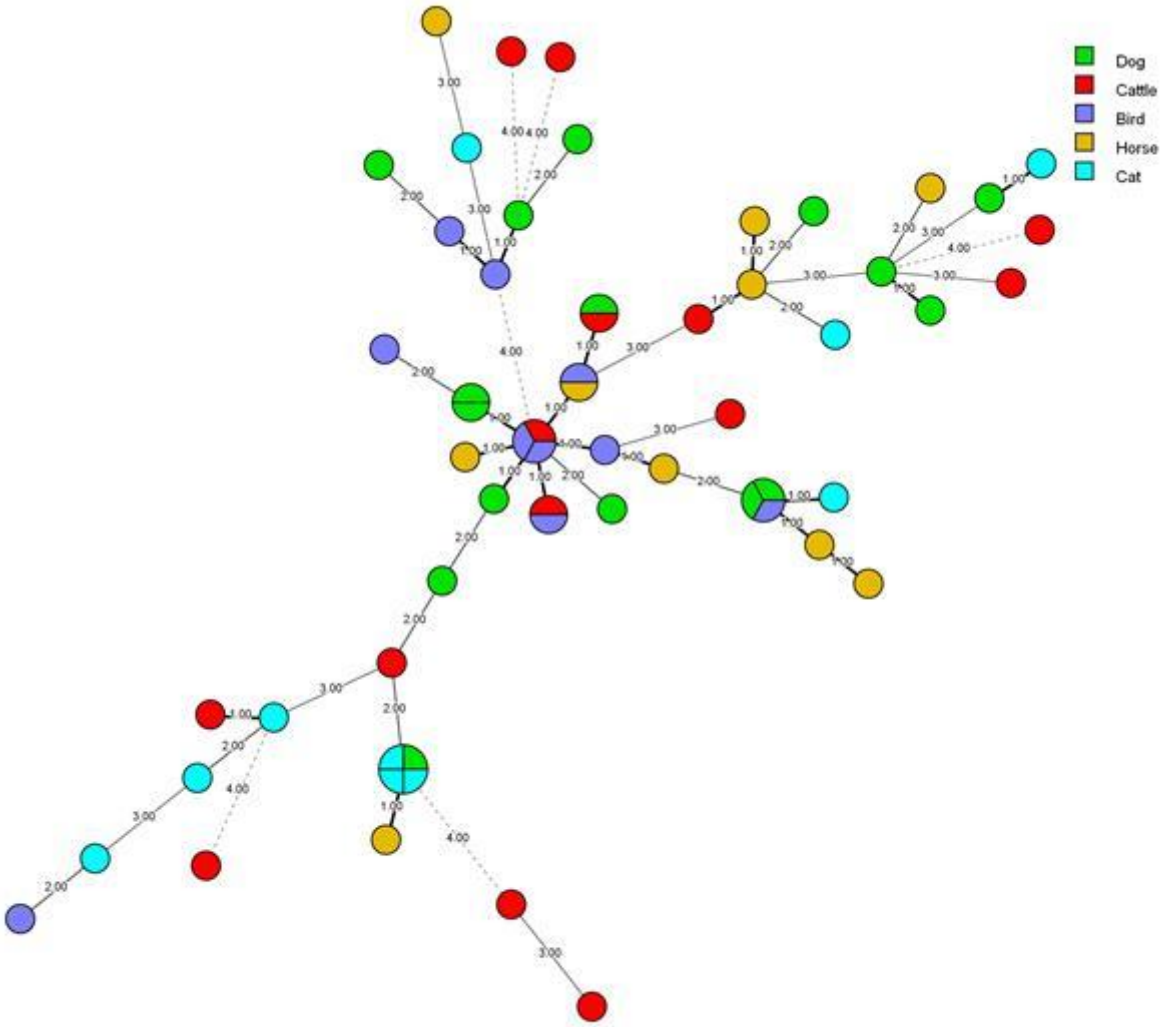
Objectives: *Candida albicans* has been shown as the most common species of *Candida* collected from different animals. This study aimed to evaluate the genetic diversity and genetic relationships among *C. albicans* isolates collected from clinical specimens of animals suffering from candidiasis using microsatellite length polymorphism (MLP).
Materials & Methods: We used MLP for a group of 60 *C. albicans* strains isolated from various animal species (dog: 16, cat: 10, horse: 10, cow: 14 ,chicken: 10), previously defined as animal clinical isolates. Three loci, including EF3, CDC3, and HIS3, were amplified, and the products ran onto an ABI XL 370 genetic analyzer, and fragment sizes were determined.
Results: Of the 60 clinical strains illustrated, 49 different genotypes were identified with a discriminatory power index of 0.991. A total of 17 alleles and 26 different combinations were identified for EF3 locus, six alleles and 13 combinations for CDC3 locus, and 17 alleles and 27 combinations for HIS3 locus. The most common genotypes were GP9 (four strains) and GP1 and GP33 (three strains). Wright's fixation index (FST) values were calculated to assess inter-group genetic diversity for all pairwise combinations of the five sub-populations of *C. albicans* isolated from the different animal hosts. The highest FST values related to *C. albicans* isolated from chicken to three sub-populations of cats (FST: 0.1397), cows (FST: 0.0639), and horses (FST: 0.0585).
Conclusions: The results indicated a moderate genetic differentiation ($0.05 < FST < 0.15$) between *C. albicans* strains isolated from cats, cows, and horses as a mammal vs. chickens.



Host	ID	Gender	City	AFT	Genotype	EF3a	EF3b	CDC3a	CDC3b	HIS3a	HIS3b
Cat	KV17	F	Tehran	S	GP15	119	128	116	124	162	162
Cattle	KV40	F	Tabriz	S	GP34	120	128	116	124	162	162
Cat	KV24	M	Tabriz	S	GP20	119	119	124	124	162	162
Cattle	KV32	F	Tabriz	S	GP26	130	146	117	125	162	162
Cat	KV26	M	Tabriz	DD	GP21	119	125	124	124	147	154
Bird	KV48	F	Tehran	S	GP39	120	125	124	124	147	166
Cattle	KV29	F	Tabriz	S	GP23	119	124	120	124	178	186
Cattle	KV34	F	Tabriz	S	GP28	129	140	120	124	194	194
Dog	KV5	M	Tabriz	DD	GP5	123	123	120	124	154	154
Bird	KV43	F	Tabriz	S	GP36	123	123	120	120	154	154
Bird	KV50	F	Tabriz	S	GP41	120	123	120	120	154	154
Dog	KV2	M	Tabriz	S	GP2	123	137	120	124	154	166
Dog	KV15	F	Tehran	S	GP14	120	123	120	120	153	153
Cat	KV18	M	Urmia	S	GP16	124	136	120	120	154	172
Horse	KV59	F	Tehran	S	GP49	124	136	120	124	153	192
Dog	KV11	M	Tabriz	R	GP11	123	129	112	116	149	162
Dog	KV13	M	Tabriz	S	GP13	123	129	112	116	149	154
Horse	KV60	F	Tehran	S	GP50	123	137	112	116	150	154
Dog	KV4	F	Tabriz	S	GP4	124	138	112	116	149	149
Cat	KV20	M	Tabriz	S	GP17	124	140	112	116	149	149
Cattle	KV38	F	Tabriz	S	GP32	129	129	112	116	162	166
Cattle	KV35	F	Tabriz	S	GP29	123	129	116	116	154	179
Horse	KV52	M	Tabriz	S	GP43	123	129	116	116	150	179
Horse	KV56	F	Tehran	S	GP46	123	123	116	116	150	179
Cat	KV21	F	Tabriz	S	GP18	123	138	116	116	179	179
Dog	KV12	M	Tabriz	S	GP12	123	129	116	124	179	179
Cattle	KV30	F	Tabriz	S	GP24	123	140	112	128	190	190
Dog	KV8	M	Tabriz	R	GP8	129	138	128	128	154	154
Dog	KV14	M	Tehran	R	GP8	129	138	128	128	154	154
Bird	KV41	M	Tabriz	S	GP35	129	138	124	128	154	154
Bird	KV44	F	Tehran	S	GP37	129	138	116	116	154	154
Horse	KV55	F	Tehran	S	GP37	129	138	116	116	154	154
Cattle	KV39	F	Tabriz	S	GP33	129	138	116	128	154	154
Bird	KV42	F	Tabriz	S	GP33	129	138	116	128	154	154
Bird	KV46	M	Tehran	S	GP33	129	138	116	128	154	154
Cattle	KV27	F	Tabriz	S	GP22	129	135	116	128	154	154
Bird	KV49	F	Tabriz	R	GP40	129	135	116	128	154	154
Dog	KV7	M	Tabriz	S	GP7	129	142	116	128	154	154
Horse	KV57	F	Tehran	DD	GP47	129	138	116	128	147	154
Dog	KV3	F	Tabriz	S	GP3	129	141	116	116	154	154
Cattle	KV28	F	Tabriz	R	GP3	129	141	116	116	154	154
Dog	KV10	M	Tabriz	S	GP10	138	138	116	140	154	154
Bird	KV47	F	Tehran	S	GP38	129	135	128	128	147	154
Dog	KV9	M	Urmia	S	GP9	129	136	116	116	149	162
Cat	KV19	M	Tabriz	S	GP9	129	136	116	116	149	162
Cat	KV23	M	Tabriz	S	GP9	129	136	116	116	149	162
Cat	KV25	M	Tabriz	S	GP9	129	136	116	116	149	162
Horse	KV53	F	Tehran	S	GP44	129	136	116	116	150	162
Cattle	KV37	F	Tabriz	S	GP31	129	137	116	116	162	162
Dog	KV6	M	Tabriz	S	GP6	129	142	116	128	162	162
Cattle	KV36	F	Tabriz	S	GP30	129	129	124	129	154	162
Horse	KV51	M	Tabriz	S	GP42	129	137	124	128	150	166
Horse	KV54	F	Tehran	DD	GP45	129	137	128	128	150	166
Dog	KV1	M	Tabriz	DD	GP1	129	137	124	128	150	167
Dog	KV16	M	Tehran	S	GP1	129	137	124	128	150	167
Bird	KV45	M	Tehran	S	GP1	129	137	124	128	150	167
Cat	KV22	M	Tabriz	R	GP19	129	137	124	128	149	167
Horse	KV58	M	Tehran	S	GP48	129	138	124	128	150	154
Cattle	KV31	F	Tabriz	S	GP25	126	126	121	129	162	214
Cattle	KV33	F	Tabriz	S	GP27	126	126	121	121	149	162


Dog
Cattle
Bird
Horse
Cat

P365

New insights on titanization process in Cryptococcus neoformans/gattii species complex (CNGSC)

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Objectives:
The aim of this study (partly supported by grant BPIDUB.3.2023) was to assess influence of physicochemical and genetic factors on the formation of giant (Titan) cells in representatives of *C. neoformans/gattii* species complex (CNGSC). The following factors were tested based on modified *in-vitro* "titanization" protocols: 1. biologically active fetal bovine serum (FBS) versus heat-inactivated (HI-FBS); 2. The influence of 5% CO_2; 3. The effect of various mutations that affect formation of polysaccharide capsule; 4. The ability of cerebrospinal fluid (CSF), in comparison to FBS, to stimulate development of Titan cells; 5. The effect of antioxidants.

Materials & Methods:
In vitro titanization was performed based on two previously established protocols, serum-dependent (doi: 10.1371/journal.ppat.1006978) and serum-free protocol (doi: 10.3390/pathogens11070768). CSF (Lee Biosolutions), FBS, or HI-FBS (Merck) were used at 10% final concentrations. To find which physicochemical factor(s) is/are critical for titanization, or to assess the inhibitory/killing activity of serum, cells of chosen representatives of CNGSC were incubated in the presence of 5% CO_2 or under standard atmosphere, at 37°C or 30°C, under different pH values or with antioxidants - glutathione (GSH) or ascorbic acid (AA). Viability of cells was evaluated quantitatively by plating on YPD medium. To determine ploidy of cells, flow cytometry was utilized with propidium iodide (PI, 5 µg/mL) or SYTOX® Green (500 nM) staining. Microscopic assessment of cell and capsule sizes were performed based on staining with India ink. Size measurements were performed using ImageJ and the data were visualized and analysed using the GraphPad Prism software. Finally, to assess the role of capsule in Titan cells formation, *CAP10*, *CAP59*, *CAP*60 and *CAP64* single gene deletion mutants were tested.

Results:
We found that exposing stationary phase cells to a serum-free low nutrient medium at pH 7.3, in the presence of 5% CO_2 at 37°C triggers Titan cell formation to the same degree as the protocol that utilized 10% HI-FBS. Various impacts of individual physicochemical factors on titanization, proliferation and viability were observed, depending on the presence of 5% CO_2. For instance, no significant differences between HI-FBS and FBS were observed in cells proliferation and efficiency of titanization at 5% CO_2. For experiments performed with FBS under standard atmosphere at 37°C, Titans were not observed, and strong inhibitory/killing effect was noticed, likely due to an inhibitory effect of the pH. The response of acapsular mutants to titanization was inconsistent with only some mutants being affected.

Conclusions:
Titans are formed equally in FBS, HI-FBS, and CSF, when cells are incubated in the presence of 5% CO_2 at 37°C. The serum-free protocol reveals minimal *in vitro* conditions necessary for titanization. While some physicochemical factors promote proliferation and inhibit Titan cells formation, the other ones induce Titans and cause decrease in cell viability. Capsule formation is not necessary for titanization. However, some aspects of capsule-relevant signalling, for instance response to low nutrients and vacuole formation, appear important for development of Titans.

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Effect of linoleic acid conjugated with zinc oxide nanoparticles in inhibiting expression MDR and CDR genes Candida albicans by PCR

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Objectives :Candida albicans is an opportunistic human fungal pathogen, the fourth most common cause of nosocomial infections, and the leading cause of systemic candidiasis with a mortality rate of 50%. Increase in resistance to antifungal drugs, especially azoles, such as fluconazole, has resulted in the failure of Candida infections treatment and hence in the useof naturally occurring nano-derived compounds which have appropriate antifungal effects,lack toxic effects on the human body, and can inhibit drug resistance genes.**Material and Methods:**Fluconazole-resistant Candida albicans isolates with MDR and CDR drug resistance genes were confirmed with molecular methods. In the first step, RNA was extracted, specific bacterial cDNA was synthesized, and real-time PCR was performed to quantify the expression rates of MDR and CDR genes. In the next stage, Candida was exposed to theMinimum Inhibitory Concentration (MIC) of the nano-complex and re-cultured. As in the first stage, RNA extraction, specific bacterial cDNA synthesis, and real-time PCR were performed. The formula was used to calculate the expression rate of MDR and CDR genesbefore and after treatment with the nano-complex, and the results were compared **Results** :A total of 20 samples of Candida albicans with MDR and CDR drug resistance genes were isolated from patients with candidal vaginitis by a gynecologist in health centers of Tehran using a vaginal swab. The samples were examined with real-time PCR in terms of MDR and CDR genes expression before and after exposure to the nano-complex, and the obtained data were analyzed with SPSS. The results showed that CDR and MDR genes expression was reduced as 97.41 and 94.36, respectively, indicating the great impact of this nano-complex in reducing the expression of MDR and CDR drug resistance genes **Conclusions**Given the inhibitory effect of ZnO-conjugated linoleic acid nano-complex on Candida albicans growth, which is accomplished through inhibition of the main drug resistance genes of MDR and CDR, this nano-complex is used for the first time in this study to reduce the expression of these drug resistance genes.

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Isolation, identification, determination of drug sensitivity and ERG11 gene mutation of Candida species isolated from vulvovaginal candidiasis

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Objectives: Vulvovaginal candidiasis (VVC) is a prevalent infection of the genitourinary tract affecting millions of women worldwide. In the present study, the importance of virulence factors, *ERG11* gene mutations, *ERG11* gene expression, and plasma membrane ergosterol content for fluconazole resistance in *Candida* species was investigated in 200 women suspected of vulvovaginitis. Methods & Materials :Isolated *Candida* species were identified using the ITS-restriction fragment length polymorphism (ITS-RFLP) technique. Antifungal susceptibility testing was performed according to the CLSI document. *ERG11* gene expression was analyzed using real-time PCR. *ERG11* gene mutation analysis was performed using sequencing methods, and the ergosterol content of the cell membrane was determined in fluconazole-resistant isolates. Furthermore, the production of phospholipase and proteinase enzymes was evaluated in recurrent and non-recurrent infections. Results :VVC was diagnosed in 101 (50.5%) of the 200 clinical cases, of which 21 (20.8%) were confirmed as RVVC. *Candida albicans* was the most prevalent species, followed by *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. guilliermondii*. Ketoconazole and fluconazole were the most effective drugs against *C. albicans* among five tested antifungals with MIC ranges between 0.06 and 16 μg/mL and 0.25–64 μg/mL. Substitutions of A114S, Y257H, T123I and A114V were detected in fluconazole-resistant *C. albicans.* The ergosterol content of the fungal cell membrane and the mean levels of *ERG11* gene expression transcript were higher in fluconazole-resistant *C. albicans* isolates obtained from RVVC than in those obtained from VVC cases. Phospholipase and proteinase were produced in different amounts in all *Candida* species isolated from VVC and RVVC cases. Conclusion :In this review, our results demonstrated that several molecular mechanisms, including *ERG11* gene expression, changes in the cell membrane ergosterol content, and mutations in *ERG11* gene alone or simultaneously involved in fluconazole resistance of *C. albicans* species and the recurrence of VVC.

P368

Longitudinal study looking at the performance of Aspergillus molecular diagnostic workflows from 2019 to 2022.

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Objective: Invasive aspergillosis remains difficult to diagnose despite the availability of antigen-based serological testing, radiological imaging, and defined protocols for nucleic acid-based diagnostic. The External Quality Assessment scheme (EQA) for the molecular detection of *Aspergillus spp.* has been provided by QCMD annually since 2009, highlighting the performance of molecular diagnostic tests in clinical use. QCMD data on workflow and performance between 2019 and 2022 was analysed to identify technical and performance trends.

Material/methods: Between 2019 and 2022 the EQA panel for molecular *Aspergillus* detection was distributed to 283 participants in 26 countries. The panels consisted of positive samples containing *Aspergillus fumigatus* or *Aspergillus niger* suspended in synthetic sputum or plasma and negative control samples. The panels were tested blind and the data, including information on test methodology, were returned to QCMD *via* an online data management system and the results analysed qualitatively.

Results: In 2019, 55 datasets were collected with most participants using an in-house developed assay (63.6%) By 2022 the number of datasets had grown to 83, while the proportion of in-house assays used had dropped annually, being 47% in 2022. Across the 4 years of this review, the high titre samples were correctly identified ≥89% in plasma samples and in ≥95% in sputum samples for *A. fumigatus*. *A. niger* was correctly identified in ≥79% of higher titre datasets. There was a higher than expected rate of false positivity observed in the negative sputum ≤11.0% and plasma samples ≤14.8%.

Conclusions: We present a longitudinal study showing the performance of molecular assays across the last 4 years based on data from the QCMD EQA schemes for the detection on *Aspergillus* species. Over the past 4 years there has been a steady shift away from the use of in-house assays by the participant population, potentially associated with lower rates of detection of *A. fumigatus* in plasma. Laboratories have continued to demonstrate proficiency in the detection of *A. fumigatus* and *A. niger* in both sputum and plasma. False positivity remains a concern in current *Aspergillus* testing and the use of negative controls is paramount. Further analysis on technical parameters associated with performance will be presented.

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Diversity of vaginal bacterial and fungal microbiome among women with RVVC in the Southern Nigeria

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Objectives
The human vaginal microbiome (HM) significantly influences women's health, changing substantially under certain conditions. In the vagina, commensal microbes provide the first line of defense against opportunistic pathogens. A healthy vaginal microbiome is associated with a less diverse community of microbes, predominantly *Lactobacilli*. In contrast, dysbiosis of the vaginal microbiota is associated with increased species diversity, with fewer *Lactobacilli* and a mixture of commensal and pathogenic organisms. Vulvovaginal candidosis (VVC) is an inflammatory process caused by an overgrowth of *Candida* species common in women in their reproductive years. Recurrent VVC (RVVC) is a history of four or more episodes of VVC in 12 months. The study aimed to investigate bacterial and fungal microbiomes to understand the changes in perceived healthy women and those with RVVC and to know how repeated and long-term use of azole antifungal drugs impacts vaginal microbiome diversity.

Materials & Methods
We conducted a prospective population-based cross-sectional case-control study across Nigeria's three southern geopolitical zones. Ninety-one vaginal swab samples from the participants from the southern zones of Nigeria were processed. We sequenced fungal and bacterial microbiomes using ITS1 16S V1-V4 amplicon libraries. We sequenced libraries on an Illumina MiSeq, and diversity metrics were calculated.

Results
The mean age of cases was 33±10 years (range 19-55) and 39±9 years (range 18-55) of the controls (p=0.012). Of 84 analysed samples, 22 were cases (RVVC), and 62 were controls (No-RVVC). In the microbiome analysis, after the initial taxa filtration at 2% of total samples and >0.2% in any samples, respectively, six samples with fewer than 5000 counts (raw data) were removed, leaving 84 samples containing 115 taxa and 47 genera. The mean genera per sample was 12, the range of genera per sample was 2-27, while the median counts were 56,125. *Lactobacilli* were the most predominant in the filtered data. No difference existed between the bacterial diversity of cases and controls (P>0.05). There was an increased abundance of *Pseudomonas* and *Streptococcus* in the controls, while a similar number of *Lactobacilli* was noticed in cases and controls. A relatively high abundance of *Lactobacillus and Gardnerella* was found among cases and controls in all regions except cases in the SS, which were *Gardnerella* dominated. In the mycobiome, there was an increased abundance of *Candida albicans* among cases and that of *A. penicillioides, C. delicatulum* and *Malassezia restricta* in the controls (No RVVC). Shannon diversity was significantly reduced in cases compared to controls (p = 1.3e-05, Wilcoxon rank sum test).

Conclusion

We found a significant difference in the dominance of *Candida* species among RVVC cases and controls, and a lactobacilli-dominated population with significant vaginal anaerobes in both RVVC (cases) and controls. There was no evidence of a significant change in bacterial diversity/dysbiosis in RVVC.

P370

Mitochondria complex I deficiency in Candida albicans arrests the cell cycle at S phase through suppressive TOR and PKA pathways

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Objectives:
Investigating effect of mitochondrial functional defects in *Candida albicans* on the cell cycle of strains

Materials & Methods:
Using genetic null mutants for ETC complex I (CI), CIII and CIV mutants, the cell cycle in each G_0/G_1, S and G_2/M stages are assessed by SYBR Green I-activated cell sorting (FACS) analysis.

Results:
An extended S phase and short G_2/M interface in 3 of the 4 CI null mutants, and this differs from our CIII mutant in that the CIII mutant shows increased cell populations in the G_1/G_0 phase. The extended S phase in the CI mutants causes a decrease in the population of cells with sizes over 10 μm. The absence of rapamycin resistance in *nuo1Δ* or *nuo2Δ* – together with gene transactional repression for Tor/Sch9/Rim15/Msn2/4 – indicate that both Nuo1p and Nuo2p are involved in TOR signaling pathway for maintaining a normal cell cycle and life span. On the other hand, the abnormal cell cycle phenotypes in *ndh51Δ* or *goa1Δ* appear to match a downregulated cAMP/PKA response reported in our previous study. The downregulation of either TOR1 or cAMP/PKA will eventually interfere with the progress of the cell cycle, thereby reducing the calorie restriction-dependent stress resistance and life span extension in these CI mutants.

Conclusions:
The mitochondrial electron transport chain (ETC) proteins are involved in cell cycle regulation via TOR signaling and cAMP signaling pathways.

Figure 1

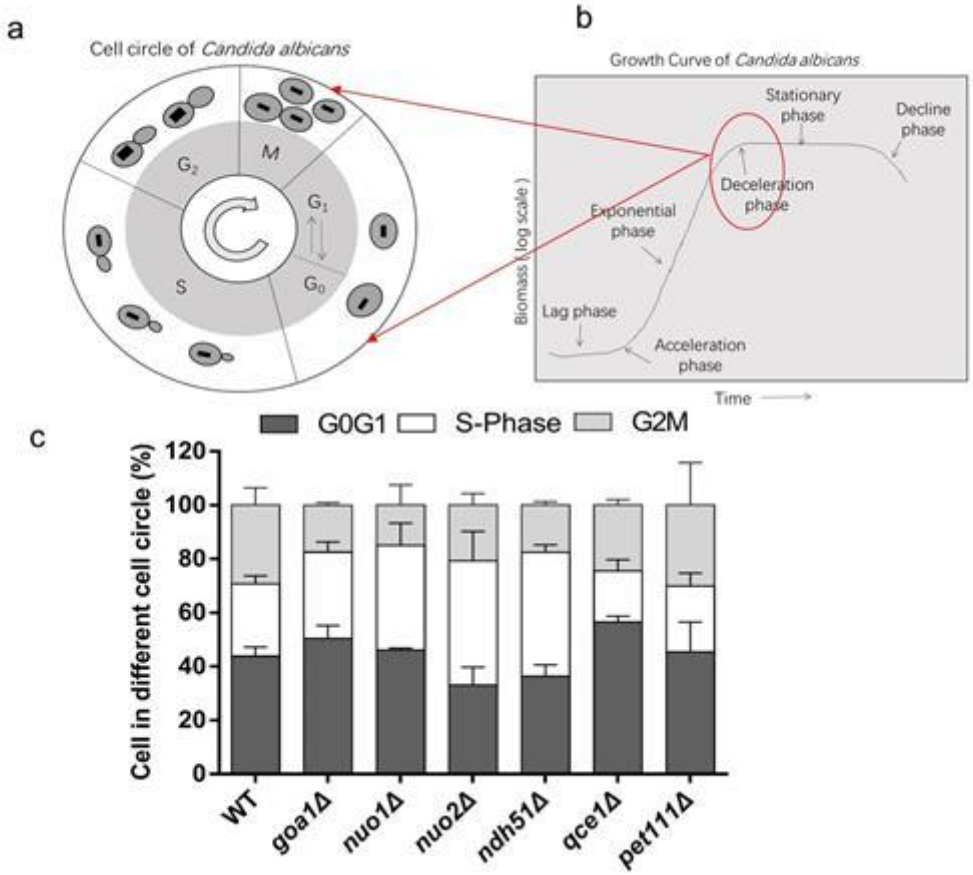
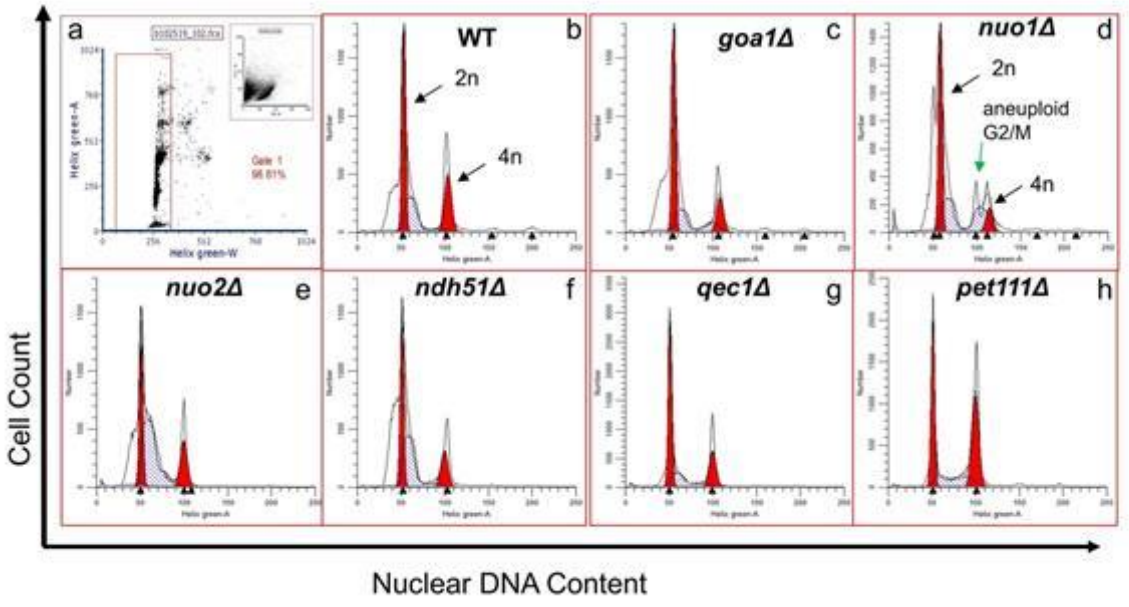


Figure 2



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Differential levels of expression of cyp51 A, B, C genes among azole resistant Aspergillus flavus clinical isolates

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Objectives:
Invasive and allergic infections by *Aspergillus flavus* are more common in tropical and subtropical countries. The emergence of panazole (itraconazole,voriconazole,posaconazole and isavuconazole) resistance in *A. flavus* impacts the management of aspergillosis, as azoles are used as the first line and empirical therapy. The extent of azole resistance in *A. flavus* and its underlying mechanism is unknown. The objective of the study is to determine the Minimal Inhibitory Concentration of conventional and newer azole antifungal agents against *A. flavus* isolates and to assess the amount of expression of the lanosterol 14-α-demethylase coding genes (cyp51A, cyp51B, and cyp51C) associated with azole resistance
Materials & Methods:
A total of 130 *A. flavus* isolated from various clinical specimens between March 2020 to June2021 in the central laboratory and mycology laboratory were used in the study. Antifungal susceptibility patterns of itraconazole, voriconazole, posaconazole, isavuconazole and ravuconazole were determined by broth microdilution method as per Clinical Laboratory Standards Institute (CLSI) M38-A2 guidelines. All the pan azole resistant *A. flavus* strains along with equivalent number of susceptible strains were subjected to expression profile analysis by reverse transcriptase quantitative realtime PCR. The total RNA was extracted using TRIzol method and the cDNA was synthesized by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), according to manufacturer's recommendations. RT-PCR was performed in a 10µl volume containing KAPA SYBR® green PCR master mix each primer pair, and 0.5µl of cDNA and RNase-free water up to the final volume. Independent assays were performed with three biological replicates, and expression levels were normalized to the GAPDH mRNA level. The threshold cycle ($2^{-\Delta\Delta CT}$) analysis method was used to determine the fold change.
Results:
Out of the 130 *A. flavus* isolates, 45.39% (59/130) were resistant to itraconazole and 6.2 % (8/130) were resistant to voriconazole and isavuconazole and 3.1% (4/130) were resistant to posaconazole. Overall, 3.08% (n=4/130) of *A. flavus* isolates had MICs above epidemiological cutoff values (≥1µg/ml) for all the azoles that were tested. cyp51A (5.78 fold), cyp51B (4.44 fold), and cyp51C (1.23 fold) genes were found to be overexpressed among pan azole resistant isolates,when compared to the susceptible isolates.
Conclusions:
These results suggest that combination of overexpression of *cyp* 51 A and *cyp* 51 B genes could simultaneously contribute to panazole resistance in *A. flavus*.

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The transcriptional and cellular responses of Candida auris to macrophage phagocytosis: variations on a theme

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Objectives

We aimed to investigate the gene expression of *Candida auris* in response to phagocytosis by macrophages. Also, to determine the fate of phagocytosed fungal cells and unravel survival mechanisms that enable this emergent pathogen to evade immune surveillance.

Methods

We studied the gene expression of *C. auris* cells that were incubated with BMDMs for 1 h via RNA sequencing analysis. For time lapse microscopy, J774A.1 murine macrophages were infected with fluorescently labelled *C. auris* strains during 3 h. Survival of *C. auris* isolates after co-incubation with macrophages was assessed via CFU formation. Macrophage survival was determined via quantification of LDH release.

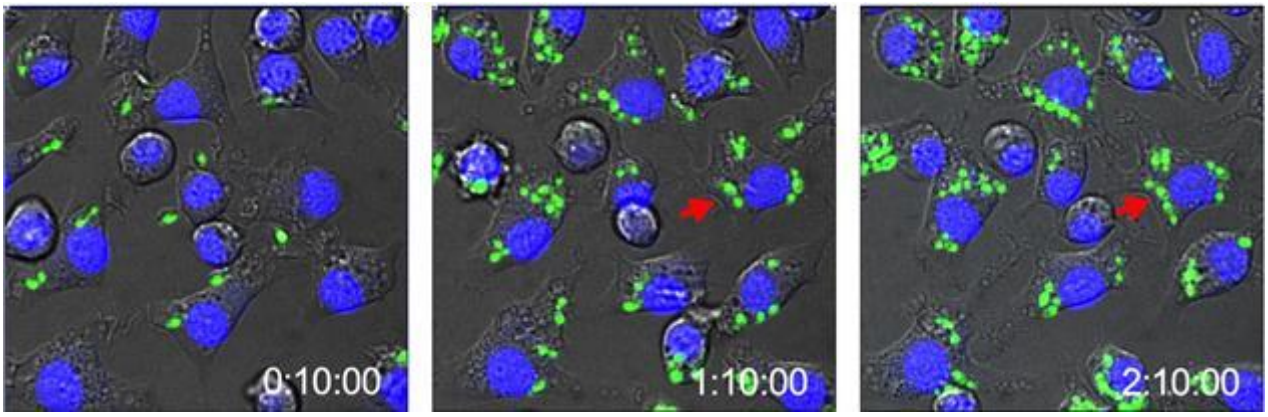
Results

Macrophages readily phagocytose *C. auris* cells within the first minutes of infection. The macrophage however is not capable of inhibit growth and replication of the fungal cells (Figure 1), suggesting that *C. auris* is equipped with detoxification mechanism to counteract the killing mechanisms imposed intracellularly. Despite the aggressive replication inside the macrophage, the cellular integrity of the phagocyte is maintained at least during the first 12 h of co-incubation, suggesting a mechanism that allows *C. auris* to dampen inflammasome activation and subsequent inflammatory signals.

At the transcriptional level, however, *C. auris* exhibits a remarkably conserved response when compared to other fungal pathogens from the CUG clade (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*), consisting of decrease in protein synthesis (downregulation of transcription and translation) and upregulation of alternative metabolic pathways (glyoxylate cycle, gluconeogenesis, beta oxidation of fatty acids, proteolysis), as well as the upregulation of transport systems and a robust oxidative stress response. Among the gene families that are prominently represented in the induced genes we found amino acid and oligopeptide transporters, as well as lipases and proteases.

Conclusions

C. auris shares the key gene expression signatures with other members of the CUG clade. However, it capitalizes on the expansion of gene families to expand the virulence attributes that allows its survival, persistence, and evasion of the immune system. Intracellular survival and replication in macrophages is conserved in this species, suggesting that this is a widespread strategy needed for successful human fungal pathogens.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P373

Diploid whole-genome MLST of Candida albicans in vulvovaginal candidiasis patients: a tool to Unravel genetic diversity and clinical phenotypes

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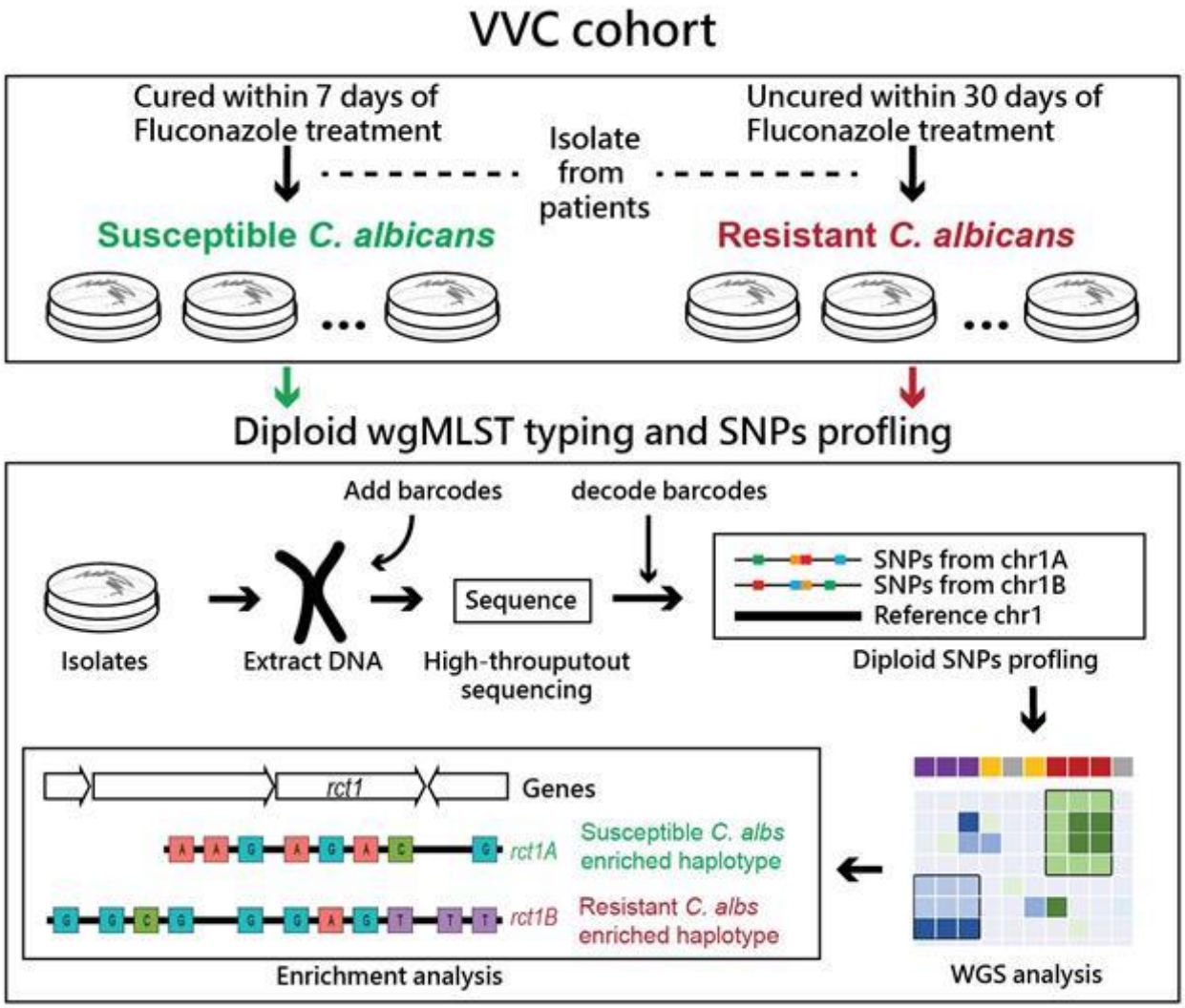
Objectives:
Candida albicans is the most common pathogen causing vulvovaginal candidiasis (VVC) and exhibits microevolution during recurrent infections, leading to the emergence of new subtypes with distinct biological characteristics. While the multilocus sequence typing (MLST) method was widely used to strain-typing *C. albicans* isolates, weak correlation between strains and clinical phenotypes were found, indicating the limitations and insufficiency of current typing methods in clinical guidelines.

Materials & Methods:
Our study presents a whole-genome multilocus sequence typing (wgMLST) method that diploid nucleotide polymorphisms (SNPs) linkage information can be distinguished. Over 100 isolates of *C. albicans* were cultured from vaginal specimens of VVC patients. A single-tube long fragment reads (stLFR) method was used for co-barcoding DNA fragments from the same chromosome during DNA library construction (Fang, C., *et al*., 2023). Sequencing reads mapped to the reference genome are grouped by the co-barcodes. Diploid profile were further generated since SNPs from the same alley chromosome sharing the same linkage information (Figure 1).

Results:
In our initial investigation, strain typing using MLST of 306 *C. albicans* isolates collected from VVC patients revealed that 66% of the isolates corresponded to the MLST 1 subtype, which exhibited a notable association with the severity of VVC. However, the non-dominant subtypes exhibited a weak correlation with clinical phenotypes, indicating the limitations and insufficiency of current typing methods in explaining clinical phenotypes. After applying stLFR co-barcoding sequencing, we obtained a diploid SNP profile that provided detailed molecular insights into the association between genetic characteristics and clinical phenotypes. For each isolate, double sets of homozygous MLST types were identified, which provided more precise strain-typing than the current heterozygous MLST approaches. By eliminating heterozygous interference, open reading frames (ORFs) were deduced without ambiguity. Notably, among all the identified SNPs, the *rct1* gene, as well as its affiliated upstream gene *chs4* and downstream gene *kip3*, depicted a conspicuous enrichment of haplotypes either in susceptible or resistant *C. albicans* strains. We also found a significant correlation between the SNPs and treatment failure, suggesting that they may contribute to a stronger resistance to antifungal drugs. However, further investigation is currently underway for validation.

Conclusions:
This study presents a new diploid whole-genome MLST method for *C. albicans* strain-typing and WGS analysis, with high-resolution at the molecular level. This new method shows great potential in addressing the limitations of current typing methods and deepening our understanding of *C. albicans* and pathogenesis of VVC.


VVC cohort
Cured within 7 days of Fluconazole treatment
Isolate from patients
Uncured within 30 days of Fluconazole treatment
Susceptible C. albicans
Resistant C. albicans
Diploid wgMLST typing and SNPs profling
Add barcodes
decode barcodes
Isolates
Extract DNA
Sequence
High-throuputout sequencing
SNPs from chr1A
SNPs from chr1B
Reference chr1
Diploid SNPs profling
rct1
Genes
rct1A
Susceptible C. albs enriched haplotype
rct1B
Resistant C. albs enriched haplotype
Enrichment analysis
WGS analysis

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Dual RNA-Seq reveals expression signatures beneficial for iron uptake and intracellular long-term interaction of Lichtheimia corymbifera (Mucorales) with macrophages

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Objectives:
Investigate the gene expression of *Lichtheimia corymbifera*, *M. circinelloides*, *Rhizopus delamar* (*Mucorales*), a pathogenic fungus, during its interaction with macrophages to understand the mechanisms of long-term intracellular survival and iron uptake.

Materials & Methods:
The following strains obtained from the Jena Microbial Resources Collection were used: *L. corymbifera*, *M. circinelloides* delta 1 and delta 2, *Rhizopus delamar*.
Cell culture was performed with Mh-S macrophages.
Using dual RNA-Seq (modified protocol of Kämmer et al. 2020[1]), we identified specific gene expression signatures associated with fungal survival in macrophages and efficient iron uptake (figure 1).

Results:
The study findings shed light on the molecular mechanisms underlying fungal pathogenesis and interplay between fungal and host cells during infection, contributing to a better understanding of how pathogens evade host immune responses (see Figure 2). Specifically, the study revealed increased expression of genes related to iron transport (FTR) in *L. corymbifera*, and to a lesser extent in *R. delamar*, while M*. circinelloides* 2 showed the highest expression of heme oxygenase 2. Furthermore, macrophages co-infected with *L. corymbifera* displayed elevated expression of nitric oxide synthase 2 (NOS2). In another hand, macrophages co-infected with R. delamar showed downregulation of NOS2 and IL1B (figure 2).

Conclusions:
Gene expression demonstrates an activation of the iron uptake pathway on the Mucorales side. On the host side, increased expression can be observed for *L. corymbifera* in NOS2, SOD2 (figure 2) which may be related to the control mechanisms used by macrophages to control infection[2]. These findings provide valuable insights into the gene expression patterns of fungal pathogens and host immune responses during infection. The downregulation of NOS2 and IL1B in *R. delamar* could be involved in immunoevasion mechanisms[2].

References

1. Kämmer, P. *et al.* Survival Strategies of Pathogenic Candida Species in Human Blood Show Independent and Specific Adaptations. *mBio* **11**, 1–21 (2020).

2. Navarathna, D. H., Lionakis, M. S. & Roberts, D. D. Endothelial nitric oxide synthase limits host immunity to control disseminated Candida albicans infections in mice. *PLoS One* **14**, e0223919 (2019).

Figure 1. Method

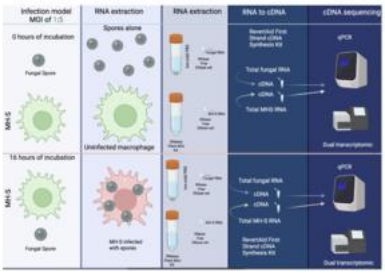

Figure 1. Performance of dual RNAseq was carried out after a modified protocol of Kämmer et al. 2020[2].

Figure 2. Results

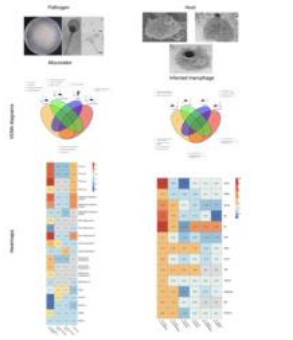

Figure 2. Expression patterns on pathogen (left) and host (right) side. Dual RNAseq VENN diagrams of the upregulated genes (top) and heatmaps of the differentially expressed genes (bottom). The heatmap on the pathogen side focuses on iron acquisition.

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Evaluation of the Whole Genome Sequencing (WGS) and Ef-1 alpha/ITS1/ITS2 sequencing for T. indotineae/mentagrophytes/interdigitale typing and identification among Belgian strains.

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Objectives: *Trichophyton (T.) indotineae* is a newly identified dermatophyte characterized as near-epidemic form on the Indian subcontinent and closely related to *T. interdigitale/mentagrophytes*. The huge rate of resistance to terbinafine of this dermatophyte is problematic and leads to extended dermatophytoses. Terbinafine resistance has been associated with mutations on the squalene epoxidase (SQLE) gene. Currently, the identification of dermatophytes at the National Reference Center for Mycoses in Liège (NRCML) is based on microscopic examination coupled with sequencing of the internal transcribed spacer 2 region (ITS2). *T. indotineae/mentagrophytes/interdigitale* cannot be distinguished by ITS sequencing. So, there is a need for efficient molecular tools to easily discriminate these species. In our study, whole genome sequencing (WGS) and Ef-1 alpha/ITS1/ITS2 sequencing have been evaluated for this purpose.

Materials & Methods: In total, 59 dermatophytes strains have been characterized. Among 53 from the NRCML collection (2018-2022), 34 were identified as *T. mentagrophytes* by conventional methods while 19 were identified as *T. interdigitale.* Six additional *T. indotineae* reference strains were included (BCCM/IHEM fungi collection, Brussels). WGS has been performed by Illumina sequencing (GIGA Genomics, Liège, NovaSeq S4 V1.5 300 cycles XP workflow). Assembly of the genome was done using SPAdes integrated in a custom made bioinformatic pipeline “WGS typer” (Hedera22, Liège). Similarity dendrogram was generated by WGS typer using the maximum likelihood method. Reference strains of *T. indotineae/mentagrophytes/interdigitale* were included on the tree. In parallel, Ef-1 alpha/ITS1/ITS2 region Sanger sequencing was evaluated as rapid identification tool in association with the generation of a dendrogram by maximum likelihood using RAxML-NG. Minimal inhibitory concentrations (MICs) of selected strains have been determined by the microdilution method EUCAST E.Def.11.0.

Results: WGS characterized 25 strains as *T. mentagrophytes*, 22 as *T. interdigitale* and 12 as *T. indotineae* including the 6 reference strains. Three distinct clades, one for each species were visible on the dendrogram based on WGS results. Ef-1 alpha gene sequencing associated with phylogenetic tree generation allowed to obtain the same discrimination power than Illumina WGS to distinguish among the three species while ITS1 and ITS2 gene sequencing didn’t permit to efficiently distinguish between *T. interdigitale* and *T. mentagrophytes*. *T. indotineae* was part of a well distinct clade considering the three studied regions. Among the *T. indotineae* clade, the SQLE coding gene has been checked for amino-acids substitutions. Seven out of 12 strains (58%) shared the F397L substitution on SQLE, and 1/12 (8,3%) presented the L393F substitution. Four out of 12 strains (33,3%) didn’t harbor any substitutions on SQLE. The MICs for terbinafine of *T. indotineae* strains have been determined: 8 (67%) out of 12 strains (also harboring SQLE substitutions) were resistant to terbinafine with MIC values >2µg/ml.

Conclusions: Our study demonstrates that since 2018, terbinafine-resistant strains of *T. indotineae* circulate in Belgium but were not correctly identified. The powerful discriminatory power of WGS for the genomic differentiation of *T. indotineae/mentagrophytes/interdigitale* species has been confirmed. Moreover, a rapid phylogenetic approach based on Ef-1 alpha gene sequencing showed to be as efficient as WGS to discriminate between the three species.

P376

A Novel Combination of mutations in Insig and Cyp51A confers multi-azole Resistance to Aspergillus fumigatus

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Objectives:
Aspergillus fumigatus is an opportunistic pathogenic fungus that causes aspergillosis. Azole antifungal agents play a pivotal role in the treatment of aspergillosis. Recently, the incidence of azole-resistant *A. fumigatus* in clinical settings and the environment is rising and is becoming a serious problem worldwide. Several genetic mutations that cause azole resistance in *A. fumigatus* have been reported, although some mechanisms of resistance remain unclear. In recent years, several reports have shown that focusing on multiple gene phenotypes is essential to elucidate the whole picture of azole drug resistance mechanisms in *A. fumigatus*. In this study, we report that mutations in Insig, a regulator of lipid metabolism, contribute to azole drug resistance in concert with Cyp51A mutations.

Materials & Methods:
To search novel factors associated with azole resistance, comparative genomic analysis was performed on *A. fumigatus* strains with different susceptibilities to azoles isolated serially from the same patient. To verify the association between newly found genetic variants and azole resistance, mutant alleles were replaced with wild-type alleles by the CRISPR-Cas9 system in clinical isolates. Antifungal susceptibility tests were performed according to CLSI-M38.

Results:
Four *A. fumigatus* strains isolated from two different patients were studied. Case A: The MICs of the 1st isolate of itraconazole (ITCZ), voriconazole (VRCZ), posaconazole (PSCZ) and isavuconazole (ISCZ) were 2, > 8, 1 and 8, whereas the ones of 2nd isolate were 8, > 8, 1 and > 8, respectively. The numbers of short tandem repeats of these two strains were identical, and same mutation (Gly448Ser) in Cyp51A was confirmed. Case B: The MICs of the 1st isolate of ITCZ, VRCZ, PSCZ and ISCZ were 1, 1, 0.25 and 0.5, whereas the ones of 2nd isolate were 4, > 8, 0.5 and 8, respectively. The numbers of short tandem repeats of these two strains are identical. 2nd isolate confirmed the mutation (Gly448Ser) in Cyp51A. The genome comparison analysis revealed mutations in the gene encoding insulin-inducible protein (Insig) in the two multi-azole resistant strains carrying the Gly448Ser mutation in Cyp51A. The mutations found in Insig were a nonsense mutation (Trp320*) and an unfinished mutation (cDNA502-533del). Replacing the mutated insig gene with the wild-type gene in these clinical isolates restored susceptibility to ITCZ and ISCZ. On the other hand, susceptibility to VRCZ was unchanged. Replacing the wild-type insig gene with the mutated insig gene in the experimental strain Afs35 did not change susceptibility to azoles.

Conclusions:
This study identified a novel genetic alteration associated with azole resistance. The Insig mutation contributes additively to azole resistance in concert with the Cyp51A mutation, but not by itself. These results indicate that focusing on the phenotypes of multiple genes is essential to gain a complete picture of the azole resistance mechanism in *A. fumigatus*.

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Assessing the importance of Iff cell wall adhesins for virulence of the pathogenic yeast Candida auris

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Infections caused by *Candida auris* are a major public health concern due to their high mortality rates. In addition, this yeast is multidrug resistant and shows a high persistence in the hospital environment.

Objectives: The aim of this work was to evaluate the involvement of six *C. auris* cell wall adhesins of the Iff family, previously identified in cell wall preparations, in the virulence *in vivo* using the nematode *Caenorhabditis elegans* and the larva of the lepidoptera *Galleria mellonella* as host models.

Materials & Methods: The virulence of 14 strains of *C. auris*, including 13 mutants with single and multiple deletions of up to six genes (*iff4Δ/iff1Δ/iff6Δ/rbr3Δ/hyr3Δ/iff6.4Δ*) of the Iff family and parental strain VPCI479/P/13 was evaluated. Mutants were generated by homologous recombination using recyclable *SAT1*-flipper constructs, aided by RNP-based CRISPR-Cas9. *In vivo* virulence was tested in the *G. mellonella* model by inoculating 10^6 *Candida* cells per larva. Post-infection, the larvae were incubated at 37°C, and survival was assessed every 24 h for 120 h. In the case of the *C. elegans* model, age-synchronized L4 larval populations were used. These nematodes were transferred to plates with *Candida* growth, and infected by feeding for 2 h. The nematodes were then incubated at 25°C and survival was assessed by visualization under a stereomicroscope every 24 h up to 96 h. Differences in survival in the two models were analyzed and compared using the log-rank test. Values of $p < 0.05$ were considered statistically significant.

Results: The parental strain showed high virulence in both models with a survival rate of 1.7% at 120 h in *G. mellonella*, and 24.6% at 96 h in *C. elegans*. In *G. mellonella*, survival at 120 h was highest in larvae infected with *iff4Δ/iff1Δ/iff6Δ* (16.7%), followed by *hyr3Δ* (13.3%), *rbr3Δ* (11, 7%), *iff6Δ* (10%) and *iff4Δ/iff1Δ/iff6Δ/rbr3Δ/hyr3Δ/iff6.4Δ* (8.3%). All these adhesin mutants were significantly less virulent than the parental strain. In the *C. elegans* model, the mutans *iff4Δ/iff1Δ/iff6Δ/rbr3Δ, iff4Δ/rbr3Δ, Iff6Δ/rbr3Δ*, and *iff4Δ/iff1Δ/iff6Δ/rbr3Δ/hyr3Δ/iff6.4Δ* showed lower virulence compared to the parental strain.

Conclusions: Deletions of *IFF6, RBR3, HYR3* and cumulative deletions of *IFF1, IFF4* and *IFF6* as well as *IFF1, IFF4, IFF6, IFF6.4, RBR3* and *HYR3* reduce the virulence of *C. auris* in *G. mellonella*. The mutant with deletions in six *IFF* genes (*iff4Δ/iff1Δ/iff6Δ/rbr3Δ/hyr3Δ/iff6.4Δ)* was less virulent in both infection models. Altogether, these results underline the importance of Iff cell wall adhesins for virulence of the multidrug resistant pathogenic yeast *C. auris*.

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Pangenome Analysis Predicts Specific Genes in Molecular Identification of Mucorales

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Abstract

Background: Mucoralean fungi could cause mucormycosis in humans, particularly in immunodeficient individuals. The morbidity and mortality of mucormycosis were increasing rapidly in developing countries. Our retrospective study showed Rhizopus species, Mucor species, and Lichtheimia species have high proportions.

Objectives: To screen the specific genes, genomic variations were analyzed between Mucorales and non Mucorales, and intraspecies of Mucorales

Methods & Materials: based on the whole genome sequences of Rhizopus arrhizus, Mucor irregularis, Mucor hiemalis, and Lichtheimia corymbifera, as well as 43 medical important fungal pathogens.

Results: Mucorales-specific genes such as STE/STE20 protein kinase, GH36, and sel1 repeat protein were found, while genus specific genes were annotated covering cellular structure, biochemistry metabolism, molecular processing, and signal transduction. Proteins related to the virulence of
Mucorales species varied with distinct significance in gene copy numbers, of which
112092, cotH3, gcn4 and igp1 exhibited differential gene copy numbers among the
included candidate species.

Conclusion: Thus, our study dug the target genes for monitoring Mucorales species, which may potentially provide convenience in molecular identifications of mucormycosis

P380

A study on function and localization of Sporothrix globosa Cyclophilin B

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Objectives

The gradual spread of *Sporothrix globosa* from the initial infection to the distal end of the lymphatic vessels is an important characteristic of this disease. The invasive behavior of *S. globosa* infection is closely related to its adhesion to lymphatic endothelial cells and the inflammation caused by the fungus. Cyclophilin B (CypB) in pathogenic fungi plays an important role in the process of fungal invasion and adhesion. This study aims to investigate the antigenicity, immunogenicity, and subcellular localization of CypB of *S.globosa*.

Materials & Methods:

Bioinformatics methods were employed to identify the *CypB* gene of *S.globosa* (*Sg.CypB*) and its encoded protein (*Sg.*CypB) from the *S.globosa* genome database. The recombinant *Sg.*CypB protein (r*Sg.*CypB) was expressed by a prokaryotic expression system. Antibodies against *Sg.*CypB were generated from rats, which were injected with r*Sg.*CypB. Titers of antibodies were evaluated with ELISA. Immunofluorescence staining of *S.globosa* was performed with antibodies to examine the subcellular localization of *Sg.*CypB.

Results:

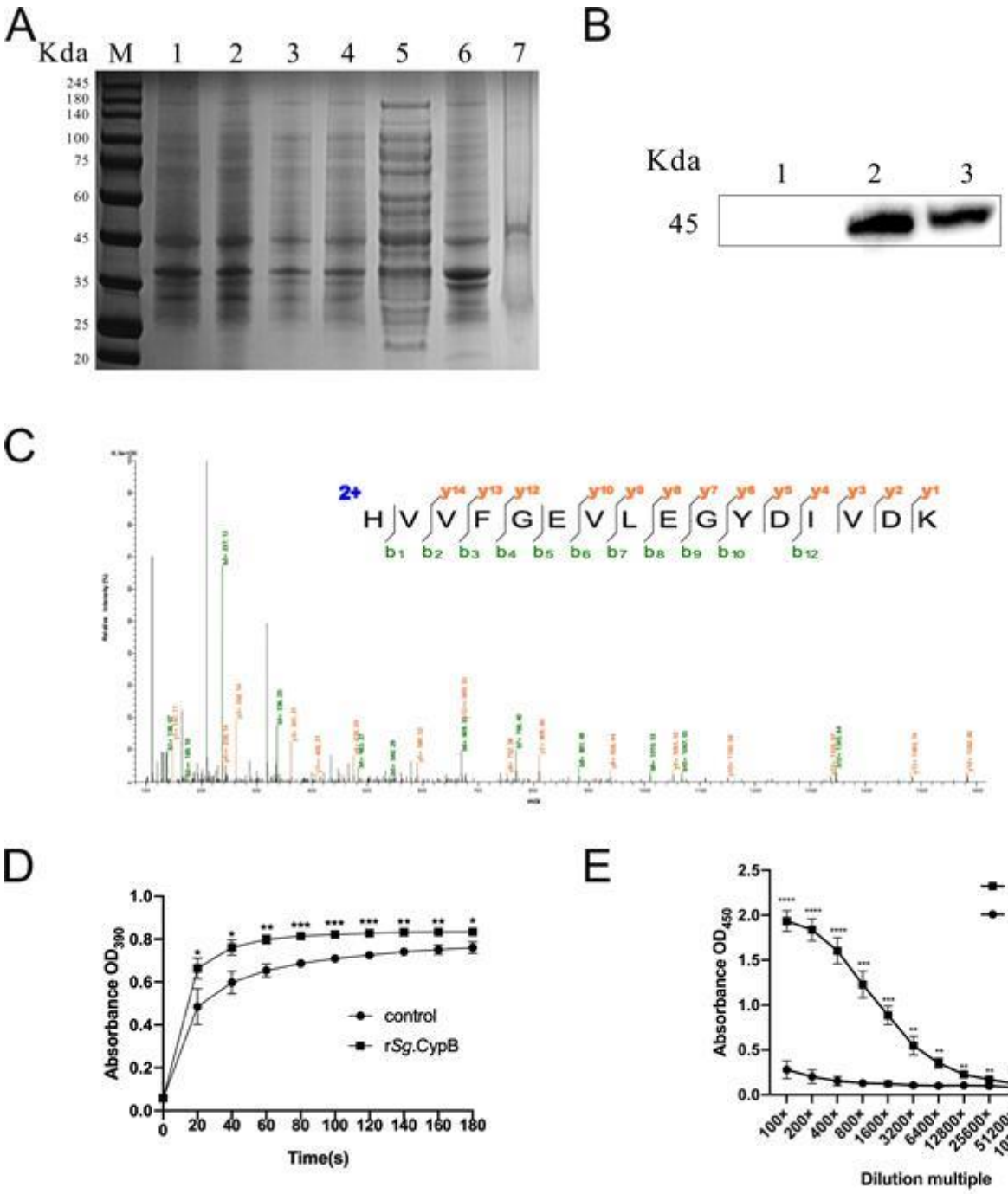
The *Sg.CypB* gene was 930-bp long, encoding a 309-amino-acid protein (*Sg.*CypB). It was predicted that *Sg.*CypB may be a membrane-associated protein with a typical signal peptide and a single transmembrane region at its C-terminus. The majority of *Sg.*CypB was exposed to an extracellular environment, processing a typical peptidyl-prolyl cis-trans isomerase (PPIase). The PPIase activity can also be detected in r*Sg.*CypB(Fig. 1). With antibodies (titer reach 1:51200, $P<0.05$), immunofluorescence staining demonstrated *Sg.*CypB localizing on the *S. globosa* cell surface(Fig. 2).

Conclusions:

*Sg.*CypB is an antigen with PPIase activity located on the *S. globosa* surface, making it possible to play an important role in fungus–host cell interaction.

Fig. 1 (A) Expression and purification of r*Sg*.CypB protein analyzed by SDS-PAGE; Lane M, protein marker; lane 1, pET-30(a) transformant without IPTG induction; lane 2, pET-30(a) transformant with IPTG induction; lane 3, pET-30(a)-*Sg.CypB* transformant without IPTG induction; lane 4, pET-30(a)-*Sg.CypB* transformant with IPTG induction; lane 5, the lysate of recombinant bacteria with IPTG induction; lane 6, the supernatant of the lysate of recombinant bacteria with IPTG induction; lane 7, purified r*Sg*.CypB protein; (B) Western blot analysis of r*Sg*.CypB protein expression. lane 1, pET-30(a)-Sg.CypB transformant without IPTG induction; lane 2, pET-30(a)-*Sg.CypB* transformant with IPTG induction; lane 3, purified r*Sg*.CypB protein;(C) LC-MS/MS analysis of r*Sg*.CypB protein;(D) PPIase activity test of r*Sg.*CypB with chymotrypsin-coupled assay;(E) Effect detection of the rat anti- rSg.CypB serum (Arabic numerals in the horizontal coordinates represent dilution);* refers to significant difference ($P < 0.05$), ** refers to significant difference ($P < 0.01$), *** refers to significant difference ($P < 0.001$), **** refers to significant difference ($P < 0.0001$)

Fig. 2 (A) Immunolocalisation of *Sg.*CypB in the mycelial phase and yeast phase of *S. globosa*; (B) Compared with antisera, the negative results of control groups with pre-immune serum and PBS. All images were taken at 630×(scale bar, 10 μm), 1260×(scale bar, 5 μm)


A
Kda M 1 2 3 4 5 6 7
245
180
140
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75
60
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35
25
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B
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1 2 3
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C
2+
H V V F G E V L E G Y D I V D K
b1 b2 b3 b4 b5 b6 b7 b8 b9 b10 b12
y14 y13 y12 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
D
Absorbance OD390
1.0
0.8
0.6
0.4
0.2
0.0
0 20 40 60 80 100 120 140 160 180
Time(s)
control
rSg.CypB
E
Absorbance OD450
2.5
2.0
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100× 200× 400× 800× 1600× 3200× 6400× 12800× 25600× 51200× 102400× 204800×
Dilution multiple
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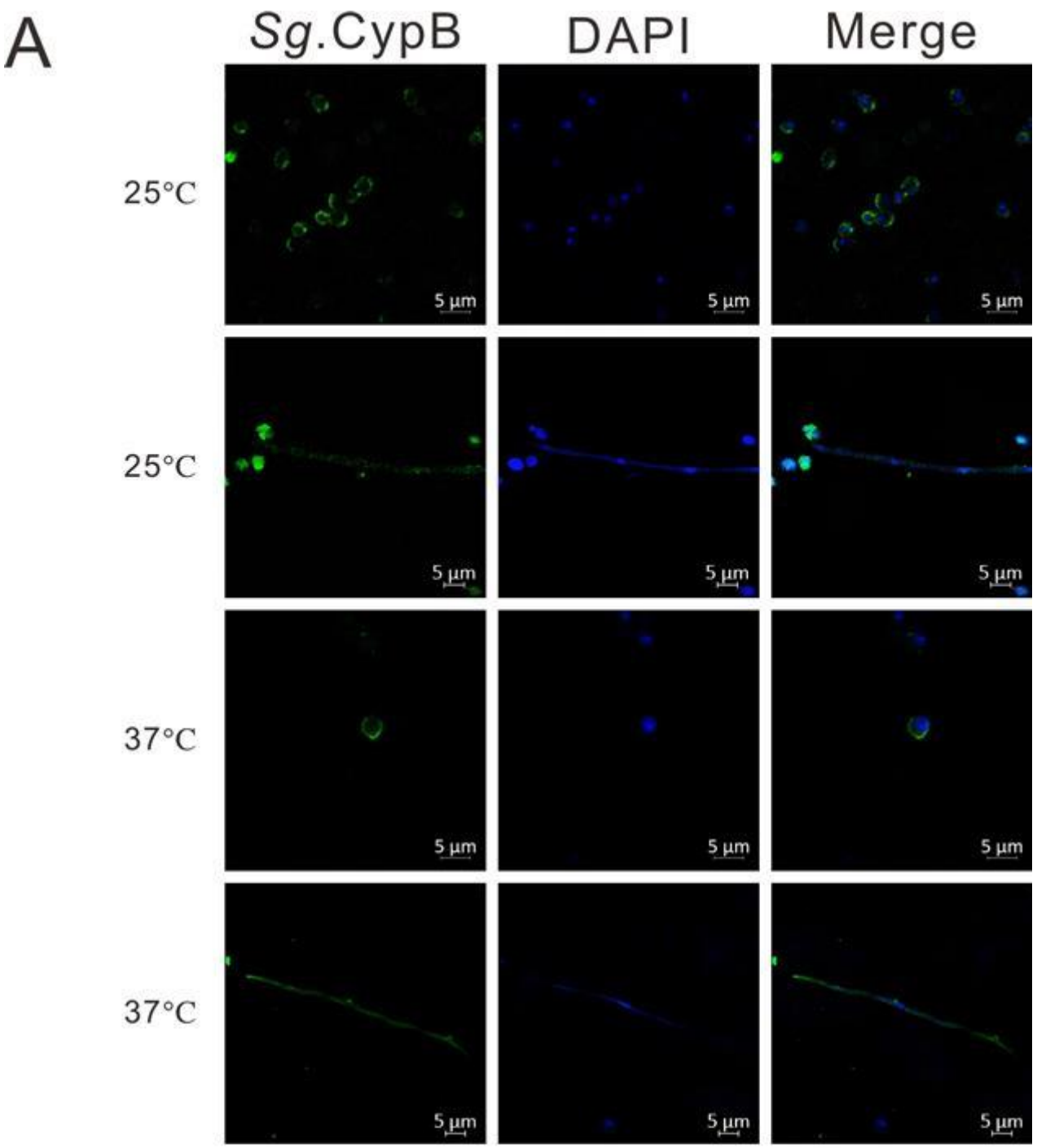
A
Sg.CypB
DAPI
Merge
25°C
25°C
37°C
37°C
5 μm
B
BF
Sg.CypB
DAPI
Merge
S.globosa +antisera
S.globosa +pre-immun serum
10 μm

P381

β-glucan masking to evade the host immunity in the emerging pathogen Candida auris

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[Objectives]
Candida auris is an emerging pathogenic yeast that has been designated as a global public health threat. Despite this, the immune response against *C. auris* infection is still not well understood. Humans fight *Candida* infections through the immune system that recognizes β-glucan and mannan on fungal cell wall. The objective of this study is to check the levels of β-glucan and mannan exposure in *C. auris* grown under different culture conditions and to determine the *in vitro* and *in vivo* effect of these changes in the cell wall on the immune response.

[Materials & Methods]
In this study, a clinical isolate, MYA 5001 and MYA 5002 of *C. auris* were used. *C. auris* strains were subjected to different physiologically relevant stimuli and changes in β-glucan and mannan levels were detected by staining and flow cytometry. To determine the effect of these changes on the immune response, a phagocytosis assay involving mammalian cell cultures was performed followed by quantification of released cytokines by ELISA and measurement of ROS production. *Bombyx mori* was used in the infection assay to check the effect of cell wall changes in the virulence of *C. auris.*

[Results]
Flow cytometric analyses of stained cells revealed that lactate and hypoxia trigger a reduction of β-glucan, while low pH triggers an increase in β-glucan. There was no inverse relationship between exposure levels of β-glucan and mannan. The reduction of β-glucan led to a decrease in the uptake of *C. auris* by PMA-differentiated THP-1 and RAW 264.7 macrophages. Furthermore, lactate-induced β-glucan masking in *C. auris* led to reduced MIP-1α production upon co-incubation with the macrophages, but TNF-α and IL-10 levels and macrophage ROS production remained the same. For the *in vivo* infection analysis using silkworm, the decrease in β-glucan on the fungal cell wall was found to increase the lethality of *C. auris*.

[Conclusions]
The results demonstrate that the β-glucan masking phenomenon in *C. auris* occurs as an escape mechanism of this fungus from immune cells. This study sheds light on the dynamics of yeast cell wall remodeling and immune cell interaction.

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Phenotypic response and RNA-seq profile of PIG1- deleted conidia in Scedosporium apiospermum

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Objectives

Virulence factors of *Scedosporium apiospermum* which contribute to its pathogenicity are still poorly characterized. Little is known about the specific role of dihydroxynaphtalene (DHN)-melanin, a key cell wall element, involved in the early recognition by the host immune system. In *S.apiospermum,* a previously identified transcription factor PIG1 may be involved in the successive enzymatic reactions supporting the DHN-melanin biosynthesis. The present work was aimed to i) characterize the conidial phenotype of Δ*PIG1* melanin-deficient strains exposed to various stressful conditions including incubation with human macrophages, and ii) to analyze their transcriptomic profile compared to parental strains, as a starting point toward the elucidation of PIG1 functions and DHN-melanin involvement in *S. apiospermum* virulence processes.

Methods

The *PIG1* deletion was performed using CRISPR Cas9 genome editing from two parental melanized *S. apiospermum* strains, the wild-type 14462 strain (WT) and the *ΔKU70* deleted 14462 strain (*ΔKU70*), in which the homologous recombination needed for transformation is facilitated. After molecular validation, different phenotypical tests were performed on *PIG*-deleted conidia, including melanin detection using electronic paramagnetic resonance (EPR), exploration of the cell wall structure using transmission electronic microscopy, quantification of polysaccharide exposure to conidial surface using fluorescent lectins and cell wall integrity assays (Congo Red, SDS). Resistance of *ΔPIG1* disruptants to oxidizing conditions (cumene hydroperoxide), ultraviolet light, high temperature (50°C) and survival to macrophage killing were also explored. The RNA-seq profile of a *ΔPIG1*- deleted strain and its parent was performed to identify differentially expressed genes (DEGs) involved in the phenotypic changes observed.

Results

The absence of functional PIG1 in the three *ΔPIG1* disruptants was phenotypically associated with the absence of DHN-melanin in the cell wall, as revealed by the unpigmented *ΔPIG1* colonies, and confirmed by EPR. The cell wall thickness was reduced in melanin- deficient Δ*PIG1* mutants by a factor 1.5 to 2.7. Unexpectedly, *PIG1* deletion triggered changes in conidia size and shape, as more than 40% of large septate conidia were observed. The absence of melanin significantly increased the exposure of polysaccharide residues compared to melanized conidia. The ability of Δ*PIG1* mutants to grow in oxidizing conditions was drastically hampered compared to the parental strains, as well as the survival to heat shock and macrophage engulfment. The transcriptomic analysis revealed 278 DEGs of which 115 and 163 were respectively up- and down-regulated in the Δ*PIG1* disruptant. Among the 198 DEGs

with a known function, the most represented classes were related to oxydoreduction processes (N=43), transmembrane transport (N=23), and carbohydrate metabolism (N=16).

Conclusion

These results confirm the regulatory role of PIG1 on melanin biosynthesis in *S. apiospermum* conidia. Structural changes and poor survival to stressful conditions of Δ*PIG1* conidia foreshadow the role of melanin/PIG1 in fungal biological processes. Supported by the transcriptomic analysis, this study highlights the pleiotropic action of PIG1 in *S. apiospermum* beyond melanin regulation, including on the cell-wall architecture and enzymatic pathways crucial in the protection of conidia against environmental damages and macrophage killing, and possibly, in *Scedosporium* virulence, that will be further explored in a murine model.

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De novo whole genome sequence of Myriodontium keratinophilum, an emerging dermatophyte pathogen

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De novo whole genome sequence of *Myriodontium keratinophilum*, an emerging dermatophyte pathogen

Amtoj Kaur[1*], Arijit Pal[2*], Sandhyarani Mahanto[2], Priya Nagpal[2], Gulnaz Bashir[3], Ashutosh Singh[1,4], Kusum Jain[1], Vivekanandan Perumal[2], Anuradha Chowdhary[1, 4]

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Objectives: Dermatophytosis is the fourth most common human disease affecting ~25% of the global population, with morbidity more than that of cardiovascular diseases. Currently, the etiological agents of these superficial fungal infections are going beyond the commonly encountered species of *Trichophyton*, *Microsporum* and *Epidermophyton*. Here, we report *de novo* whole genome sequence of a rare keratinophilic fungi, *Myriodontium keratinophilum*, causing an outbreak of dermatophytosis in Kashmir, India.

Materials & Methods: Isolate was retrieved from a patient with tineae capitis and was identified through sequencing of the internal transcribed spacer (ITS) region. Genomic DNA was isolated using the phenol-chloroform-isoamyl alcohol extraction and ethanol precipitation method. An Oxford Nanopore Technologies (ONT) library was constructed using the Native Barcoding Kit 24 (SQK-NBD112.24) which was sequenced using Flow Cell R10.4 on a GridION Mk1 instrument. Base-calling was performed using Guppy v6.0.7. Quality check of the raw reads was done using FastQC version 0.11.5. High quality sequence reads (Q score≥ 10) were considered for *de novo* assembly construction using Flye 2.9.1. Medaka v1.8.1 was used to polish the generated *de novo* assembly and the quality of the generated consensus was assessed using QUAST toolkit.

Results: 379 ng/µL of extracted genomic DNA was yielded. BLAST analsysis of ITS sequence confirmed the isolate to be *M. keratinophilum* with 100% similarity to CBS strain 256.81. FastQC data indicated ~99.9% base-calling accurary (Phredscore: 29) with no error flag and no overrepresentation of genomic region in the constructed library. QUAST demonstrated generation sufficient number (536146) of ONT

reads with satisfactory N50 of ~0.71Mb and over-all coverage of 44x. The polished consensus of *de novo* assembly of *M. keratinophilum* was found to be 24.79 Mb long covered by 80 contigs with largest contig size being 1.8 Mb. The GC content of the assembly was estimated 47.12% with 48 contigs having size greater than 50 kb.

Conclusions: With increasing worldwide reports of resistant dermatophytes like *Trichophyton indotineae*, it becomes extremely important to study rare pathogenic fungi causing difficult-to-treat recalcitrant dermatophytosis at an early stage. This study will help to get important genomic insights into the cause of high virulence of *M. keratinophilum*, so that, an extensive spread can be avoided with accurate treatment strategies.

This work was supported in part by research grant from Science and Engineering Research Board [SERB File No. CRG/2020/001735], Department of Science and Technology, Government of India, New Delhi, India.

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Zataria multiflora-loaded nanostructured lipid carrier topical gel as a new approach in onychomycosis treatment: a randomized double-blind placebo-controlled clinical trial

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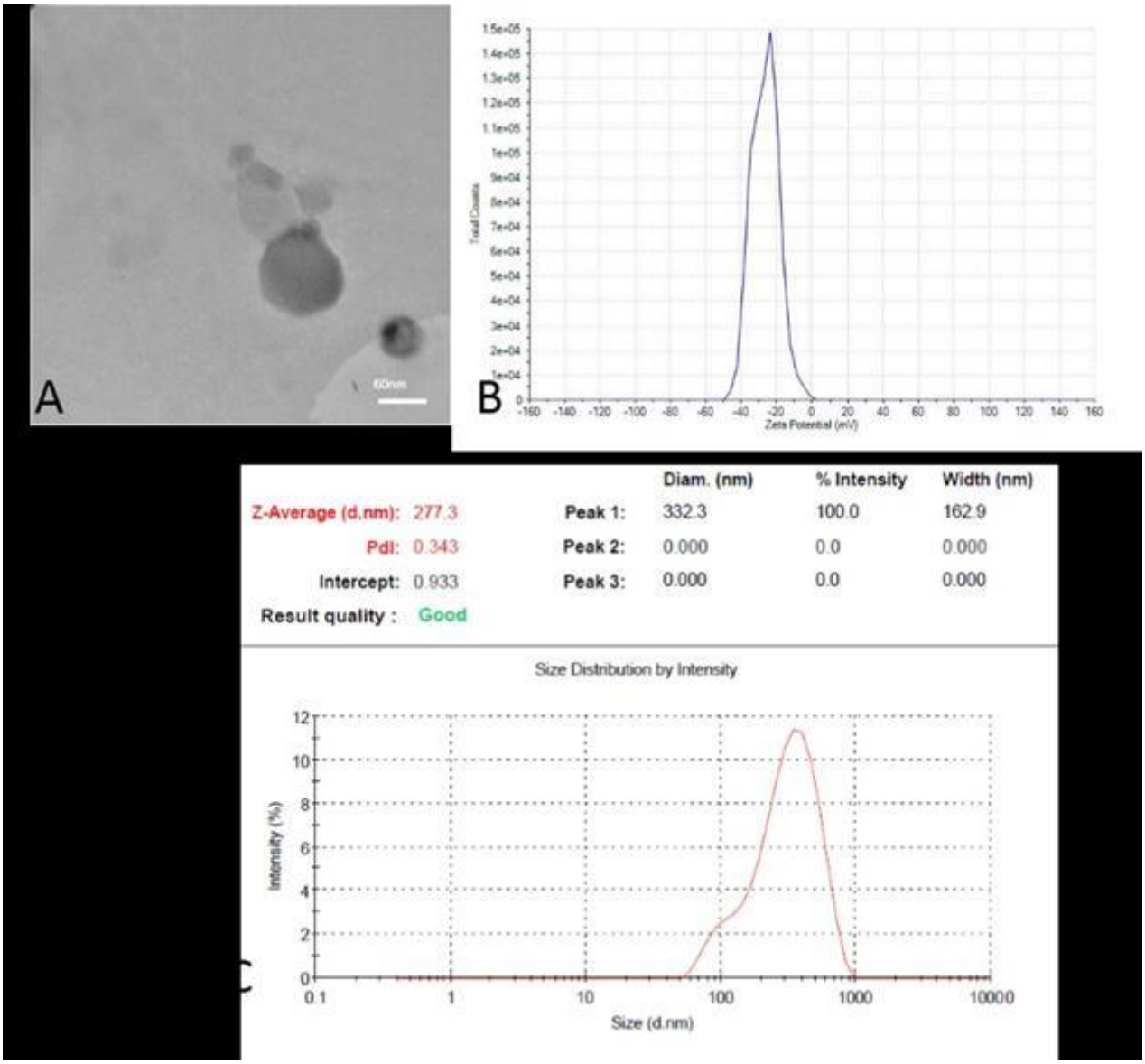
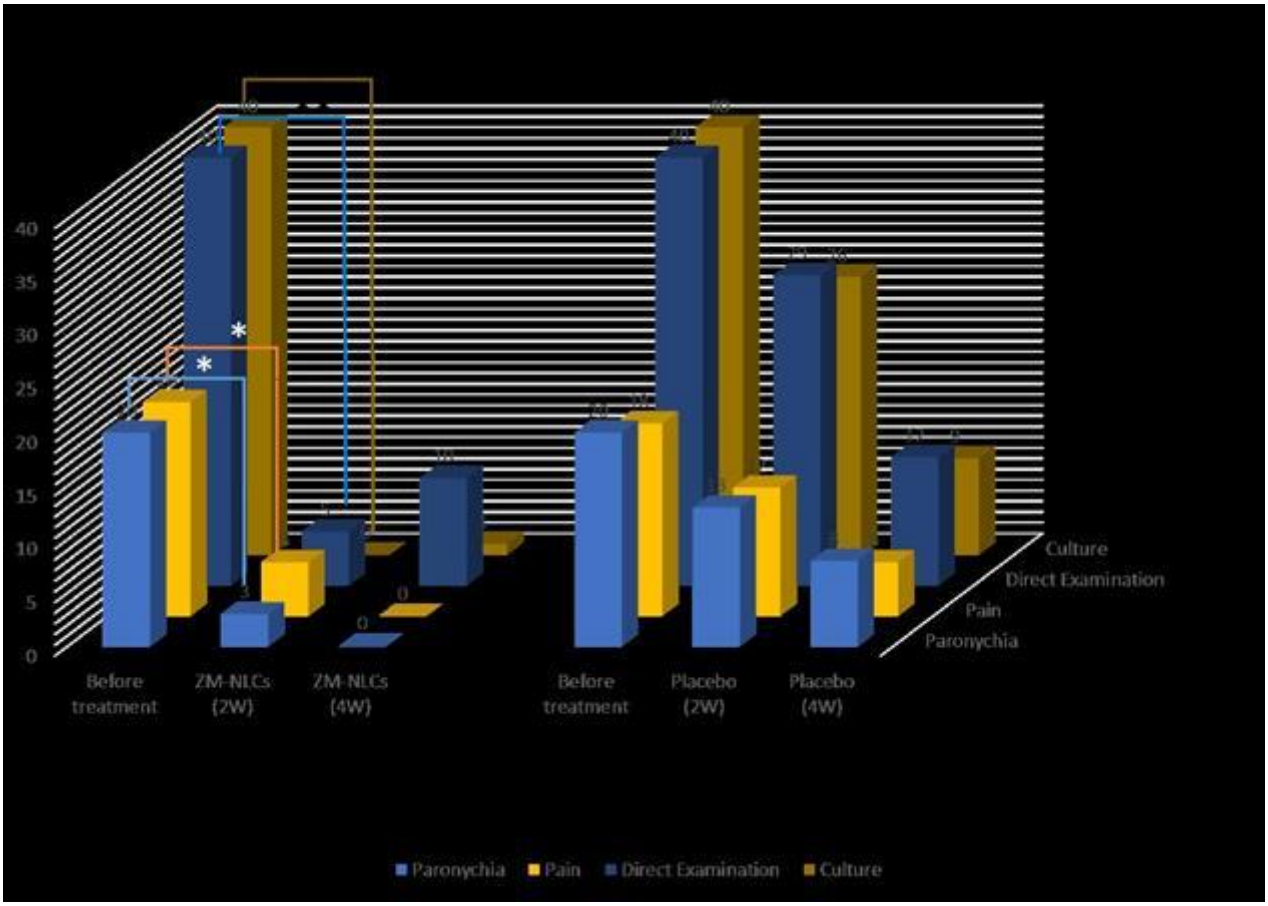
Objectives:
The increasing resistance to onychomycosis treatment globally is a major concern, resulting in treatment failure. Therefore, there is a significant need for alternative and effective antifungal agents to replace or complement the current front-line treatment. The objective of this research was to investigate the efficacy of nanostructured lipid carriers (NLCs) incorporated in a gel formulation for the treatment of mild to moderate *Candida*-associated onychomycosis using *Zataria multiflora* (ZT) essential oils
Materials & Methods:
Zataria multiflora-loaded nanostructured lipid carriers (Zt-NSLCs) were formulated as a 1% w/w carbopol gel, and the properties of the NLCs were confirmed. *In vitro* antifungal susceptibility testing was performed on 10 commonly encountered dermatophyte species according to the CLSI M60 guidelines. A clinical study was conducted with 80 volunteers, who were randomly assigned to two groups (Zt-NSLCs gel and placebo) in a double-blind, placebo-controlled design. The objective was to assess the clinical manifestations and mycological findings following topical application for 2 and 4 weeks. The species of the causative agents were identified using a PCR-RFLP method.
Results:
The preparation of Zt-NSLCs gel yielded a uniform suspension of spherical nanoparticles, exhibiting favorable characteristics and no cytotoxic effects. Zt-NSLCs demonstrated a significant inhibitory effect on fungal growth and led to effective improvement in clinical and mycological criteria compared to the placebo group ($p < 0.005$) even after 2 weeks of treatment. C. albicans complex was identified as the predominant species isolated from the patients using PCR-RFLP.
Conclusions:
This randomized controlled clinical trial demonstrates the promising strategy of integrating an alternative medication with a nano-scaled colloidal system to achieve a safer, faster, and more effective treatment for *Candida*-associated onychomycosis. The use of Zt-NSLCs gel for a duration of only two weeks resulted in significant improvement in disease management according to both dermatologists and mycologists.

Figure legends:

Fig. 1. (a) Presents TEM images of Zt-NSLCs, revealing the presence of spherical nanoparticles with an estimated size of 200-300 nm. The scale bar represents 60 nm. (b) Illustrates the zeta potential of Zt-NSLCs, showing a negative zeta potential of -26.6 ± 7.7 mV, indicating the favourable stability of the nanoparticles. (c) displays the particle size distribution and PDI index graph, demonstrating satisfactory results with a PDI index of 0.34 ± 0.03 and a particle size of 277.3 ± 3 nm.

Fig. 2. Patients who received Zt-NSLCs gel exhibited significant negative results in both KOH 10% direct examination after a 2-week prescription period. Clinical criteria were also reduced meaningfully, nevertheless, more significant result were observed after 4 weeks of application.




40
35
30
25
20
15
10
5
0
*
*
Before treatment
ZM-NLCs (2W)
ZM-NLCs (4W)
Before treatment
Placebo (2W)
Placebo (4W)
Culture
Direct Examination
Pain
Paronychia
Paronychia
Pain
Direct Examination
Culture

P385

Antivirulence drug discovery to disarm Candida albicans with metabolites from myxobacteria.

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Only four classes of antifungals are available to physicians to treat and prevent severe Candidiasis. These drugs directly kill or inhibit the growth of *C. albicans*, thereby exerting a strong selective pressure towards drug resistance. Antivirulence drugs, by contrast, target virulence traits of pathogens without affecting their growth, making drug resistance less likely to evolve. We seek to discover novel antivirulence compounds against *C. albicans* that keep it at, or return it to, its baseline commensal state even under conditions that otherwise support pathogenicity.

To find novel antivirulence drugs, we screened ≈2,800 extracts from an untapped clade of bacteria – myxobacteria – in an *in situ C. albicans*-mammalian epithelial cell infection model. Based on the readouts of the host cell damage, and fungal growth and morphology, we prioritized the extracts based on their antivirulence and antifungal properties. From the top-ranked hits, we selected antivirulence hits that did not significantly affect the fungal metabolic activity, but reduced host cell damage as confirmed by an independent propidium-iodide based assay. A combination of biological assays as well as dereplication of these myxobacteria crude extracts via LC-MS analysis identified myxoquaterines, which were recently described to show moderate antifungal activity (Popoff, 2020), as potentially promising compounds.

With our combined host-pathogen screening pipeline, we were therefore able to identify several compounds that protected mammalian epithelial cells from *C. albicans*. Efforts to characterize the antifungal and antivirulence mechanisms of myxoquaterines using large-scale *Candida* spp. knock-out libraries and multi-omics approaches are underway.

References

Popoff, A., 2020. Exploiting the biosynthetic potential of myxobacteria for natural product discovery. https://doi.org/10.22028/D291-31279

P386

Determination of toxicity of compounds with antifungal activity on a fruit fly model

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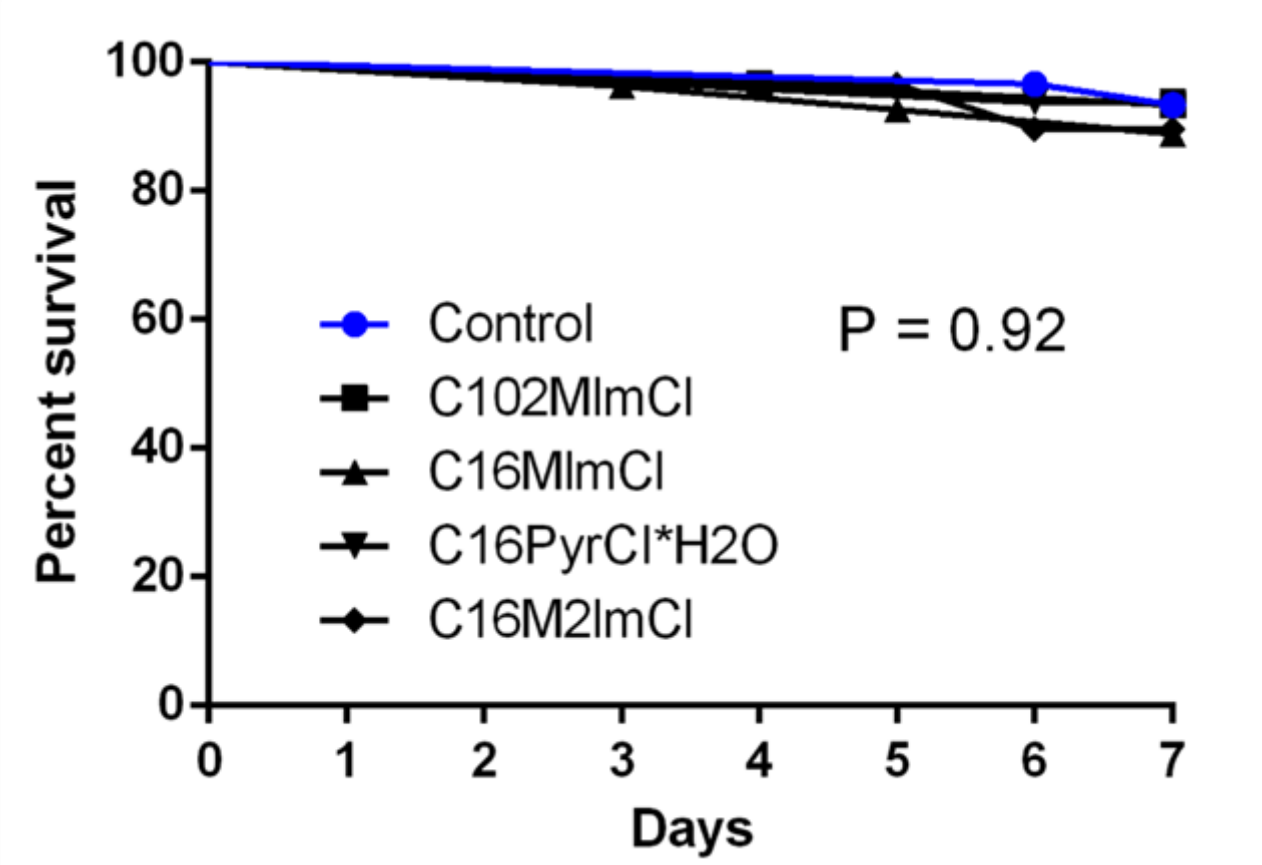
Objectives: To evaluate the toxicity of imidazolic compounds on a fruit fly model.
Materials & Methods: The molecules, identified as 3: (C10)2MImCl; 5: C16MImCl; 7: C16PyrCl*H2O and 8: C16M2ImCl, were subjected to a toxicity test, in which the model used was the fruit fly (*Drosophila melanogaster*). After a 6 hour fasting, Oregon R *wild-type* flies (n = 30) were placed in sterile bottles containing food, to which a 100 µL suspension of molecule at 1mg/mL was added. The process was done with each molecule. After 7 days the survival curves for each molecule were generated using the Kaplan-Meier method, and then analysed using logrank's test. Negative control was made with a sterile 0,85% saline solution.
Results: None of the molecules showed toxicity to the flies, due to the fact that there was no significant diference in the survival curves of the molecules compared to the negative control.
Conclusions: There was no difference observed in the mortality between the treatments and the negative control, thus the molecules showed no signicant signs of toxicity on the fruit fly model.



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Design and evaluation of nanoencapsulated oregano essential oil as alternative treatment to Candida albicans infection

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Objectives:

Vulvovaginal candidiasis (VVC) is characterized as a very common fungal infection with a huge negative impact on women's health worldwide. The limited effective and safe therapies available and the consequent increase in resistance to antifungal agents, make the development of new fundamental therapies crucial. Natural products, such as essential oil (EOs), are currently being evaluated regarding their antimicrobial activity. However, the EOs effect depends on several factors such as photosensitivity, high volatility, low water-miscibility, and degradability when exposed to temperature, decreasing their bioavailability. To overcome these limitations, micro or nanoencapsulation has emerged as an efficient technique to protect and control the release of EOs, improving the water-solubility and bioavailability of lipophilic compounds. Thus, the main goal of this study was to produce and characterize nanoparticles of keratin (KNP´s) loaded with oregano essential oil (OO-KNP´s) as an alternative treatment for VVC.

Materials & Methods:

The OO- KNP's were produced by ultrasound cycles through a high-intensity ultrasonic and characterized regarding morphological and physicochemical parameters (particle stability,

OEO encapsulation efficiency and release profile). First, the OO-KNP´s effect against *C. albicans in vitro* was evaluated by broth microdilution and diffusion in agar. The activity against biofilm was quantified by colony forming units' enumeration (CFUs). Then, the efficacy of OO-KNP's on *in vivo* VVC mouse model was also studied. For this, 20 female BALB/C female mice (18.7±1.2 gr of weight) were infected with $1.33x10^8$ CFU/mL of *C. albicans* and 24h after the infection, 11 animals recived single dose of OO-KNP's intravaginally and the rest of the animals recived saline solution, remaining as controls. Vaginal fluid were collected in all the animals 24h and 48h after the treatment to quantify *C. albicans* and *Lactobacillus* species growth in culture medium (CFUs/ mL).

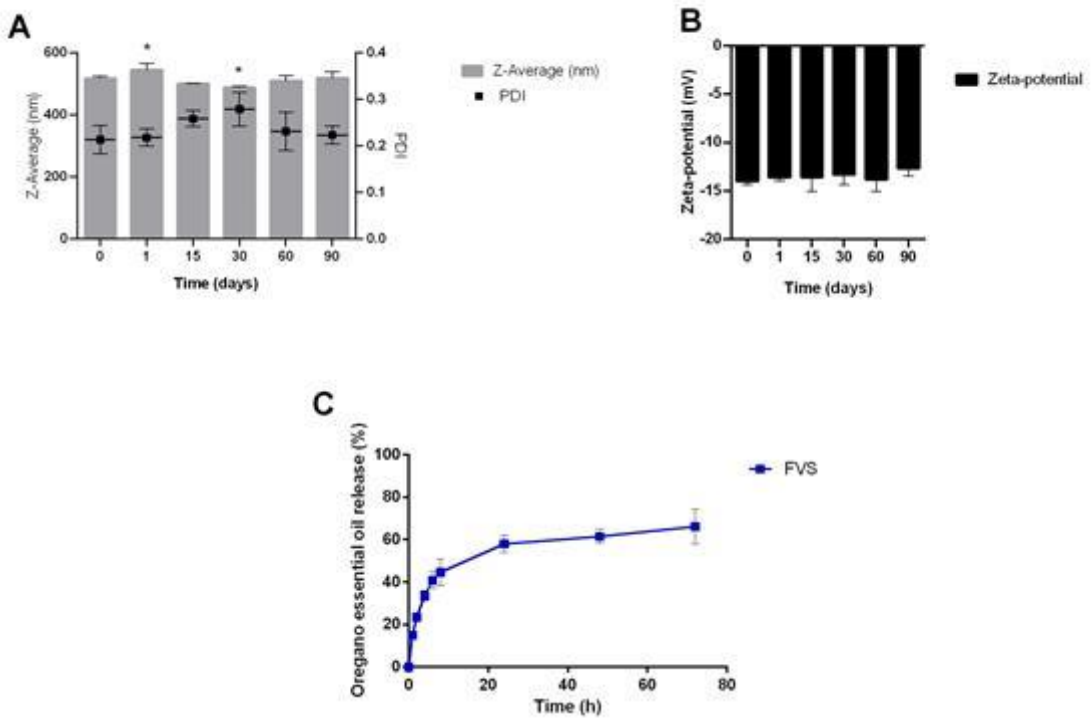
Results:

The OO-KNP´s remained stable over time and exhibited high encapsulation efficiency (99.42 %). Furthermore, a controlled rate of OEO release was also observed during the first 24h in the synthetic vaginal fluid, due to the destabilization of the particles in this medium. The size of these particles, approximately 500 nm, which is suitable for penetration of delivery systems inside fungal cells. In fact, a total inhibition of the planktonic growth of *C. albicans* was obtained. Furthermore, the results showed that, *in vitro*, the application of only 2.5% OO-KNP´s eradicates mature *C. albicans* biofilms while preserving the *Lactobacillus* species. In *in vivo*, a single intravaginal application of OO-KNP´s induced a reduction of *C. albicans* growth (0.6 Log CFU/mL). Furthermore, one of the most important factors, this therapy keeps intact the remaining microflora in relation to the *Lactobacillus* species, confirming previuos *in vitro* results.

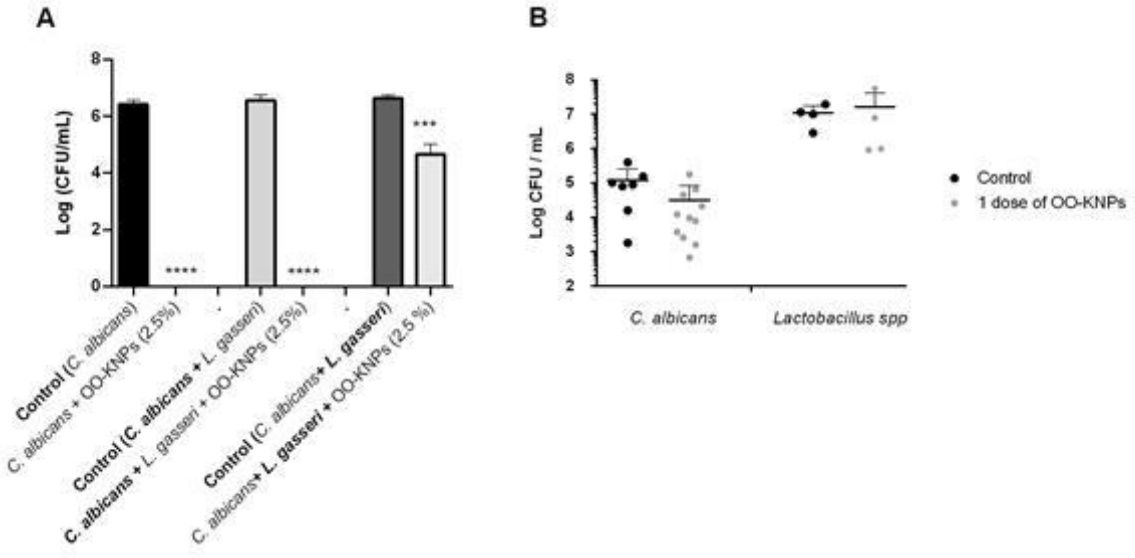
Conclusions:

The stability of OO-KNP´s over time and its effect, *in vitro,* against *C. albicans* infection was verified. Despite the need to complement our *in vivo* study, our preliminary results showed that this new OEO therapeutic approach can be a promising alternative or complementary

therapy for the treatment of VVC. In addition, OO-KNP´s may have a less harmful effect on women's health, due to their natural characteristics.



Characterization of nanoparticles of keratin loaded with oregano essential oil (OO-KNP´s). (A) particle size (Z-average) and polydispersity (PDI); * indicate statistical difference in particle size when compared to the results obtained at time 0 (* p< 0.05); (B) surface charge (æ-potential) and (C) *In vitro* release profiles of oregano essential oil from keratin-based particles in simulated vaginal fluid, over 72 h.



Effect of the nanoparticles of keratin loaded with oregano essential oil (OO-KNPs) on infection of *Candida albicans* and the induced effect on *Lactobacillus* species. (**A**) I*n vitro* assay; (B) *In vivo* assay. * indicate statistical difference in comparison with respective control (*** p<0.001, **** p< 0.0001).

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In vitro inhibitory effect of some local medicinal plants from the southern part of Nigeria on the growth of Candida

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Candidiasis is one of the most common fungal infections and due to the increasing prevalence of multi-resistant candidiasis, there is an urgent need for the development of new antifungal agents. Worldwide, there has been an increase in the prevalence of species other than *Candida albicans* is emerging, particularly *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida auris*.
Objective: To evaluate the *in vitro* antimicrobial activity of extracts and fractions of some Nigerian medicinal plants commonly used for various ethnomedicinal purposes.
Materials & Methods: Five different extracts/ fractions were analysed: ethylacetate (CP1) and dichloromethane (CP2) fractions of the stem bark of *Spondias mombin (Anacardiaceae* family); methanol (CP3) and dichloromethane (CP4) fractions of the stem bark of *Detarium microcarpum*; and dichloromethane:methanol (50:50; CP5) extract of *Picralima nitida* leaf. A total of 25 clinical isolates and reference strains of *Candida* were tested, including six *Candida albicans,* five *Candida auris,* five *Candida krusei*, three of each of *Candida glabrata, Candida parapsilosis* and *Candida tropicalis.* Anticandidal activity was determined by the broth microdilution method according to EUCAST guidelines (document 7.3.2). The extract or fractions were evaluated in monotherapy at concentrations ranging from 2 to 32 mg/L. In addition, the combination of fractions CP1 and CP4 with fluconazole was evaluated in a checkerboard test using concentrations of 0.25 to 16 mg/L fluconazole and 0.25 to 128 mg/L CP1 or CP4. Minimum inhibitory concentrations (MICs) were calculated as the lowest concentration that inhibited ≥ 50% of yeast growth compared with the control growth. Antifungal drug interactions were evaluated using the fractional inhibitory concentration index (FICI) method and by the Bliss independence as a surface response model.
Results: The extracts exhibited *in vitro* antifungal activity against 13 out of 25 *Candida* isolates (CP1), 16 out of 25 isolates (CP4), 2 out of 15 isolates (CP3) and 1 out of 15 isolates (CP5). The dichloromethane fraction of the stem bark of *Spondias mombin* (CP2) showed no activity against *Candida*. The most active fraction was the dichloromethane fraction (CP4) obtained from *Detarium microcarpum*, followed by the ethylacetate fraction (CP1) from *Spondias mombin* with appreciable activity against isolates of all *Candida* species studied. The species, *C. glabrata* and *C. krusei* were more susceptible to CP1 and CP4 fractions with MICs ranging from 4 to 16 mg/L. The synergistic interactions of fluconazole (1 mg/L) with CP1 (4 mg/L) and fluconazole with CP4 (8 mg/L) were further investigated against fluconazole-resistant isolates of *C. albicans* (UPV 15-157), *C. glabrata* (UPV 22 008) and *C. krusei* (UPV 05 054) and the fluconazole-susceptible isolate of *C. tropicalis* (UPV 22 090). The combination of fluconazole and CP4 resulted in MIC values up to 128 times lower than those observed in the monotherapy study. Absorption spectra from HPLC chemical fingerprints of the CP1 and CP4 fractions revealed several peaks representing important bioactive phytochemicals that that may be related to their overall antifungal activity.
Conclusions: Ethylacetate and dichloromethane fractions of *Spondias mombin* and *Detarium microcarpum*, respectively showed appreciable growth inhibitory properties against *Candida* species and isolates resistant to conventional treatment and may therefore be promising anticandidal candidates.

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Successful Treatment of Refractory Invasive Aspergillosis with a Novel Antifungal Agent Olorofim in a Leukemia Patient: A Case Report

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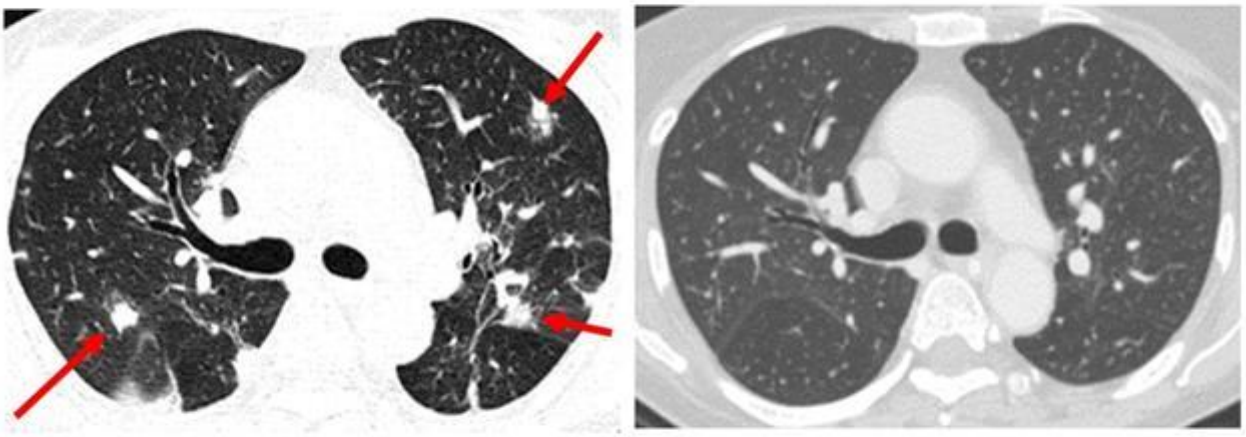
Objectives: To demonstrate the favorable treatment outcomes of invasive pulmonary aspergillosis in a patient with acute promyelocytic leukemia treated with a novel orotomide antifungal agent, olorofim.
Materials & Methods: This is a case report of a patient from F2G Study 32 (NCT03583164) from a tertiary university hospital from Thailand.
Results: We report a case of a 63-year-old woman who was diagnosed acute promyelocytic leukemia (APL) in August 2020. She was treated with all-trans retinoic acid (ATRA) and idarubicin on 5th August 2020, complicated by febrile neutropenia two weeks after chemotherapy. Three weeks later, she was diagnosed with invasive pulmonary aspergillosis and *Aspergillus terreus* sinusitis. Voriconazole was given for 3 months with good response. In December 2020, she had relapsed APL and received ATRA, idarubicin and arsenic trioxide for treatment. One month after chemotherapy, she developed fever and her chest computed tomography (CT) revealed new consolidation and irregular nodules with peripheral ground glass opacity halos in both lungs. Serum and bronchoalveolar lavage fluid galactomannan was positive (OD = 0.89 and 4.99, respectively). Probable invasive pulmonary aspergillosis was diagnosed and she was treated with voriconazole. Ten days after voriconazole treatment, her symptoms deteriorated with high-grade fever, dyspnea, and hypoxemia (oxygen saturation 88%). Chest CT scan showed new right middle and lower lung atelectasis, heterogenous enhancement with air-bronchogram, few hypodense foci at medial segment of right middle lung. Antifungal therapy was switched to olorofim for treatment of refractory invasive aspergillosis. The patient improved and became afebrile within 2 weeks. Her neutrophil counts increased and neutropenia was resolved after 4 weeks of olorofim treatment. Clinical improvement was likely attributed to antifungal therapy and neutrophil recovery. Six weeks later, she was diagnosed hepatosplenic candidiasis by abdominal CT scan that showed new discrete and conglomerate hypodense enhancing lesions both hepatic lobes and a positive serum beta-D-glucan. As olorofim does not have activity against yeast, we therefore added fluconazole to the treatment regimen. She dramatically improved and olorofim was stopped after 3 months of treatment (Figure 1). She is doing well until now after 2 years of treatment without disease relapse.
Conclusions: Olorofim is a promising novel antifungal agent for treatment of refractory invasive aspergillosis.

Figure 1 : Chest computed tomography before and after olorofim treatment revealed improvement and disappearance of pulmonary nodules



Before olorofim treatment

Three months after olorofim treatment

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Brazilian Brown Propolis shows in vitro antifungal activity against Paracoccidioides brasiliensis.

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[1]Universidade Federal de Alfenas, Alfenas, Brasil, [2]Universidade Estadual Paulista, Botucatu, Brasil

Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis with high prevalence in South America and especially in Brazil. The etiological agent of this mycosis is a thermodimorphic fungus belonging to the genus *Paracoccidioides* spp. The lesions caused by the fungus cause serious clinical consequences for the patient. The treatment of PCM is long and the drugs with antifungal activities used have serious collateral effects. Propolis is a natural bee resin used in traditional medicine and there are reports of its use for the treatment of oral and systemic diseases due to its anti-inflammatory and antimicrobial activities. Propolis in Brazil is generally classified according to its color. The brown type, Brazilian Brown Propolis (BBP) contains mainly phenolic acids and diterpenes, but as it is produced in different regions of Brazil, and contains compounds from different botanical origins, its chemical composition varies. BBP presents antioxidant, anti-inflammatory, antibacterial, antileishmania and antimycoplasma properties. Objectives: To investigate the direct *in vitro* effect of BBP on *Paracoccidioides brasiliensis* (Pb). Material and methods: Antifungal activity of BBP at a concentration of 500 mg/mL was tested against the virulent PB18 isolate. The antifungal activity was evaluated using the microdilution technique. In another set of experiments, mice splenocytes co-cultured with Pb18 were treated with BBP at different times. Negative controls consisted of only Pb. The absolute number of splenocytes and viable Pb were stained respectively with Trypan Blue and Vital Janus Green B dyes and counted in Neubauer chambers. Mitochondrial activity was determined by the MTT [3-(4,5-dimethylthiazole-2yl)-2,5-diphenyl tetrazoline bromide] test and the number of viable fungal cells by counting colony forming units (CFU) after 72 hours of cultivation in adequate medium. Results: The i*n vitro* experiments showed remarkable direct antifungal activity of BBP, significantly reducing the number of viable Pb in relation to the original inoculum after 72hs incubation. Splenocyte co-culture assays showed that BBP had no cytotoxic effect on these cells; on the contrary, it exerted a stimulatory effect. Thus, BBP added to splenocytes resulted in marked inhibition of fungal growth at all times of cultivation. The CFU data showed that there was no fungal growth after 72 hours of cultivation, strongly suggesting fungicidal effect. Conclusion: Our results suggest that BBP has a direct antifungal effect and is able to prevent fungal growth. An important observation was that BBP acts in combination with the immune system cells. This data allows us to suggest that Brazilian Brown Propolis can be a new natural alternative to enrich the therapeutic options for PCM treatment.

Grants: CNPq 309917/2020-4, FAPEMIG PPM-00497-18 and FAPEMIG BPD-00341-22. L.A. Santos holds a scholarship from CNPq within the Young Doctors Fixation in Brazil Support Program. B.J.N. Gomes was and J.C.Dutra is recipient of CAPES scholarship. E.M.Picoli is recipient of Institutional Program of Scientific Initiation Scholarships from CNPq-PIBIC.

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Antifungal Activity of a New Derivative of 5-Aminoimidazole-4-Carbohydrazonamide

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Objectives: Fungal infections are among the most worrying challenges considered by the World Health Organization (WHO) due to their high incidence, recurrence, and the emergence of resistance to the few available drugs and therapies. The discovery of new molecules with antifungal activity, exhibiting new mechanisms of action and less side effects, represents an important step forward in the development of alternative treatments [1].

Methods and Results: In a previous work, an imidazole-based amidrazone (5-amino-*N*'-phenyl-1*H*-imidazole-4-carbohydrazonamide, 2h) was synthesized and exhibited great antifungal activity evaluated against *Candida krusei*, *Candida albicans* and *Cryptococcus neoformans* [2]. A trimeric derivative compound was prepared from spontaneous oxidation of 2h in contact with the air and exhibited equivalent or higher fungicidal activity against the yeasts tested, with Minimal Fungicidal Concentration of 2-64 µg/mL. Considering the instability of 2h and the rapid conversion of 2h to the trimeric derivative observed in contact with the air, we can conclude that the antifungal activity observed for the compound 2h may result from the activity verified in the trimeric derivative.

Effects on yeast's metabolic activity and mitochondrial function were evaluated using the resazurin reduction and JC1 assays, respectively. The observed decrease in resazurin reduction indicate a decrease in cell's metabolic activity. Noteworthy, an impaired mitochondrial function seems to be involved in the antifungal activity of the trimeric derivative, as observed by the hyperpolarization of the mitochondrial membrane of *C. albicans*.

Cytotoxicity of the compounds was evaluated in immortalized human keratinocytes - the HaCaT cell line - by the neutral red uptake, resazurin reduction and sulforhodamine B binding assays, 24 h after exposure. For 2h a significant and concentration-dependent cytotoxic effect was detected, for concentrations ≥16 µg/mL. Remarkably, the trimeric derivative did not significantly affect cell viability for all the concentrations tested (4-64 µg/mL). From this perspective, this trimeric derivative could constitute a safer antifungal with possible application in therapy.

Conclusions: The high activity registered against *C. neoformans* and *C. krusei* and the low toxicity of trimeric derivative are particularly important, as these fungal pathogens were included in critical priority group and medium priority group, respectively, by WHO in 2022.

The application of these compounds in biomedical materials, namely in the development of smart and functional textiles with antimicrobial properties, is currently being tested, opening new perspectives in this emergent field of research.

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Effect of subcutaneous or systemic administration of Celecoxib in mice infected with Paracoccidioides brasiliensis

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Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis, caused by the fungus *Paracoccidioides brasiliensis* (Pb). The treatment of this disease includes the use of antifungals that, in addition to the prolonged time of administration, may have several side effects. One of the challenges of PCM treatment is to reduce the intense inflammatory response present in the affected organs. Therefore, the use of anti-inflammatory drug such as Celecoxib may represent a new alternative to complement the antifungal therapy currently in use in PCM. Objective: In this work, we evaluated the effect of Celecoxib (6mg/Kg) either introduced directly into the air pouch or administered systemically in mice. Methods: Two experimental models were employed: In the first one, used to evaluate the effect of Celecoxib treatment in the initial aspects of the immune response, mice were inoculated via subcutaneous(sc) air pouch with the virulent Pb18 strain of *P. brasiliensis*. Treatment was performed with Celecoxib at 5, 6 and 7 days post-infection. At the 8th h day, the cells present in the air pouch were collected to evaluate their cytotoxic activity and supernatants were obtained to determine the concentration of catalase and peroxidase produced at 2, 6 and 18 hours of cell culture. In the second experimental model, used to evaluate the effect of Celecoxib treatment on acquired immunity, mice were infected intraperitoneally with Pb18 and after three days of infection, Celecoxib treatment by gavage was initiated and maintained daily for 15 or 120 days, during which, survival rate and body weight were monitored daily. At 15-120 days post-infection, the mice were sacrificed and lungs, spleen, liver, and epiploon/pancreas were collected. The organs were macerated and nitric oxide (NO) concentration and numbers of viable fungi (CFU) were assayed. Results: In the initial phases of the infection, treated mice showed higher levels of catalase and peroxidase production at 6- and 18-hours incubation than controls. At his time, Celecoxib also increased the antifungal activity of PMNs, mainly in relation to their phagocytic capacity, showing an upward trend at 90 minutes of incubation. Survival and weight gain after intraperitoneal infection were not affected by Celecoxib-treatment, either at 15 or 120 days. However, NO concentration was higher in the liver in Celecoxib-treated mice than in the controls and there was a significant CFU reduction in all organs. Conclusions: Celecoxib increases the synthesis of oxidative products, reflecting on the improvement of the fungicidal capacity of PMNs and conferring a more effective immune response against Pb. In the long term, it does not affect weight gain or survival, despite improving NO production and reducing the number of viable fungi. It can be concluded that Celecoxib has an important protective effect both in the acute and chronic phases of experimental PCM.

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Determination of antifungal activity of new compounds against agents of cromoblastomycosis

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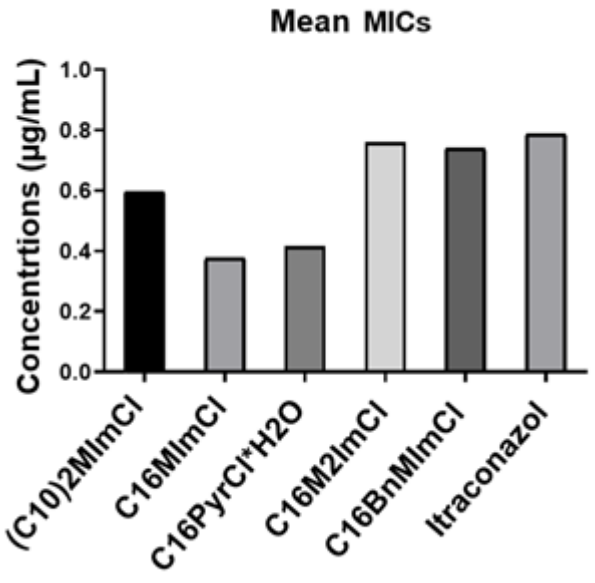
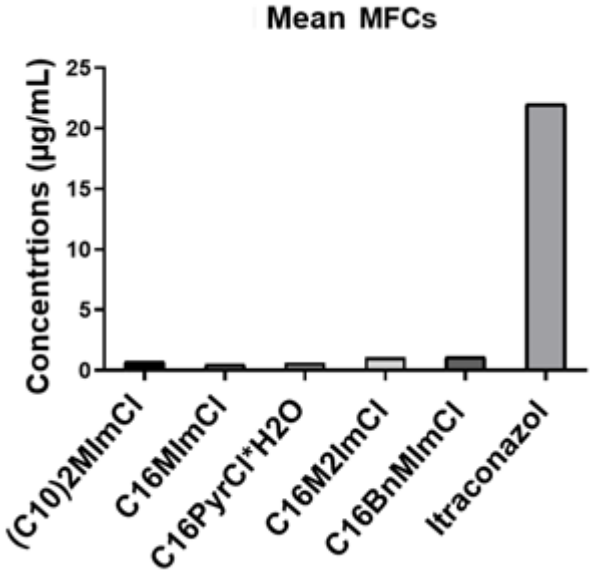
Objectives: -To determine the antifungal activity of imidazolic compounds against cromoblastomycosis fungi samples from multiple regions of Brazil;
-To evaluate the MICs of the tested concentrations of the new molecules against Fonsecaea spp., based on antifungal susceptibility tests, comparing the MICs obtained with the MICs of itraconazole (standard antifungal);
-To determine whether the new molecules have fungistatic or fungicidal activity.

Materials & Methods: Antifungal activity tests were performed following the document M38-A2 of the Clinical and Laboratory Standards Institute protocol. Samples of cromoblastomycosis agents from the fungi collection of the Laboratory of Pathogenic Fungi, Department of Microbiology, ICBS, UFRGS. The most prevalent species as etiological agents of CBM will be included. No calculation was made to define the sample size as it depends on the number of strains available in the aforementioned collection (n = 78). The fungi were grown for 14 days over 30°C on potato-dextrose agar. Conide suspensoins were collected using sterile 0,85% saline solution to make a smear, then filtered on sterile filters to remove any mycelium fragments. The molecules, identified as 3: (C10)2MImCl; 5: C16MImCl; 7: C16PyrCl*H2O, 8: C16M2ImCl and CB: C16BnMImCl were solubilized on Dymethilsulfoxide (DMSO) and then diluted to a concentration of 64µg/mL for use in the tests. Itraconazole (ITZ) was used for negative control on equivalent concentrations. Tests were made in 96 pits plates and incubated for 7 days, then read and aliquots colected for realization of MFC test, which was incubated for 10 days and then read. For statistical analysis the mean, geometric mean, MIC50 and MIC90 will be used to compare the results of susceptibility to itraconazole and the new molecules tested.

Results: From a pool of 12 molecules, 5 presented significant antifungal activity againts cromoblastomycosis agents, having lower MICs and MFCs than ITZ, which is the current standard for cromoblastomycosis treatment. Each molecule that showed activity possesed distinct MICs and MFCs.

Conclusions: The 5 molecules that presented antifungal activity at lower concentrations than ITZ. The molecules have both fungistatic and fungicidal activity, the latter being significantly more effective when compared to the activity of ITZ.


Mean MICs
Concentrtions (µg/mL)
1.0
0.8
0.6
0.4
0.2
0.0
(C10)2MImCl
C16MImCl
C16PyrCl*H2O
C16M2ImCl
C16BnMImCl
Itraconazol


Mean MFCs
Concentrtions (µg/mL)
25
20
15
10
5
0
(C10)2MImCl
C16MImCl
C16PyrCl*H2O
C16M2ImCl
C16BnMImCl
Itraconazol

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In vitro antifungal activity of 3-bromopyruvate (3-BP) against etiological factors of pityriasis versicolor

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Objectives:
Pityriasis versicolor (PV) is one of the three most common superficial fungal infections besides candidiasis and dermatophytosis. The aim of this study was to assess *in vitro* antifungal activity of 3-bromopyruvate (3-BP), used solely and in combination with chosen drugs, against *Malassezia* spp. It was also important to preliminarily determine the molecular mechanism of action of 3-BP, possible phenotypes of resistance and level of cytotoxicity against chosen mammalian cell lines, including epithelial cells.

Materials & Methods:
Determination of MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) values of 3-BP was performed according to microdilution assay in agreement with the Clinical Laboratory Standards Institute (CLSI 2008) protocol (M27-A3). Susceptibility tests were performed in RPMI 1640 medium supplemented appropriately to ensure good growth of *Malassezia* spp. Standard 2D checkerboard assay was involved to define type of interactions between tested compounds. These were determined based on Fractional Inhibitory Concentration Indexes and visualized by isobolograms. The cytotoxicity of 3-BP was evaluated with the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Invitrogen). Studies on 3-BP transport were performed using [^{14}C]-labelled 3-bromopyruvate, as previously described for the radioactive L-lactate uptake assay. ATP concentration was determined in cells cultivated in medium with 3-BP at different concentrations. The intracellular concentration of glutathione (GSH) was determined by using Ellman's reagent and metabolite extraction was carried out with buffered boiling ethanol (75% ethanol, 70 mM HEPES, pH 7.5).

Results:
Importantly, we noted fungicidal effect of 3-BP against *Malassezia* spp. The MIC and MFC values of 3-BP for the most of 69 tested strains were in the range of 8-30 µM and 12-40 µM, respectively. Our study suggests that the hypersensitivity of *Malassezia* fungi results at least in part from a relatively high uptake and accumulation of 3-BP inside the cells, what was shown in experiments with [^{14}C]-labelled pyruvate analogue. Interestingly, 3-BP used at concentrations corresponding to $^{1}/_{4}$ and $^{1}/_{8}$ of MIC values, resulted in a rapid (within 2 hours) decrease of intracellular ATP levels by 64% and 38%, respectively. Thus, fungicidal effect of 3-BP may result from drastic changes in the cellular energy metabolism. Furthermore, we found a significant correlation between MIC/MFC values and the natural level of intracellular glutathione (GSH). Moreover, concentration of GSH decreases in the presence of 3-BP suggesting that this metabolite is involved in cell detoxification. We have also shown that 3-BP is not a substrate for the proteins of ABC transporters family, what allow to exclude this resistance phenotype. Finally, both epithelial and fibroblast cells retained high viability upon treatment with 3-BP at concentrations equivalent to the highest MIC recorded (30 µM) and 50-fold higher, with the mean cell viability exceeding 85%.

Conclusions:

The results from these *in vitro* studies emphasize the high activity of 3-BP against the *Malassezia* yeast-like fungi, its synergistic effect when used in combination with tacrolimus, and the safety of the compound toward the tested mammalian cells. Due to known satisfactory physicochemical and pharmacokinetic properties, 3-BP may be considered as an effective and safe promising agent against PV.

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Revitalizing our antifungal arsenal with natural products isolated from Arctic bacteria

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Objectives:
In the search for new antifungal agents, one approach is to mine the biodiversity of poorly characterized microbial ecosystems such as the Arctic. Our team screened the secretomes of a panel of newly discovered polar bacteria from Axel Heiberg Island, Nunavut, Canada. This screen identified a natural product produced by an Arctic *Streptomyces* species with potent antifungal activity *in vitro.* Our objective of this project is to investigate A28 as an antifungal therapeutic and the potential of this compound to treat infections caused by drug-resistant fungal pathogens.

Materials & Methods:
The therapeutic index of A28 was evaluated using two approaches. First, its antifungal activity was defined using our *in vitro* microbroth dilution assay against a range of fungal pathogens. This panel included drug-resistant clinical isolates. Secondly, A28 cytotoxicity was investigated using *in vitro* assays with human cell lines. Testing of the antifungal activity of A28 derivatives and the potentiation of existing antifungals were performed using standard microbroth dilution assays. The Fractional Inhibitory Concentration Index was used to define the interaction between the combined drugs.

Results:
A28 exhibited potent activity against all pathogens tested, including drug-resistant pathogens. However, significant cytotoxicity was observed at levels of A28 required for antifungal activity, suggesting its use as a systemic drug would be limited. A medicinal chemistry approach to studying A28 derivatives found that these derivatives exhibit lower cytotoxicity while conserving antifungal activity. A28 and its derivatives were also found to potentiate the activity of the echinocandin antifungal caspofungin against *Aspergillus fumigatus* and *Candida albicans* at levels lower than the cytotoxicity threshold. The interaction between caspofungin and A28 or its derivatives was determined to be synergistic at these concentrations of A28.

Conclusions:
Further investigation into the target of A28 and the mechanism of caspofungin activity potentiation are ongoing. These studies suggest that the current arsenal of antifungals can be augmented with natural products from unexplored biomes. This project has the potential to advance the development of an urgently needed solution for drug-resistant fungal infections.

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In vitro study of the activity of cannabidiol in monotherapy and combined with anidulafungin and fluconazole against Candida clinical isolates

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Objectives: In recent years, there has been an increase in candidiasis, accompanied by an increase in multidrug-resistant isolates from different *Candida* species. This situation, together with the emergence of *Candida auris*, a multidrug-resistant species that causes hospital outbreaks with higher mortality rates, has raised concerns due to the difficulty in treating infections caused, which represents a major clinical challenge. For this reason, it is necessary to look for alternatives such as the use of natural compounds and their combination with antifungal drugs to find synergies. Cannabidiol (CBD), extracted from *Cannabis sativa*, is a compound with numerous properties, and some authors have described antimicrobial properties, so the aim of this work was to evaluate the effect of CBD in monotherapy and in combination with anidulafungin (ANI) and fluconazole (FLZ) against clinical isolates of *Candida*.

Materials & Methods: We studied five different *Candida* species: *C. albicans* SC5314, *C. auris* UPV 22-100, *C. glabrata* UPV 17-236, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019. The minimum inhibitory concentration (MIC) was determined according to the EUCAST. The tested ANI concentration ranged from 0.062 to 4 µg/ml; FLZ from 1 to 64 µg/ml and CBD from 1 to 512 µg/ml. The *in vitro* interaction of the drug combinations was interpreted in terms of the fractional inhibitory concentration index (FICI) as follows: FICI < 1, synergistic; FICI equal to 1, additive; and FICI > 1, indifferent or antagonistic. In addition, the effect of CBD on biofilm development was evaluated at concentrations ranging from 16 to 512 µg/ml. Four *Candida* isolates with high biofilm production capacity were selected for this study: *C. dubliniensis* UPV 11-366, *C. glabrata* UPV 17-236, *C. guilliermondi* UPV 05-078 and *C. tropicalis* UPV 12-366.

Results: The combination of CBD with ANI reduced the MIC of both compounds against *C. auris* and *C. glabrata*. The MIC of CBD decreased from > 512 µg/ml to 1 - 2 µg/ml against three of the five isolates included. The combination of CBD with FLZ had a similar effect to that obtained with the ANI combination, reducing the MIC of FLZ by half in two cases. The effect of the combination of CBD with ANI and FLZ against two of the isolates studied was interpreted as additive (*C. glabrata* for both combinations and *C. auris* and *C. krusei* species for ANI with CBD and FLZ with CBD, respectively) and indifferent against the other three. On the other hand, CBD in monotherapy did not reduce preformed biofilms or prevent their formation at the concentrations used, except in the case of *C. glabrata*, where both metabolic activity and biomass were reduced in both types of biofilms at concentrations between 16 µg/ml and 128 µg/ml.

Conclusions: The combination of CBD with ANI and FLZ reduced the MICs of both antifungals drugs, achieving an additive effect against two of the five isolates, one of them being the *Candida auris* isolate. Finally, CBD was able to reduce the formation of biofilm produced by *C. glabrata*.

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Pandemic Response Box® library as a source of antifungal drugs against Scedosporium and Lomentospora species

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Objectives: The search for new compounds that could work as promising candidate antifungal drugs is an increasing field of interest. In this context, in the present study we screened the Pandemic Response Box® library (Medicines for Malaria Venture [MMV], Switzerland) to identify compounds with antifungal activity against *Scedosporium* and *Lomentospora* species.

Materials & Methods: An initial screening of the drugs from this collection at 5 µM was performed using a clinical *Scedosporium aurantiacum* isolate according to the EUCAST protocol. Compounds with activity against this fungus were also tested against four other species (*S. boydii*¸ *S. dehoogii*, *S. apiospermum* and *L. prolificans*) at concentrations ranging from 0.078 to 10 µM. The selected compounds were also tested against fungal biofilm, which was measured using violet crystal, safranin and XTT reduction assay. Fluorescent probes, such as DCFDA, JC-1, calcofluor white, concanavalin A and nile red, were used to investigate the following cellular parameters, respectively: ROS production, mitochondrial membrane polarization, chitin content, sugar residues and neutral lipids. Scanning electron microscopy was performed to check alterations on fungal cell surface in the presence of the selected compounds. The interaction between the selected compounds and antifungal drugs used in clinical settings was evaluated using the FICI and BLISS methods. To analyze whether the compounds are toxic to mammalian cells, a citotoxicity assay was performed usign three cell lineages: A549, RAW 264.7 and HaCaT.

Results: Seven compounds inhibited more than 80% of *S. aurantiacum* growth, three of them (alexidine, amorolfine and olorofim) were selected due to their differences in mechanism of action, especially when compared to drugs from the azole class. These compounds were more active against biofilm formation than against preformed biofilm in *Scedosporium* and *Lomentospora* species, except alexidine, which was able to decrease preformed biofilm about 50%. Analysis of the potential synergism of these compounds with voriconazole and caspofungin was performed by the checkerboard method for *S. aurantiacum*. The analysis by Bliss methodology revealed synergistic effects among selected drugs with caspofungin. When these drugs were combined with voriconazole, only alexidine and amorolfine showed a synergistic effect, whereas olorofim showed an antagonistic effect. Scanning electron microscopy revealed that alexidine induces morphology alterations in *S. aurantiacum* biofilm grown on a catheter surface. Reactive oxygen species production, mitochondrial activity and surface components were analyzed by fluorescent probes when *S. aurantiacum* was treated with selected drugs and revealed that some cell parameters are altered by these compounds.

Conclusions: In conclusion, alexidine, amorolfine and olorofim were identified as promising compounds presented in Pandemic Response Box to be studied against scedosporiosis and lomentosporiosis.

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Brazilian Brown Propolis enhances fungicidal activity of neutrophils against Paracoccidioides brasiliensis and Paracoccidioides lutzii

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Introduction: Paracoccidioidomycosis (PCM) is a severe systemic mycosis caused by fungi of the *Paracoccidioides* spp complex. Persistence of the inflammatory infiltrate composed mainly by polymorphonuclear neutrophils (PMNs) is characteristic of PCM. Literature data shows that PMNs from PCM patients have decreased activity. In a murine model, PMNs from strains susceptible to this mycosis produce less reactive oxygen species (ROS) and have less fungicidal capacity than those from resistant strains. For this reason, our group has been studying ways to stimulate PMNs in order to reverse their lower activity. Propolis, a natural product from bees is traditionally employed as a natural therapy and has drawn attention of the scientific community due to its antioxidant, anti-inflammatory and antimicrobial properties. Brazilian Brown Propolis (BBP) is the second most produced Propolis type in Brazil and has great economic and medicinal importance. Therefore, we will study BBP employing an experimental murine model of PCM. Objectives: To evaluate whether BBP improves PMNs activation state and antifungal activity against *Paracoccidioides brasiliensis* (Pb18) and *Paracoccidioides lutzii* (Pl). Material and methods: Mice were inoculated via subcutaneous (sc) air-pouch with Pb18 or PI. On the 5th day of infection, treatment with 500 mg/mL BBP via sc air pouch was initiated and maintained for 3 days until the collection of the PMNs, at 8 days of infection and treatment. The following parameters were analyzed: absolute number of cells at the air-pouch and their viability, mitochondrial activity, ROS, catalase, peroxidase, nitric oxide and total proteins production, as well as the number of viable fungi (Pb or Pl) present at the infection site. Results: BBP treatment did not alter the viability of the PMNs collected from the air pouch. However, it caused a decrease in the influx of PMNs, regardless of whether the infection was caused by Pb 18 or by PI. When comparing control groups (Pb18 and Pl), we found that Pl attracted fewer PMNs than Pb18. Mitochondrial activity was higher in mice treated with BBP than in controls. ROS production was lower in mice treated with BBP than in non-treated controls. Catalase and peroxidase production was not significant in mice treated with BBP. Mice treated with BBP and infected with PI had increased NO and protein production. BBP was able to reduce the number of viable Pb18 and Pl in treated mice. Conclusion: Our results suggest that BBP was able to increase PMNs activation as confirmed by increased mitochondrial activity as well as by NO production. This increased metabolic activation state resulted in improvement of the fungicidal activity of PMNs, rendering these cells able to reduce the number of viable fungi. Therefore, we can suggest that BBP may be a potential therapeutic agent to treat PCM, in addition to the therapies in use.

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Celecoxib reduces antibody titers and inflammatory cytokines in mice infected with Paracoccidioides brasiliensis

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Introduction: Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Brazil. In order to understand the interaction of *Paracoccidioides spp.* (Pb), the causative agent of PCM, with the host, the experimental murine model was used to classify several strains of mice in terms of susceptibility or resistance to PCM and in correlating with the characteristics of the humoral and cellular immune responses developed, as well as with the type of granulomatous inflammatory immune response originated. Objective: The aim of this study was to verify the influence of treatment with the anti-inflammatory Celecoxib on the levels of specific antibodies IgM, IgG, IgG1a, IgG2a, IgG2b isotypes in the serum as well as of IL-4, IL-12 and KC cytokines in the supernatant of macerated organs of with *P brasiliensis*-infected mice. Methods: Swiss mice intraperitoneally infected with the virulent Pb18 *P. brasiliensis* strain were employed at 15 and 120 days post-infection, simulating respectively recent and late PCM infections. The treatment by gavage was carried out daily with 6mg/Kg Celecoxib. Delayed-type hypersensitivity test (DTH) was performed injecting paracoccidioidin antigen preparation in the paw of mice and after 48 hours measuring paw edema with a caliper. Antibody determination was performed using the ELISA. Both the DTH test and the serum collection for antibody measurement were performed at 7, 15, 30, 60, 90 and 120 days post- infection and treatment. At the end of the treatment period, lungs, spleen, liver and epiploon/pancreas were collected, macerated and their supernatants obtained for cytokine quantification. Results: After 60 days of infection and treatment, DTH results showed that Celecoxib-treated mice had less reactivity compared to that of the untreated controls. Regarding antibody titers, lower concentrations of IgM, IgG and IgG1a were observed at both 15 and 60 days of infection and treatment in the sera of infected and treated mice as compared to only infected controls. The levels of IL-4 and KC were lower in all organs supernatants at all treatment times. However, reduction of KC levels was more expressive in the lungs of mice that received the treatment for 120 days. On the other hand, levels of IL-12 increased in all organs of Celecoxib-treated mice. Conclusion: The results obtained showed that treatment with Celecoxib was able to reduce serum antibody titers and the concentration of IL-4 and KC cytokines in all organ supernatants. Our results allow us to suggest the treatment of PCM with the anti-inflammatory drug Celecoxib in combination with antifungal agents that are already used in clinical practice, as this therapeutic strategy reduces the intense inflammatory response in patients.

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Impact of the COVID-19 pandemic on ReSTORE: Phase 3 trial of rezafungin and caspofungin to treat invasive candidiasis and candidaemia

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Objectives:
The COVID-19 pandemic posed many challenges for clinical trials including changes in treatment practices and shortages in medical staff and equipment.[1] Rezafungin is a new FDA-approved echinocandin to treat candidaemia and invasive candidiasis (IC), and is in development to prevent invasive fungal diseases caused by *Candida*, *Aspergillus*, and *Pneumocystis* spp after blood and marrow transplantation. In contrast to current echinocandins (administered daily [QD]), rezafungin is administered once weekly (QW) due to its long-acting pharmacokinetic profile. ReSTORE (NCT03667690) is a Phase 3 double-blind, randomized clinical trial of rezafungin in candidaemia and IC that was ongoing during the COVID-19 pandemic (patient recruitment: October 2018 to August 2021).[2] As individuals with candidaemia and IC may utilize similar resources to critically ill patients with COVID-19, the impact of the COVID-19 pandemic on ReSTORE was assessed.
Materials & Methods:
The ReSTORE study design was reported previously; participants received rezafungin QW (Week 1: 400 mg; 200 mg thereafter) and caspofungin QD (Day 1: 70 mg; 50 mg [weight/hepatic impairment adjusted] thereafter) for ≥14 days (up to 4 weeks).[2] Individuals randomized to study treatment on or before 29-Feb-2020 (pre-cohort) and during the COVID-19 pandemic from 01-Mar-2020 (peri-cohort) were grouped. Thirty-day all-cause mortality (ACM) was the primary endpoint required by the US FDA.
Results:
Demographic and disease characteristics were balanced in the rezafungin QW and caspofungin QD arms. Patients enrolled in the pre- vs peri-COVID period had higher rates of IC (50.9 vs 21.2%) and lower rates of neutropenia (1.8 vs 8.5%). Baseline disease severity markers were similar in the pre- and peri-COVID cohorts (Table 1). Three patients (randomized to caspofungin) had COVID-19 co-infection at the time of enrolment.
While 30-day ACM was comparable with rezafungin QW and caspofungin QD in the pre-COVID (11.1% vs 10.7%; difference [95% CI] 0.4 [-18.2, 19.4) and peri-COVID (28.8% vs 25.8%; difference 3.0 [-12.3, 18.2]) cohorts, 30-day ACM rates were over 2-fold lower pre- vs peri-COVID (all patients: 10.9 vs 27.3%). In the pre- vs peri-COVID cohorts, deaths due to infections were 33.3 vs 50.0%, and deaths due to respiratory, thoracic and mediastinal disorders were 0 vs 13.9%. Several prognostic risk factors were less prevalent in patients

enrolled pre- vs peri-COVID including use of invasive interventions, antibiotics, and active cancer (Table 2). Similar incidences of adverse events (87.3 vs 88.7%) and serious adverse events (54.5 vs 54.6%) were seen in the pre- and peri-COVID cohorts.

Conclusions:

The COVID-19 pandemic likely contributed to the differing 30-day ACM rates observed in ReSTORE over time. This may be due to the overwhelming impact of COVID-19 on hospital resources and potential later presentation of patients requiring medical care. Patients enrolled during the peri-COVID period had higher incidences of some risk factors for poor prognosis, with a similar pattern seen in the rezafungin QW and caspofungin QD arms.

References:

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Table 1. Baseline demographic and disease characteristics of patients recruited prior to and during the COVID-19 pandemic (mITT population[a])

n (%)	Pre-COVID			Peri-COVID		
	RZF (N=27)	CAS (N=28)	All (N=55)	RZF (N=66)	CAS (N=66)	All (N=132)
Age, years, mean (SD)	59.2 (18.2)	63.2 (16.4)	61.2 (17.3)	59.7 (14.9)	61.3 (13.8)	60.5 (14.4)
Female	10 (37.0)	11 (39.3)	21 (38.2)	21 (31.8)	27 (40.9)	48 (36.4)
Geographic region						
Europe[b]	7 (25.9)	13 (46.4)	20 (36.4)	31 (47.0)	24 (36.4)	55 (41.7)
US[c]	15 (55.6)	13 (46.4)	28 (50.9)	11 (16.7)	11 (16.7)	22 (16.7)
Asia–Pacific	5 (18.5)	2 (7.1)	7 (12.7)	24 (36.4)	31 (47.0)	55 (41.7)
Final diagnosis						
Candidaemia only	13 (48.1)	14 (50.0)	27 (49.1)	51 (77.3)	53 (80.3)	104 (78.8)
IC	14 (51.9)	14 (50.0)	28 (50.9)	15 (22.7)	13 (19.7)	28 (21.2)
Modified APACHE II score <20	23 (85.2)	24 (85.7)	47 (85.5)	57 (87.7)	53 (80.3)	110 (84.0)
Neutropenia[d]	1 (3.7)	0	1 (1.8)	6 (9.5)	5 (7.6)	11 (8.5)
Dialysis within 3 days	2 (7.4)	3 (10.7)	5 (9.1)	8 (12.1)	9 (13.6)	17 (12.9)
Mechanically ventilated	4 (14.8)	7 (25.0)	11 (20.0)	12 (18.2)	21 (31.8)	33 (25.0)
Pancreatitis within 10 days	3 (11.1)	2 (7.1)	5 (9.1)	3 (4.5)	4 (6.1)	7 (5.3)

APACHE, Acute Physiology and Chronic Health Evaluation; CAS, caspofungin; IC, invasive candidiasis; mITT, modified intent-to-treat; RZF, rezafungin

[a]Participants with documented *Candida* infection ≤4 days prior to randomization who received ≥1 dose of study medication

[b]Includes Israel

[c]Includes one patient enrolled in Colombia during the peri-COVID period

[d]Baseline absolute neutrophil count <500/μL

Table 2. Selected risk factors for poor prognosis in patients recruited prior to and during the COVID-19 pandemic (mITT population[a])

n (%)	Pre-COVID			Peri-COVID		
	RZF (N=27)	CAS (N=28)	All (N=55)	RZF (N=66)	CAS (N=66)	All (N=132)
Invasive interventions for disease management						
Central venous catheter	14 (51.9)	17 (60.7)	31 (56.4)	41 (62.1)	41 (62.1)	82 (62.1)
Mechanical ventilation	4 (14.8)	7 (25.0)	11 (20.0)	12 (18.2)	21 (31.8)	33 (25.0)
PICC	6 (22.0)	3 (10.7)	9 (16.4)	16 (24.2)	11 (16.7)	27 (20.5)
Active malignancy	4 (14.8)	3 (10.7)	7 (12.7)	19 (28.8)	20 (30.3)	39 (29.5)
Broad-spectrum antibiotic therapy	17 (63.3)	19 (67.9)	36 (65.5)	53 (80.3)	44 (66.7)	97 (73.5)
Diabetes mellitus	7 (25.9)	7 (25.0)	14 (25.5)	19 (28.8)	21 (31.8)	40 (30.3)
Immunosuppression	4 (14.8)	2 (7.1)	6 (10.9)	11 (16.7)	15 (22.7)	26 (19.7)
Major surgery	11 (40.7)	14 (50.0)	25 (45.5)	21 (31.8)	19 (28.8)	40 (30.3)

CAS, caspofungin; mITT, modified intent-to-treat; PICC, peripherally inserted central catheter; RZF, rezafungin
[a]Participants with documented *Candida* infection ≤4 days prior to randomization who received ≥1 dose of study medication

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Miltefosine as a potential repurposing drug that affects cell biology of mucormycosis agents

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Objectives: To evaluate the antifungal effect of miltefosine against mucormycosis causing agents and its effects on fungal cells
Materials & Methods: Five Mucorales species (*Rhizopus oryzae*, *R. microsporus*, *R. stolonifer*, *Cunninghamella* sp. and *Mucor velutinosus*) were used in this work. Minimal inhibitory concentration (MIC) was determined according to EUCAST protocols. After MIC experiments, aliquots were plated in potato dextrose agar to determine minimal fungicidal concentration (MFC). Miltefosine activity against preformed biofilms was measured by quantifying biomass (crystal violet), extracellular matrix (safranin) and metabolic activity (XTT-reduction assay). Susceptibility of Mucorales species against membrane stressors (SDS and NaCl) in the presence or absence of miltefosine was evaluated by XTT-reducing assay. In order to assess miltefosine interaction with important fungal lipids, MICs were determined in the presence of ergosterol and glucosylceramide (GlcCer). To assess miltefosine effects on fungal cells, different parameters were evaluated with fluorescent stains: Nile Red for neutral lipids, Concanavalin A for mannose residues, Calcofluor White for chitin, JC-1 for mitochondrial membrane potential and DCFDA for reactive oxygen species (ROS) production . The *in vitro* efficacy of miltefosine combined with amphotericin B or posaconazole was also evaluated and synergic effect was analysed by both fraction inhibitory concentration index (FICI) and Bliss independence method.
Results: Miltefosine MIC_{70} ranged from 2 to 8 µg/ml, lower values than those observed for amphotericin B (25 to >50 µg/ml) and similar to posaconazole (1 to 8 µg/ml). Metabolic activity and extracellular matrix of mature biofilms were significantly affected by miltefosine at 4x and 8x MIC in all species. Biofilms biomass of all species was affected at 8x MIC. Miltefosine did not kill Mucorales species. With the exception of *R. oryzae*, susceptibility to SDS was increased in the presence of miltefosine for all species, suggesting membrane alterations. The presence of GlcCer and ergosterol increased miltefosine MICs against all species, suggesting interaction with those fungal lipids. ROS production, reduced mitochondrial membrane potential and chitin content reduction were observed in some species. However, reduction of both neutral lipids and mannose residues was observed in all species, which suggest surface alterations. FICI analysis revealed that the only synergic combination was miltefosine and amphotericin B against *Cunninghamella* sp. In contrast, Bliss indepence method revealed that all combinations were synergic with two exceptions such as miltefosine with amphotericin B against *M. velutinosus* and miltefosine with posaconazole against *R. microsporus*.
Conclusions: *In vitro* activity of miltefosine against Mucorales species as well as the effects on fungal cells described in this work show the potential of the drug repurposing for mucormycosis treatment.

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In vitro repurposing drugs against Sporothrix brasiliensis

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Given the urgent necessity of new therapeutic options for sporotrichosis caused by *Sporothrix brasiliensis*, repurposing drugs holds great promise as field of research. Dihydropiridines and benzoyilureas have been already evaluated against other fungal pathogens, instigating the investigation of their anti-*S. brasiliensis* activity. **Objectives:** To evaluate the *in vitro* activity against *S. brasiliensis* of each drug, a dihydropiridine and a benzoyilurea, as well their association with itraconazole (ITZ), the first-choice drug for sporotrichosis. **Materials & Methods:** It was included in this study a reference strain (CFP 00722) and 19 *S. brasiliensis* clinical isolates from the two main hyperendemic areas of sporotrichosis in Brazil, Rio Grande do Sul (n=10) and Rio de Janeiro (n=9) states. *In vitro* activity of both reporpusing drugs were evaluated through a microdilution assay, following the M38A-2 protocol from the Clinical and Laboratory Standards Institute (CLSI, 2008). Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were determined for each drug/isolate, as well as MIC/MFC50, MIC/MFC90 and geometrical mean (GM) were determined for drugs. Ten isolates were randomly selected to test drug interactions of the two repurposing candidates with ITZ using the checkerboard assay. Fractional inhibitory concentration index (FICi) was used to classify drug interactions as strong synergism (SS) when <0.5, weak synergism (WS) when 0.5 to <1, additive (AD) when 1 – <2, indifferent (IND) when 2, and antagonistic (ANT) when >2. **Results:** Dihydropiridines appear to have more potent anti-*S. brasiliensis* activity than benzoyilureas. The former showed inhibitory and fungicidal activity against all isolates included (n=20), with MIC values ranging from 32 to 256 µg/mL (GM:100.43 µg/mL; MIC50 and MIC90: 128 µg/mL), and MFC values ranging from 64 to 256 µg/mL (GM: 174.85 µg/mL; MFC50: 128 µg/mL, and MFC90: 256 µg/mL). The benzoyilurea was unable to inhibit *S. brasiliensis* isolates at concentrations lower than 64 µg/mL. Benefic interactions occurred in ITZ association with the dihydropiridine in 60% of the strains (10% of SS, 40% of WS, and 10% of AD), and in ITZ association with the benzoyilurea in 40% (all WS). No antagonistic effect occurred with the two studied combinations. **Conclusions:** One candidate repurposing drug evaluated in our study, a dihydropiridine molecule, showed a promising fungicidal activity against *S. brasiliensis* isolates from the two main epidemiological Brazilian areas, which are genotipically distinct. In addition, both drugs tested potentialized the inhibitory activity of ITZ, instigating future studies to evaluate their potential to be used as an adjuvant therapy to sporotrichosis.

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Evaluation of the in vitro activity of anidulafungin and tacrolimus in combination against Candida parapsilosis

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Introduction: The increase of invasive candidiasis caused by the species *Candida parapsilosis* represents a major medical challenge due to the resistance of this pathogen to conventional antifungal treatments. In addition, the limited antifungal drugs available makes necessary to search for new therapeutic strategies such as drug repurposing. Tacrolimus, a calcineurin inhibitor, is an immunosuppressive drug whose antifungal activity has been investigated *in vitro* against several *Candida* species. In this context, we hypothesised that tacrolimus might be able to reverse the resistance that *C. parapsilosis* develops to echinocandins due to a polymorphism in the *FKS1* gene.

Objective: To study the *in vitro* interaction of the combination of anidulafungin and tacrolimus against *Candida parapsilosis*.

Materials & Methods: The *in vitro* effect of the combination of tacrolimus and the echinocandin anidulafungin was investigated by the checkerboard method against 22 clinical isolates of *C. parapsilosis* (13 wild-type -WT- isolates and 9 isolates with a mutation in the *FKS1* gene). The minimum inhibitory concentration (MIC) was determined, following EUCAST guidelines, for both drugs in monotherapy and in combination. Drug-drug interactions were analysed using the fractional inhibitory concentration index (FICI) and Bliss models.

Results: The checkerboard assays showed that the combination of both drugs resulted in a 38.25-fold decrease in the MIC of anidulafungin (GM A_{alone}: 1.4116 mg/L; GM A_{comb}: 0.0369 mg/L) and a 4.21-fold decrease in the MIC of tacrolimus (GM T_{alone}: 0.4132 mg/L; GM T_{comb}: 0.0982 mg/L). Therefore, the addition of tacrolimus reduced the concentration of anidulafungin required to inhibit the growth of *C. parapsilosis*. A synergistic interaction between the two drugs was observed against 19 out of 22 (86.4%) *C. parapsilosis* isolates tested by FICI and/or Bliss models. No significant difference was observed between the synergy found in WT isolates (synergy in 84.6% of isolates) and *FKS1* mutant isolates (synergy in 88.9% of isolates).

Conclusions: The combination of anidulafungin and tacrolimus was synergistic against 86.4% of the *C. parapsilosis* isolates tested. The checkerboard results against *C. parapsilosis FKS1* mutants suggest that the addition of tacrolimus may increase the susceptibility of these isolates to anidulafungin. This study provides a starting point to be considered for future studies on the antifungal activity of tacrolimus and/or structurally analogous molecules.

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A Glimpse into the Treatment of Candida auris: A study of the Combination of Pentamidine and Auranofin.

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Objectives: *Candida auris* (*C. auris*) is an emerging fungal species that is capable of developing multidrug resistance and causing outbreaks of invasive infections worldwide, with high mortality rates. This study aimed to evaluate the *in vitro* and *in vivo* efficacy of the combination of pentamidine and auranofin, which has previouly demonstrated antifungal activity against *Candida albicans* isolates, in inhibiting the growth of azole-resistant *C. auris* isolates and expanding the treatment options for *C. auris* infection.
Materials & Methods: The antifungal activity of pentamidine and auranofin was evaluated using the broth microdilution assay (CLSI-M27-A3). The interpretation of the checkerboard results was done using the fractional inhibitory concentration index and response surface analysis based on the Bliss model. An *in vivo* infection model using *Caenorhabditis elegans* was employed to assess the activity and toxicity of this combination.
Results: The minimum inhibitory concentration (MIC) values for pentamidine and auranofin ranged from from 8 to 64 µg/ml and 2 to 32 µg/ml, respectively. The combination of auranofin and pentamidine exhibited a synergistic inhibitory effect against *C. auris*. The fractional inhibitory concentration index values for the pentamidine and auranofin combination ranged from 0.37 to 0.50. Both drug-susceptible and drug-resistant *C. auris*, as well as biofilm, showed a significant reduction in the minimum inhibitory concentration of each drug. Analysis of the synergism (Zero Interaction Potency model) revealed a dose-response matrix with a high synergy score (63.9). This activity was further confirmed *in vivo* using a *Caenorhabditis elegans* model of *C. auris* infection. The combination of pentamidine and auranofin increased the survival rate of nematodes infected with *C. auris* by nearly 70%.
Conclusions: Our findings suggest that the combination of pentamidine and auranofin holds potential as a therapeutic strategy for the treatment of fungal infections caused by the emerging pathogen *C. auris*. Despite the favorable effect of combining pentamidine with auranofin, further clinical studies are necessary to confirm the therapeutic advantage of this combination in the treatment of *C. auris* infections.

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In vitro anticandidal effect of amino acid substitutions on the Jelleine-II peptidic sequence

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Jelleines, from the royal jelly of the honeybee *Apis mellifera*, are a family of four peptides with antimicrobial activity. Amino acidic substitutions are a common approach used to alter the properties of a peptide, usually modifying its antimicrobial activity, toxicity and/or stability.

Objectives: To evaluate the *in vitro* effect of the amino acid substitutions on the Jelleine-II peptide sequence and to compare two of these derivatives with the wild-type peptide against different species of *Candida*.

Materials & Methods: The Jelleine-II peptide and the T-Jell-II and T-R1 derivatives were synthesized and obtained from Proteogenix (France). The substitutions of the original amino acids by an arginine or a tryptophan were made to modify the charge and/or hydrophobicity of the peptide. A total of 14 *Candida* clinical isolates and reference strains with different fluconazole susceptibilities belonging to five different species were tested: *Candida auris*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei* and *Candida albicans*. The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined by the broth microdilution method as described in the EUCAST definitive document 7.3.2. Final peptide concentrations ranged from 8 to 128 µM. The hemolytic activity of the three peptides at 128 µM was determined by a hemoglobin release assay, using 10% Triton X-100 and PBS as controls. Finally, the ability to permeabilize model vesicles mimicking the human, bacterial and *Candida* lipid composition was tested to measure the membrane activity of the peptides. Vesicles containing the ANTS/DPX pair were incubated with the peptides and the permeabilization of the vesicles was detected by fluorescence.

Results: The Jelleine-II peptide showed antifungal activity against all *Candida* isolates tested at a concentration between 32 and 128 µM. The T-Jell-II peptide at 128 µM was able to inhibit the growth of 5/14 isolates while the T-R1 peptide at 32 µM and 64 µM was effective against 5/14 and 9/14 isolates, respectively. The Jelleine-II peptide exerted a fungicidal effect against 6/14 isolates, the T-Jell-II peptide was not able to show this effect, and the T-R1 peptide enhanced the results obtained with the wild-type peptide showing a fungicidal effect against 11/14 isolates. All peptides exhibited hemolytic activity ranging from 14.4 to 27.4% compared to the control. Finally, membrane activity assays showed an increased ability of the T-R1 peptide to permeabilize *Candida* and bacterial vesicles compared to Jelleine-II. This increased activity is due to increased peptide charge (arginine), which facilitates vesicle approach, and increased hydrophobicity (tryptophan), which facilitates insertion of the peptide into the target membrane. Unfortunately, this membrane activity is also increased in membranes that mimic the lipid composition of human cells, limiting its therapeutic use.

Conclusions: Jelleine-II peptide and its derivatives exhibited candidacidal activity against *C. albicans*, *C. parapsilosis*, *C. auris*, *C. glabrata* and *C. krusei*, damaging their membranes. However, the hemolytic activity of these two derivatives and the permeabilization of human-like membranes caused by them lead to the consideration of further modifications of the amino acid sequences.

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Effect of Celecoxib on the morphology and expression of cytokines in the granulomas of P. brasiliensis-infected mice

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Objectives: Paracoccidoidomycosis (PCM) is a highly prevalent granulomatous mycosis in Latin America, caused by the fungus *Paracoccidioides brasiliensis*. Infection occurs by inhaling conidia and/or mycelial fragments present in the soil. It mainly affects male individuals who carry out agricultural activities. The initial infection can be exudative or granulomatous. Therefore, the presence of granulomas characterizes this disease. The most used model to study PCM is the murine, with mouse strains being classified as very resistant, resistant, intermediate and susceptible. The murine model of PCM is considered a very useful an instrument to aid the elucidation of immunopathological mechanisms and the development of new treatment strategies in this mycosis. The effectiveness of PCM treatment with Celecoxib (anti-inflammatory) and the immunomodulation on the host infected with *P. brasiliensis* is seldom studied.This study aimed to verify the influence of Celecoxib treatment through histological analysis and detection of IL-17 and GM-CSF cytokines in the paracoccidioidomycotic granulomas present in the epipolon/pancreas of mice infected with *P. brasiliensis* and their untreated controls.
Materials & Methods: The mice were intraperitoneally infected with the virulent Pb18 *P. brasiliensis* strain and after three days of infection, treatment was started with Celecoxib (6mg/Kg) by gavage and continued daily for 15 and 120 days. On the last day of treatment, the mice were sacrificed and the spleen, lungs, liver and epiploon/pancreas were collected. Part of each organ was macerated to measure IL-17 and GM-CSF cytokines in the supernatants and the other part was processed and stained with H/E for general histology and immunohistochemistry analyses.
Results: Observation of the histological aspects showed that Celecoxib treatment reduced the number of lesions and the severity of the granulomatous response for all organs, as most granulomas in treated animals were compact and delimited. In the epiploon/pancreas of the animals treated with Celecoxib, a smaller number of fungi with preserved morphology was detected in both treatment times as compared with the control group. Immunohistochemistry results showed strong reactivity for IL-17 and GM-CSF in the epiploon/pancreas at both stages of infection analysed. Immunostained cells consisting mainly of polymorphonuclear cells were present around the lesions.
Conclusions: Since there was an improvement in the granulomatous lesions as well as in the immunoexpression of cytokines that play an important role in the induction of protective immune responses in the Celecoxib-treated mice, it can be concluded that treatment with this anti-inflammatory drug may constitute a new therapeutic strategy, since it elicited very positive effects on the immunopathological responses in experimental murine PCM.
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In vitro and in vivo effect of the Ca37 monoclonal antibody against Candida auris

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Objectives:
The multiresistance to antifungals of the emergent pathogen *Candida auris* is one of the main reasons for the worldwide health alert that this fungus has created. In fact, infections caused by this yeast are sometimes untreatable due to its resistance to all available antifungal drugs. Consequently, new drugs need to be developed. Our research group has generated a monoclonal antibody against the alcohol dehydrogenase (Adh) protein of *Candida albicans*, called Ca37, with antifungal activity against this yeast. Since the Adh of *C. albicans* shows high homology with the Adh of other species of the genus, including *C. auris*, the aim of this study was to test the effect of the monoclonal antibody Ca37 against the fungus *C. auris in vitro* and *in vivo*.
Materials & Methods:
The ability of the monoclonal antibody to bind to the cell wall proteins of *C. auris* was studied by two-dimensional western blotting (2D-WB) and these results were analysed by Image Master 2D Platinum Software. In order to observe the growth-inhibition *in vitro,* the monoclonal antibody was incubated with the yeast for 18 hours and then cultured in Sabouraud Dextrose Agar. Furthermore, to study the possible opsonizing effect, *C. auris* was co-incubated with the murine RAW 264.7 macrophages in the presence and absence of the antibody at 1, 2 and 4 hours. The inhibition of biofilm development was also tested quantifying the biofilm mass with violet crystal after 24 hours of incubation in RPMI medium. Finally, a *Galleria mellonella* animal model was used to study the protective effect of the antibody *in vivo*, monitoring the survival of larvae for 7 days.
Results:
Firstly, the results from 2D-WB revealed that the antibody was able to recognize protein spots with similar molecular weight and isoelectric point to the Adh of *C. auris*. Moreover, in the growth-inhibition assay a decrease in fungal growth up to 70% was observed in the presence of the Ca37 antibody. Regarding the phagocytosis, in the first hour after the addition of the antibody, a significant increase was observed in comparison with the condition without antibody. In addition, the antibody decreased the development of the biofilm after 24 hours. Finally, the treatment with the Ca37 antibody exerted a protective effect on *G. mellonella,* which was similar to that of the micafungin, increasing the survival of infected larvae by one day.
Conclusions:
In conclusion, the Ca37 monoclonal antibody shows activity against *C. auris in vitro* and *in vivo*, showing to be a potential candidate to be studied as a therapeutic alternative against infections caused by this yeast.

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Evaluation of the therapeutic potential of CD5-based CAR-NK adoptive cell transfer in experimental models of Aspergillus fumigatus infection

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Introduction: *Aspergillus fumigatus* is a ubiquitous filamentous fungus that can cause severe infections in immunocompromised patients, especially those with hematological diseases. The most serious condition is invasive aspergillosis (IA), which mortality can reach to 90%. Increasing azole resistance limit seriously the treatment of any form of aspergillosis, thus having a critical impact in IA patients and making imperative the search for novel therapeutic approaches. The development of immunotherapeutic strategies involving adoptive transfer of engineered immune cells harboring Chimeric Antigen Receptors (CARs) are gaining interest, not only in the field of hematological malignancies but also for the treatment of infectious diseases. We have previously reported the ability of the scavenger receptor CD5 to recognize with high affinity β–glucans marking it as a good candidate for anti-fungal CAR design.
Objective: The aim of this study was to evaluate the *in vitro* and in vivo efficacy of NK-lymphoblastoid KHYG-1 cells stably expressing a CD5-based CAR for *A. fumigatus* targeting.
Methods: KHYG-1 cells were subjected to lentiviral transduction for expression of a second generation CD5-based CAR. Transduced KHYG-1 cells were selected for high and stable CD5CAR expression (CD5CAR-KHYG1) by cell sorting (FACS Aria; BD Biosciencies). Un-transduced KHYG-1 (UT-KHYG1) cells were used as negative controls.
The antifungal activity of CD5CAR cells was assessed in vitro by co-culture at different effector:target ratios with *A. fumigatus* (ATCC 46645), alone or in the presence of antifungals (capsofungin and voriconazole). Activity against *Aspergillus* hyphae was assessed by means of the colorimetric assay with XTT. Dynamic Fungal-cell interactions were analysed by real-time confocal microscopy.
In vivo efficacy experiments were performed by infusing (i.v.) CD5CAR-KHYG1 or UT-KHYG1 cells into NSG mice at 24 h post challenging with A*. fumigatus* conidia. Mice were euthanized on day 3 post infection for analysis of antifungal immune response, fungal burden and histological features in lung tissue sections.
Results: *In vitro* co-culture assays showed a statistically significant higher antifungal activity of CD5CAR-KHYG1 cells with regard to UT-KHYG1 controls. Addition of casponfungin or voriconazole increased antifungal activity of CD5CAR-KHYG1 cells without cell viability losses.
The qPCR analysis of fungal burden from lung homogenates of infected NSG mice showed that, relative to untreated mice (saline group), infusion of both CD5CAR-KHYG1 and UT-KHYG1 cells significantly reduced fungal burden ($p < 0.05$), with the former excelling among them ($p < 0.01$). The

later also agreed with cytokine and histology analyses from lung homogenates and tissue sections, respectively, of treated mice.

Conclusions:

The superior antifungal properties of immune cells expressing CD5-based CARs pave the way to novel off-the-shelf adoptive cellular immunotherapies against invasive fungal infections, alone or in combination with conventional antimycotics.

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Real-world observational study of olorofim: data from compassionate use in France in 15 patients

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Objectives:

The incidence of invasive fungal diseases (IFD) has increased recently with rising rates of naturally-resistant mold species and acquired antifungal resistance. Olorofim, the sole representative of the new orotomide class, has shown potent efficacy against resistant molds in animal models, but clinical data is still scarce.

Materials & Methods:
Retrospective multicenter national cohort study in France including every patient prescribed olorofim as a compassionate treatment for proven or probable IFD between 2020 and 2023. Olorofim introduction criteria were proven non-Mucorales mold infection refractory to standard antifungal agents, intolerance to such agents or IFD with no effective antifungal therapy available. We assessed efficacy – defined by mycological and clinical control of IFD – and safety of olorofim. Olorofim was provided by F2G laboratory as oral therapy.

Results:
Fifteen patients aged 39 (22.5-58.5) years received olorofim. Primary immunodeficiency, mostly chronic granulomatous disease (CGD), and lung transplantation were the main risk factors (40% and 50%, respectively). IFD localizations included lung (80%), central nervous system (CNS) (20%), with dissemination in 4 patients (26.6%). Twenty strains were identified, mostly *Aspergillus fumigatus* (n=7, 46.6%) and *Microascus* spp. (n=3, 15%)[1]. Other fungi included *Lomentospora prolificans*, *Rasamsonia aegroticola*[2], *Scopulariopsis alboflavescens* and different species of *Aspergillus* (*A. flavus*, *A. calidoustus*, *A. latus, A. tubingensis*, *A. nidulans* and *Neosartorya udagawae*). Forty percent of strains displayed high minimum inhibitory concentrations (MICs) for azoles. All patients received prior antifungal therapy for the IFD of interest (average of 2.53 ± 0.91 therapeutic lines) for a median time of 334 (138-487) days.
Olorofim was mainly started for refractory IFD (66.7%). When available (N=7), olorofim MICs were ≤0.5mg/L. Olorofim therapy was mostly given as a combination therapy (86.7%), especially with echinocandins and/or azoles. Two patients died within 15 days of olorofim introduction and were not considered for efficacy analysis. Mycological failure was reported in 3/13 individuals (23%) who died with refractory IFD: (i) disseminated lomentosporiosis in a lung transplant recipient, (ii) *Scopulariopsis* lung infection in a lung transplant recipient and (iii) lung and CNS aspergillosis caused by *N. udagawae* and *A. latus* in a CGD liver transplant recipient. All 3 received a combination of azole and olorofim. Ten patients (77%) achieved mycological remission under olorofim therapy: 6 cases of aspergillosis including lung, CNS and disseminated infections, 3 cases of *Microascus* lung infection and one *Rasamsonia* lung infection. 1/10 patient died of a non-related cause. Three-month mortality was 30.8% (N=4/13). Olorofim was stopped in 2 live patients: one post-traumatic *Microascus melanosporus* lung infection after 6 weeks, and one disseminated aspergillosis in a liver transplant recipient after 13 months. All other 7 live patients were still on olorofim therapy (mean duration 7.9 months), including 4 with other concomitant antifungals. No increase in alanine aminotransferase levels was reported in any patients.

Conclusions:
Prolonged olorofim therapy seems an effective and well-tolerated option in *Aspergillus* and *Microascus* refractory IFD, with low MICs against resistant molds.
The authors thank F2G for providing olorofim.

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Activity of Rezafungin Against Non-albicans Candida Isolates Causing Bloodstream Infections in European Medical Centres (2014–2022)

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Objectives: Candidemia is one of the most common bloodstream infections (BSI) in European hospitals. Progressive shifts from *Candida albicans* to non-*albicans Candida* spp. have been observed globally but regional differences are reported. Rezafungin is a new echinocandin approved for the treatment of candidemia and invasive candidiasis by the US FDA. In this study, we evaluated the activity of rezafungin, according to CLSI, against non-*albicans Candida* isolates causing BSI in European medical centres from 2014–2022 as part of the rezafungin surveillance program.
Methods: 907 non-*albicans Candida* isolates were collected (1/patient) in 2014–2022 from 26 medical centres located in Western Europe (W-EU; *n*=638; 20 centres) and Eastern Europe (E-EU; *n*=269; 6 centres). Isolates were identified by MALDI-TOF MS and tested by CLSI broth microdilution. CLSI M27M44S Ed3 (2022) breakpoints were applied (provisional values for rezafungin). CDC-tentative MIC breakpoints were applied to *Candida auris* (CAU).

Results: Isolates included *Candida parapsilosis* (CP; 339; 37.4%), *Candida glabrata* (CG; 329; 36.3%), *Candida tropicalis* (CT; 148; 16.3%), *Candida krusei* (CK; 49; 5.4%), *Candida dubliniensis* (CD; 34; 3.8%), CAU (4; 0.4%), and *Candida orthopsilosis* (CO; 4; 0.4%). Rezafungin inhibited all CP, CK, and CT at the susceptible breakpoints, regardless of region (Table). CP, CK, and CT isolates displayed 100% S rates against anidulafungin, caspofungin, and micafungin, except for anidulafungin vs. CP (93.5% and 96.8% S in W-EU and E-EU, respectively), and caspofungin vs. CK (96.9% in W-EU). Rezafungin ($MIC_{50/90}$, 0.06/0.12 mg/L; 98.3% susceptible in W-EU and $MIC_{50/90}$, 0.03/0.12 mg/L; 97.9% susceptible in E-EU) showed similar activity to other echinocandins ($MIC_{50/90}$ range, 0.015–0.06/0.03-0.12 mg/L; 97.0–97.8% susceptible in W-EU and $MIC_{50/90}$, 0.015–0.06/0.03–0.12 mg/L; 96.9–97.9% susceptible in E-EU) against CG. Only 6 CG (4 W-EU and 2 E-EU) isolates were nonsusceptible to rezafungin, while 8, 8, and 9 CG isolates were non-susceptible to anidulafungin, caspofungin, and micafungin, respectively. All CD isolates were inhibited by rezafungin, anidulafungin, caspofungin, and micafungin at 0.5, 0.5, 2, and 1 mg/L, respectively. Susceptible breakpoint criteria was only available for rezafungin, and only 3 CD (W-EU) isolates were nonsusceptible (88.9% susceptible). All CAU isolates were inhibited by the echinocandins at their respective breakpoints. Although no breakpoints are available against CO, all isolates were inhibited by rezafungin, anidulafungin, caspofungin, and micafungin at 2, 2, 0.25, and 0.5 mg/L, respectively. All CAU were resistant to fluconazole. Overall, fluconazole resistance was detected in 22.4% (27.8%/8.5% in W-EU/E-EU), 6.4%

(6.0%/7.2% in W-EU/E-EU), and 0.7% (1.0%/0.0% in W-EU/E-EU) of CP, CG, and CT isolates, respectively.

Conclusions: Rezafungin was very active against CP, CG, CT, CK, CD, CAU, and CO isolates causing candidemia in European medical centres. Echinocandins, including rezafungin, displayed similar activity against different *Candida* species. High resistance rates were noted for fluconazole against CAU, CP, and CG, and differences in resistance rates between European regions were observed, mainly for CP and CAU.

Table. Activity of rezafungin and other echinocandins against non-*albicans Candida* isolates causing bloodstream infections in Western and Eastern Europe

Organism (no. of isolates from W-EU/E-EU)	MIC_{50}/MIC_{90} (mg/L) CLSI %S							
	W-EU				E-EU			
	RZF	ANF	CSF	MCF	RZF	ANF	CSF	MCF
C. parapsilosis (245/94)	1/2 100.0	2/2 93.5	0.25/0.5 100.0	1/1 100.0	1/2 100.0	2/2 96.8	0.25/0.5 100.0	1/1 100.0
C. glabrata (232/97)	0.06/0.12 98.3	0.06/0.12 97.0	0.03/0.06 97.8	0.015/0.03 97.0	0.03/0.12 97.9	0.06/0.12 96.9	0.03/0.06 96.9	0.015/0.03 97.9
C. tropicalis (98/50)	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0
C. krusei (32/17)	0.03/0.12 100.0	0.06/0.12 100.0	0.12/0.25 96.9	0.06/0.12 100.0	0.03/0.06 100.0	0.06/0.06 100.0	0.12/0.25 100.0	0.06/0.12 100.0
C. dubliniensis (27/7)	0.06/0.25 88.9	0.03/0.12 NA	0.03/0.25 NA	0.015/0.12 NA	0.06/- 100.0	0.06/- NA	0.03/- NA	0.03/- NA
C. auris (0/4)	NA NA	NA NA	NA NA	NA NA	0.5/- 100.0	0.25/- 100.0[a]	0.25/- 100.0[a]	0.12/- 100.0[a]
C. orthopsilosis (4/0)	0.25/- NA	0.5/- NA	0.25/- NA	0.5/- NA	NA NA	NA NA	NA NA	NA NA

Abbreviations: RZF, rezafungin; ANF, anidulafungin; CSF, caspofungin; MCF, micafungin; NA, not applied.
[a] Applying CDC-tentative MIC breakpoints published at https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html.

Table. Activity of rezafungin and other echinocandins against non-*albicans Candida* isolates causing bloodstream infections in Western and Eastern Europe

Organism (no. of isolates from W-EU/E-EU)	MIC_{50}/MIC_{90} (mg/L) CLSI %S							
	W-EU				E-EU			
	RZF	ANF	CSF	MCF	RZF	ANF	CSF	MCF
C. parapsilosis (245/94)	1/2 100.0	2/2 93.5	0.25/0.5 100.0	1/1 100.0	1/2 100.0	2/2 96.8	0.25/0.5 100.0	1/1 100.0

C. glabrata (232/97)	0.06/0.12 98.3	0.06/0.12 97.0	0.03/0.06 97.8	0.015/0.03 97.0	0.03/0.12 97.9	0.06/0.12 96.9	0.03/0.06 96.9	0.015/0.03 97.9
C. tropicalis (98/50)	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0	0.03/0.06 100.0
C. krusei (32/17)	0.03/0.12 100.0	0.06/0.12 100.0	0.12/0.25 96.9	0.06/0.12 100.0	0.03/0.06 100.0	0.06/0.06 100.0	0.12/0.25 100.0	0.06/0.12 100.0
C. dubliniensis (27/7)	0.06/0.25 88.9	0.03/0.12 NA	0.03/0.25 NA	0.015/0.12 NA	0.06/- 100.0	0.06/- NA	0.03/- NA	0.03/- NA
C. auris (0/4)	NA NA	NA NA	NA NA	NA NA	0.5/- 100.0	0.25/- 100.0[a]	0.25/- 100.0[a]	0.12/- 100.0[a]
C. orthopsilosis (4/0)	0.25/- NA	0.5/- NA	0.25/- NA	0.5/- NA	NA NA	NA NA	NA NA	NA NA

Abbreviations: RZF, rezafungin; ANF, anidulafungin; CSF, caspofungin; MCF, micafungin; NA, not applied.
[a] Applying CDC-tentative MIC breakpoints published at https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html.

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Outcomes of invasive fungal disease in a subgroup of patients receiving olorofim/azole combination in an open label salvage study.

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Objectives:
A phase IIb trial (S32 - NCT03583164) evaluated efficacy of olorofim in patients with invasive fungal diseases (IFD) with imited/no treatment options. As in vitro data suggest that azole antifungals can raise the MIC of olorofim by 1-2 fold dilutions under certain static testing conditions, this analysis describes clinical outcomes in the subset of the first 100 S32 patients who received olorofim in combination with azoles for ≥2 days (ConAzole) in comparison with those who received olorofim either as monotherapy or in combination with a non-azole antifungal or who received concomitant treatment with azoles for <2 days (NoConAzole).

Materials & Methods:
Patients with Proven IFD (any fungus) or probable pulmonary aspergillosis received oral olorofim 150mg BID (loading dose) followed by 90mg BID. Because azole-mediated CYP3A4 inhibition reduces olorofim clearance, olorofim maintenance dose was reduced to 60mg BID when co-dosing with any azole other than isavuconazole. An independent Data Review Committee (DRC)- adjudicated Overall Success defined as Complete or Partial response per EORTC-MSG criteria[1]. All-cause mortality (ACM), as an objective measure of efficacy, was assessed through D84.

Results:
Of 100 patients in S32, 29 received olorofim with ConAzole (most often fluconazole; n=13). Mo-st patients had invasive aspergillosis (IA) (N=16, including a case of co-infection with *Aspergillus* spp. and *Scopulariopsis* spp.), 8 had extrapulmonary coccidioidomycosis, 2 each had IFD due to *Lomentospora prolificans* and *Scopulariopsis* spp., and 1 due to *Scedosporium* spp.
Of the 71 remaining patients (NoConAzole), 69 received olorofim either as monotherapy or in combination with a non-azole antifungal, and 2 with azoles for <2 days. Thirty-seven had IA. In both groups, lung was the most frequently involved body site, and at similar rates (55% (16/29) and 46.5% (33/71), respectively). Disseminated and/or central nervous system (CNS) disease was reported in 44.8% (13/29) ConAzole and 17% (12/71) NoConAzole. Fifteen NoConAzole (21.1%; 15/71) had bone and/or joint disease compared wit no subject among ConAzole group.
High immunosuppression (Haem Malignancy and/or HSCT) was recorded in 51% (15 /29) ConAzole and 42.2% (30/71) NoConAzole patients.
Similar efficacy outcomes were observed in ConAzole and NoConAzole groups, and in the respective subsets with IA (Table 1). At D42 (primary endpoint) Overall Success was seen in 41.4% (12/29) and 45.0% (32/71); at D84 in 44.8% (13/29) and 40.8% (29/71); ACM at D90 was 20.7% (6/29) and 19.7% (14/71) in ConAzole and NoConAzole, respectively.

Conclusions:
Olorofim is an oral, novel anti-mould agent with activity against a range of fungi including pan-drug resistant species. Beside limitations due to small sample size, in patients treated with a combination of olorofim and azoles for at least 2 days (ConAzole), mirrors efficacy outcomes of patients treated with olorofim either as monotherapy or in combination with a

non-azole antifungal or who received concomitant treatment with azoles for <2 days (NoConAzole), and of the full cohort of 100 patients enrolled in S32[2]. These results indicate there is no meaningful impact on clinical efficacy from a treatment combination of olorofim with azole drug class and no evidence of any clinical antagonism.

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Table 1. Efficacy Outcomes in ConAzole and NoConAzole patients

ConAzole patients N=29	DAOS	Failure	Day 90 ACM	NoConAzole patients N=71	DAOS	Failure	Day 90 ACM
Day 42	12/29 (41.3%)	17/29 (58.6%)	5 /29 (17.2%)	Day 42	32/71 (45.0%)	39/71 (54.9%)	10/71 (14.0%)
Day 84	13/29 (44.8%)	16/29 (55.2%)	6/29 (20.7%)	Day 84	29/71 (40.8%)	40/71 (56.3%)	14/71 (19.7%)
ConAzole IA patients N=16	Overall Success	Failure	Day 90 ACM	NoConAzole IA patients N=37	Overall Success	Failure	Day 90 ACM
Day 42	8/16 (50%)	8/16 (50%)	4/16 (25.0%)	Day 42	16/37 (43.2%)	21/37 (56.7%)	9/37 (24.3%)
Day 84	9/16 (56.2%)	7/16 (43.8%)	5/16 (33.2%)	Day 84	14/37 (37.8%)	23/37 (62.1%)	11/37 (29.7%)

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Efficacy Assessment of SF001, a Third-Generation Polyene Antifungal, in the Immunosuppressed Mouse Model of Invasive Pulmonary Aspergillosis

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Objectives: Invasive pulmonary aspergillosis (IPA) is associated with high mortality and morbidity. While most of IPA is caused by *Aspergillus fumigatus,* other species that show resistance to current antifungal therapies (e.g. *A. lentulus* and *A. calidoustus*) are increasingly reported to cause infection. Thus, novel therapeutic modalities are needed. SF001 is a rationally designed 3rd generation polyene with reduced nephrotoxicity and broad-spectrum fungicidal activity. We sought to compare the efficacy of SF001 to liposomal amphotericin B (LAMB) in a neutropenic mouse model of IPA due to either *A. fumigatus, A. lentulus,* or *A. calidoustus.*

Methods & Materials: *In vitro* susceptibility was conducted by the CLSI M38 method using clinical isolates *A. fumigatus* AF293, *A. lentulus* AL1, and *A. calidoustus* AC6. ICR mice were immunosuppressed with cyclophosphamide and cortisone acetate on days -2, and +3, relative to infection challenge by intranasal inhalation with *A. fumigatus* or through intratracheal instillation for *A. lentulus* or *A. calidoustus*. Treatment (iv, qd), with diluent (placebo control), SF001 (0.3, 1.5, 5, 7.5, or 30 mg/kg, or LAMB (5 mg/kg), began 16 h post challenge and continued for 4 days for *A. fumigatus* and *A. lentulus* or for 7 days for *A. calidoustus*. Survival (n=10/group) through Day +21 and tissue fungal burden (n=10/group) on Day +4 (by qPCR) served as primary and secondary endpoints, respectively.

Results: The MIC_{100} values defined as the concentration that resulted in 100% growth inhibition for SF001 were 0.5 µg/mL for *A. fumigatus*, 2 µg/mL for *A. lentulus,* and 1 µg/mL for *A. calidoustus.* In contrast, the MIC_{100} values for LAMB were 0.06 µg/mL for *A. fumigatus*, and >16 µg/mL for either *A. lentulus,* or *A. calidoustus*. MIC value for a panel of clinical *Aspergillus* species are provided in Table 1. All mice receiving placebo died, at day 8 (*A. fumigatus*), day 12 (*A. lentulus*) and day 16 (*A. calidoustus*). SF001 at doses >1.5 mg/kg, improved median survival time and overall percent survival compared to placebo (Table, $p < 0.05$ for any treatment vs. placebo). LAMB at 5 mg/kg improved survival of *A. calidoustus*, but less for *A. lentulus*. SF001 demonstrated dose-dependent improvement in survival, with the highest dose (30 mg/kg) being well-tolerated and resulting in enhanced survival compared to LAMB (Table 2). SF001 at 30 mg/kg also resulted in 6 log and 5 log reductions in lung fungal burden of mice infected with *A. fumigatus* when compared to placebo and LAMB (5 mg/kg) treated mice, respectively.

Conclusions: SF001 is a 3rd generation polyene that has more potent *in vitro* activity compared to LAMB against select "polyene-resistant" *Aspergillus* species. SF001 demonstrated dose-dependent efficacy, was well-tolerated and improved survival in a mouse model of IPA caused by *Aspergillus* species that exhibit polyene resistance. Continued investigation and development of SF001 as a novel agent against IPA is warranted.

Table 1: Comparison of the MIC of SF001 vserus clinically used antifungal agents against *Aspergillus* species.

Species	Isolate No.	SF001 (AM-2-19Ac)		LAMB		Isavuconazole		Posaconazole		Voriconazole	
		50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
Aspergillus calidoustus	AC1	0.5	1	1	>16	1	4	2	>16	4	8
	AC2	0.25	1	1	>16	1	4	2	>16	4	8
	AC3	0.25	1	0.5	>16	1	4	2	>16	4	8
	AC4	0.25	0.5	0.125	>16	1	4	2	>16	2	8
	AC5	0.25	1	0.5	>16	1	4	2	>16	4	8
	AC6	0.25	1	0.25	>16	1	4	2	>16	2	8
Aspergillus flavus	Aflav1	1	1	1	1	0.5	2	0.125	0.5	0.5	2
	Aflav2	0.25	1	1	8	0.5	1	0.125	0.25	0.5	1
	Aflav3	0.5	1	0.25	0.25	0.5	1	0.125	0.25	0.5	1
	Aflav4	2	2	>16	>16	0.5	2	0.125	0.25	1	2
	Aflav5	0.5	0.5	0.125	0.125	0.5	1	0.125	0.125	0.5	1
	Aflav6	0.5	1	0.125	0.125	0.25	0.5	0.06	0.06	0.25	1
Aspergillus fumigatus	AF293	0.25	0.5	0.06	0.06	0.5	1	0.25	0.5	0.5	1
	AF1	0.5	1	0.125	0.125	>16	>16	0.5	1	>16	>16
	AF2	0.25	0.5	0.125	0.125	4	16	0.5	1	4	8
	AF3	0.25	0.5	0.06	0.06	0.25	0.25	≤0.03	≤0.03	0.25	0.25
	AF4	0.25	0.5	0.125	0.125	0.25	1	0.06	0.125	0.25	0.5
	AF5	0.25	0.5	0.125	0.5	0.25	2	0.125	0.125	0.5	1
Aspergillus lentulus	AL1	1	2	>16	>16	1	2	0.25	0.5	4	4
	AL2	1	4	>16	>16	1	2	0.25	0.25	2	4
	AL3	2	4	>16	>16	1	4	0.25	0.5	2	8
	AL4	1	2	0.5	4	1	4	0.25	0.5	2	4
	AL5	1	2	>16	>16	1	1	0.25	0.5	4	4
	AL6	0.5	2	>16	>16	1	2	0.125	0.25	2	4
Aspergillus niger	AN1	0.125	0.25	≤0.03	≤0.03	0.5	1	0.06	0.125	0.5	1
	AN2	0.125	0.25	≤0.03	0.06	1	2	0.125	0.25	1	2
	AN3	0.25	0.25	≤0.03	0.06	1	2	0.125	0.125	0.5	1
	AN4	0.125	0.125	≤0.03	≤0.03	0.5	1	0.06	0.125	0.5	0.5
	AN5	0.25	0.25	≤0.03	≤0.03	1	2	0.125	0.25	1	1
	AN6	0.25	0.25	≤0.03	0.06	1	2	0.125	0.25	1	1
Aspergillus terreus	AT1	0.5	1	0.5	1	0.5	8	0.125	0.25	1	2
	AT2	0.5	1	1	4	0.5	4	0.06	0.25	0.5	2
	AT3	0.5	2	2	>16	0.5	4	0.125	0.25	1	2
	AT4	0.5	1	1	2	0.25	4	≤0.03	0.25	0.5	2
	AT5	0.5	0.5	>16	>16	0.25	2	≤0.03	0.125	0.5	1
	AT6	0.5	0.5	2	4	0.25	1	≤0.03	0.06	0.5	0.5

Table 2	*A. fumigatus*		*A. lentulus*		*A. calidoustus*	
Drug dose (mg/kg)	**Median survival (day)**	**% Survival**	**Median survival (day)**	**% Survival**	**Median survival (day)**	**% Survival**
Placebo	7	0	8	0	12	0
SF001 0.3	7	0	9.5	10	15.5	20
SF001 1.5	8	0	9.5	10	16.5	30
SF001 5.0	ND	ND	12	30	>21	70
SF001 7.5	10	20	ND	ND	ND	ND
SF001 30	16	50	16.5	40	>21	70
LAMB 5	8.5	10	10.6	20	>21	60

P415

In-vitro Antifungal Activity of Five Novel Anti-Fungal Compounds Against Talaromyces marneffei

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Objectives

Talaromyces marneffei (Tm) is a thermally dimorphic fungus causing invasive mycosis in human immunodeficiency virus (HIV) infected patients in Southeast Asia and is a leading cause of AIDS-related deaths in this region with mortality on anti-fungal therapy as high as 30%. The limited options, routes of administration, and toxicity of antifungals with established efficacy against Tm calls for assessment of efficacy for additional antifungals against Tm. Here we evaluate the in-vitro efficacy of 5 novel anti-fungal agents against 32 clinical strains of Tm. The agents tested include ibrexafungerp, two manogepix compounds (APX001A and APX2039), olorofim, and oteseconazole.

Methods

32 Tm isolates were cultured from clinical samples from patients in Vietnam and identified as Tm by standard morphological characteristics. Inocula were prepared from these clinical samples in conjunction with CLSI standards and plated onto 96-well microplates. Concentrations for each drug ranged from 0.008 µg/mL to 4 µg/mL. AlamarBlue™, a resazurin-based cell viability indicator dye, was added to the drug-inoculum solution. Use of this reagent enabled utilization of a microplate fluorescence intensity(FI) reader to objectively identify the designated MIC endpoint of 50% inhibition of growth.

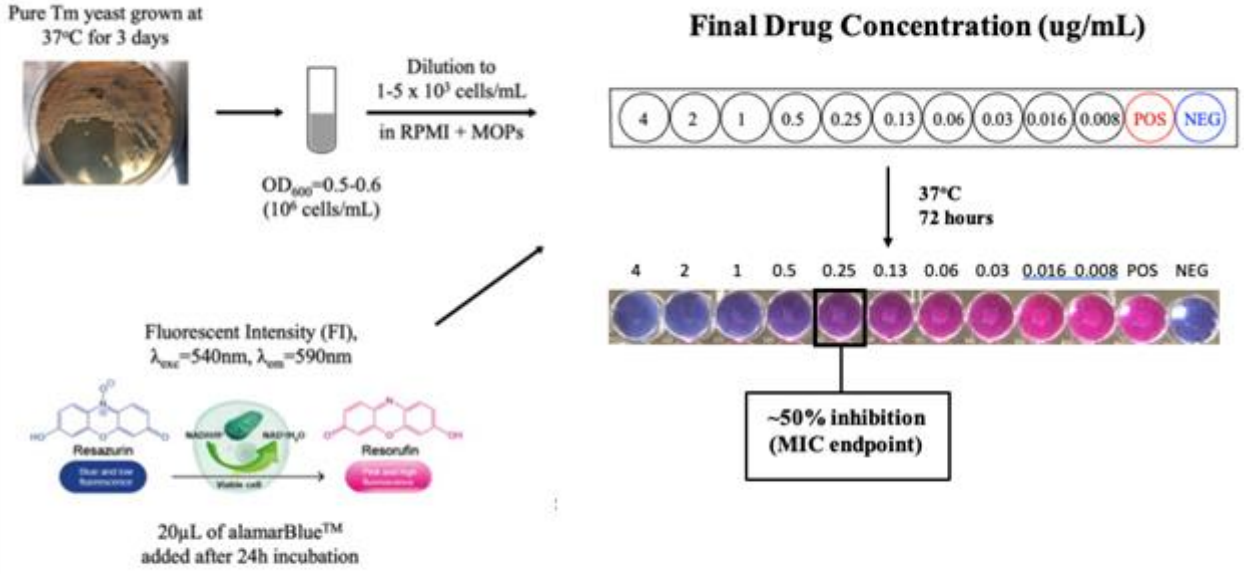
Results

Of the drugs tested, olorofim showed the highest activity against the 32 isolates, with MICs consistently ≤0.008 µg/mL. This was followed by oteseconazole with MICs between ≤0.008 and 0.031 µg/mL APX001A with MICs between 0.031 and 0.25 µg/mL and APX2039 with MICs between 0.25 and 4 µg/mL. Ibrexafungerp had the broadest MIC distribution with MICs between 0.063 and 4 µg/mL. Figure 2 and Table 1 summarize the results of the MIC distribution.

Conclusions

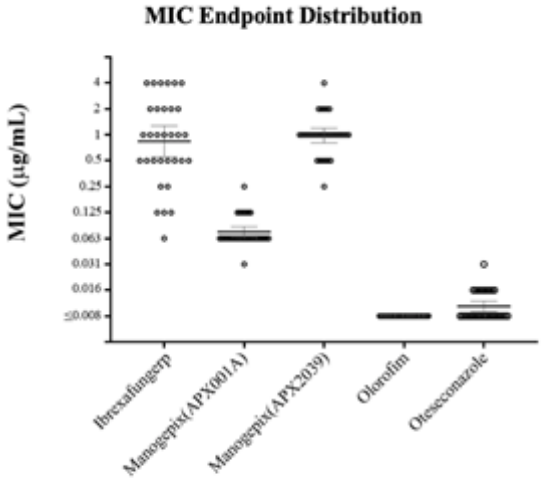
In-vitro activity of the 5 drugs tested against the 32 Tm strains was variable, but all should be considered potential candidates for treating Tm infection. To further evaluate the potential role of each drug in treating Tm infection, the next step should be to evaluate the efficacy of each in in-vivo models.

Figure 1.



Methods (Figure 1). MIC endpoint determination using AlamarBlue™ indicator dye method

Figure 2.



Results (Figure 2/Table 1). MIC Endpoint Distribution. Figure 2 shows a scatter plot of MIC endpoints for each drug. Error bars demonstrate the geometric mean and 95% confidence intervals. Table 1 illustrates the MIC endpoint distribution in a graded heat map format. In addition, the absolute values of the geometric mean (GM), mode, MIC50, and MIC90 are displayed at the right side of the table. Both Figure 2 and Table 1 illustrate high efficacy for olorofim and oteseconazole, with low MIC endpoints consistent across the 32 isolates. The manogepix compounds(APX001A and APX2039) demonstrate surprisingly contrasted performance against Tm, despite the similar chemical structure and identical mechanism of action of the drugs. APX001A had significantly lower MIC endpoints compared to APX2039. Ibrexafungerp had the broadest range of MIC endpoints from 0.063 to 4µg/mL and the highest MIC50 and MIC90 of all the drugs. However, a significant proportion of isolates were highly susceptible to the drug.

Table 1.

Antifungal Agent	MIC (µg/mL)													
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	GM	Mode	MIC50	MIC90
Ibrexafungerp				1	3	2	8	7	5	6	0.84	0.5	1	4
Manogepix(APX001A)			1	23	7	1					0.075	0.063	0.063	0.125
Manogepix(APX2039)						1	6	18	5	1	0.98	1	1	0.5
Olorofim	32										≤0.008	≤0.008	≤0.008	≤0.008
Oteseconazole	21	10	1								0.01	≤0.008	≤0.008	0.016

Note: MIC50 and MIC90 refer to the MICs that inhibit at least 50% and 90% of the sample population, respectively.

P416

Once-weekly rezafungin versus daily caspofungin to treat candidaemia and invasive candidiasis: pooled analysis of clinical trial participants in Europe

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Objectives:
Rezafungin is a new FDA-approved echinocandin to treat candidaemia and invasive candidiasis (IC), and is in development to prevent invasive fungal diseases caused by *Candida*, *Aspergillus*, and *Pneumocystis* spp after blood and marrow transplantation. In contrast to available echinocandins (administered daily [QD]), the long-acting pharmacokinetic profile of rezafungin enables once weekly (QW) administration with front-loaded dosing. Rezafungin QW was compared with caspofungin QD to treat candidaemia and/or IC in two double-blind randomized clinical trials of similar design: Phase 2 STRIVE (NCT02734862) and Phase 3 ReSTORE (NCT03667690).[1,2] ReSTORE showed rezafungin QW was non-inferior to caspofungin QD.[2] This pooled analysis of STRIVE and ReSTORE reports outcomes for participants from Europe.

Materials & Methods:
STRIVE and ReSTORE study designs are previously described.[1,2] This pooled analysis included STRIVE and ReSTORE participants recruited in Europe who received rezafungin QW (Week 1: 400 mg; 200 mg thereafter) and caspofungin QD (Day 1 70 mg; 50 mg thereafter [weight and hepatic impairment adjusted]) for ≥14 days (up to 4 weeks). Efficacy endpoints included Day 14 Global response rate (comprising clinical cure [STRIVE: investigator assessed, ReSTORE: data review committee assessed], radiological cure [ReSTORE], and mycological eradication [ReSTORE and STRIVE]; primary for European Medicines Agency), 30-day all-cause mortality (ACM; primary for US FDA), mycological eradication at Days 5 and 14 (secondary), and time to negative blood culture (TTNBC; exploratory).

Results:
Baseline characteristics were balanced between the rezafungin (N=67) and caspofungin (N=76) arms. Most participants had candidaemia only (82.1 and 77.6%) and *Candida albicans* was the most frequent *Candida* spp (47.8 and 40.8%; Table 1).
Efficacy outcomes for rezafungin QW vs caspofungin QD, including Day 14 Global response rate (70.1 vs 67.1%; difference [95% CI] 4.2 [-11.4, 19.1]), 30-day ACM (14.9 vs 19.7%; difference [95% CI] -6.6% [-19.2, 6.1]), and mycological eradication on Day 5 (82.1 vs 64.5%; difference 16.9 [2.5, 31.1]) and Day 14 (76.1 vs 68.4%; difference 8.4 [-6.0, 22.9]) were

comparable (Table 2). Median (25, 75 percentile) TTNBC for rezafungin was 21.7 h (14.3, 51.0) vs 28.0 h (18.1, 169.3) for caspofungin; p=0.011 (stratified log–rank).
With rezafungin QW vs caspofungin QD, treatment-emergent adverse events occurred in 89.9 vs 79.3% (15.9 vs 12.2% were considered treatment related) and serious AEs occurred in 46.4 vs 45.1% (2.9 vs 3.7% were considered treatment related). Outcomes according to *Candida* spp will be presented.

Conclusions:

This integrated analysis of STRIVE and ReSTORE participants recruited in Europe provides regional insight into the efficacy of rezafungin for the treatment of candidemia and IC. Once-weekly rezafungin was comparable to daily caspofungin for Day 14 Global response, 30-day ACM, TTNBC, and mycological eradication, with early efficacy signals and numerical improvements with rezafungin vs caspofungin seen from Day 5. Efficacy outcomes in patients from Europe broadly reflected findings from an integrated analysis of the primary populations of STRIVE and ReSTORE.[3]

References:

1. Thompson III GR, et al. *CID* 2021;73:e3647–e3655.
2. Thompson III GR, et al. *Lancet* 2023;401:49–59.
3. Soriano A, et al. Abstract 04673, presented at ECCMID, 23–26 April 2022.

Table 1. Baseline demographic and disease characteristics of patients recruited in Europe[a] (mITT population[b])

n (%)	Rezafungin QW (N=67)	Caspofungin QD (N=76)
Age, years, mean (SD)	63.4 (14.1)	63.6 (15.1)
Female	22 (32.8)	25 (32.9)
Race, White	62 (96.9)	71 (93.4)
Weight, kg, mean (SD)	72.9 (21.3)	74.6 (17.5)
Final diagnosis		
Candidaemia only	55 (82.1)	59 (77.6)
IC	12 (17.9)	17 (22.4)
Modified APACHE II score <20	58 (86.6)	59 (80.6)
Creatinine clearance ≥60 mL/min	35 (57.4)	41 (59.4)
Frequent *Candida* spp[c]		
Candia albicans	32 (47.8)	31 (40.8)
Candida glabrata	17 (25.4)	14 (18.4)
Candida parapsilosis	11 (16.4)	20 (26.3)
Candida tropicalis	8 (11.9)	7 (9.2)

APACHE, Acute Physiology and Chronic Health Evaluation; IC, invasive candidiasis; mITT, modified intent-to-treat; QD, once daily; QW, once weekly

[a]Includes participants in STRIVE and ReSTORE from Europe and Israel

[b]Participants with documented *Candida* infection ≤4 days prior to randomization who received ≥1 dose of study medication

[c]*Candida* spp >10% in either treatment arm

Table 2. Efficacy outcomes in patients recruited in Europe[a] (mITT population[b])

n (%)	Rezafungin QW (N=67)	Caspofungin QD (N=76)
Day 14 Global response rate[c]	47 (70.1)	51 (67.1)
Difference (95% CI)[d]	4.2 (-11.4, 19.2)[c]	
30-day all-cause mortality	10 (14.9)	15 (19.7)
Difference (95% CI)[d]	-6.6 (-19.2, 6.1)[c]	
Mycological eradication[e]		
Day 5	55 (82.1)	49 (64.5)
Difference (95% CI)[d]	16.9 (2.5, 31.1)[c]	
Day 14	51 (76.1)	52 (68.4)
Difference (95% CI)[d]	8.4 (-6.0, 22.9)[c]	
TTNBC	(N=60)	(N=63)
Patients with negative blood culture[f]	53 (88.3)	51 (81.0)
Time to first negative blood culture, h, median (25, 75 percentile)[g]	21.7 (14.3, 51.0)[g]	28.0 (18.1, 169.3)

CI, confidence interval; mITT, modified intent-to-treat, QD, once daily; QW, once weekly

[a]Includes participants in STRIVE and ReSTORE from Europe and Israel

[b]Participants with documented *Candida* infection ≤4 days prior to randomization who received ≥1 dose of study medication

[c]Global response comprised clinical cure (per investigator [STRIVE] or Data Review Committee [ReSTORE] assessment), radiological cure (ReSTORE, only), and mycological eradication (ReSTORE and STRIVE)

[d]Difference is rezafungin minus caspofungin (analysis stratified by study and study part [STRIVE])

[e]Failure following prior mycological eradication may be due to fungal persistence or recurrence (documented or presumed), change of antifungal therapy to treat invasive candidiasis/candidaemia, or death (or study discontinuation) due to any cause

[f]Patients with candidaemia and no positive culture following the first dose of study treatment. Patients were censored if they received an antifungal other than study drug for candidaemia, died, or were lost to follow-up prior to a negative blood culture

[g]From first dose of study medication

[g]p=0.011 (stratified log–rank test)

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Nikkomycin Z for the treatment of sporotrichosis caused by Sporothrix brasiliensis

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Sporothrix brasiliensis is a dimorphic fungus of global concern due to its high virulence causing feline and cat-transmitted human sporotrichosis. Therapeutic options to treat this mycosis are few, thus new antifungals are urgently needed. **Objectives:** Given promising activity of nikkomycin Z (NikZ) *in vitro*, we evaluated NikZ monotherapy and combination with itraconazole (ITZ) in the treatment of experimental sporotrichosis caused by *S. brasiliensis* in a murine model. **Materials & Methods:** Male BALB/cJ mice were subcutaneously infected in one paw with inoculum obtained from a single spore-derived culture of *S. brasiliensis* (final concentration of 10^7 yeasts per animal) and treated orally for thirty days, followed by 2 days' observation to allow drug washout from tissues. Animals were divided (nine/group) into a control group (untreated), ITZ group (50 mg/kg/day), two NikZ monotherapy groups (200 and 400 mg/kg/day), and one combination of NikZ (400 mg/kg/day) and ITZ (50 mg/kg/day). ITZ and placebo were given by gavage once/day, and NikZ by drug in drinking water, as described by Sass et al. (2021). Efficacy of treatments was analyzed via body weight gain, mortality rate, and fungal burden in tissues (brain, liver, lung, kidneys, testicles). The experiment was conducted according to the Animal Use Ethics (CEUA-FURG P003/2021). **Results:** All animals developed a local disease that progressed to a disseminated form of sporotrichosis. At the end of the treatment 100% survival occurred only in the group treated with NikZ 400 mg/kg; mortality rate in other groups ranged from 10% to 20%, without statistically significant differences. Animals from the untreated and the ITZ group lost weight (16 and 2%, respectively) during the experiment, otherwise, all groups treated with NikZ with or without ITZ gained weight ranging from 3% to 13%. At the end of the experiment no local clinical cure was achieved, and fungi in the footpad (site of inoculation) was recovered from all mice (p=0.5). With respect to residual fungal burdens (summated from internal organs), animals treated with NikZ (200 mg/kg) showed a similar fungal burden of *S. brasiliensis* (p >0.05) compared to ITZ treatment; mice treated with NikZ (400 mg/kg) had lower residual *S. brasiliensis* (p=0.016) than the ITZ group; the group treated with the combination had significantly reduced burden compared to each as monotherapy (p = 0.002 vs. ITZ; p =0.039 vs NikZ 400 m/kg). The superiority of the combination regimen was also apparent in the quantitative burdens in each of the organs. **Conclusions:** Our study is the first to describe the in vivo activity of NikZ against sporotrichosis caused by *S. brasiliensis*, showing the high potential of this compound to be used in monotherapy or in combination with ITZ to treat this mycosis. Our study also expands the scant data available for pre-clinical studies using *in vivo* models applied to sporotrichosis treatment.

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Anti-melanin activity of diphenyl diselenide against Cryptococcus neoformans

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Melanization is an essential virulence factor of *Cryptococcus neoformans*, being directly related to the pathogenesis of cryptococcosis. The organic compound diphenyl diselenide (DD) has inhibitory activity against *C. neoformans* proven in recent *in vitro* studies, but its mechanism of action is still unclear. A desirable approach for new drugs is anti-virulence activity, where virulence factors, such as melanin, are the target of action of the potential drug. **Objectives**: Evaluate the anti-virulence activity of diphenyl diselenide at distinct concentrations on *in vitro* melanin production by clinical isolates of *C. neoformans.* **Materials & Methods:** Ten clinical isolates of *C. neoformans* with a minimum inhibitory concentration (MIC) of 16µg/mL for diphenyl diselenide previously determined by microdilution assay (Benelli *et al.,* 2021) were selected. An inoculum solution was standardized with young colonies according to CLSI guidelines, and 100µL of each strain were plated on Agar Niger (AN) and on AN plus four distinct concentrations of DD (1.6, 4, 8 and 16µg/mL). Visual reading was performed on days 3 to 7 post-incubation at 30°C, to analyze number of colonies forming units (CFU), size and color of the colonies in each plate. To assess melanization, a semi-quantitative scale in crosses (1+ to 5+) was created, ranging from beige to dark brown. Data were tabulated and analyzed, and the averages of the outcome parameters as well as period to maximum melanization (in days) were calculated, comparing the results of isolates plated on the control AN (without DD) with their growth on AN plus distinct concentrations of DD. **Results**: DD was fungicide in 7/10 isolates in the highest concentration tested (16µg/mL), and at subinhibitory concentrations a reduction of >50% of CFU in comparison with control plate was shown ($5{,}2.10^5$ *versus* $2{,}0.10^5$ cells/mL). It was also possible to show a progressive decrease in the size of the colonies (ranging between 0.75mm to 0.25mm) and an increase in the maximum melanization period comparing strains growth in control plates (AN without DD) and in plates with DD exposures (Table 1). As the concentration of diphenyl diselenide increases, there was a progressive decrease in the melanization of the colonies observed by staining (Figure 1). **Conclusions:** Exposure to diphenyl diselenide decreases and delays growth and melanization of *C. neoformans* isolates, showing that this compound has a potential anti-virulence action in addition to inhibitory activity, instigating the continuity of the studies in this field.

Table 1. Maximum period (number of days) necessary to a complete melanization of the colonies in each isolate of *C. neoformans* (n=10) plated on Agar Niger (AN) and on AN plus distinct concentrations of DD.

Isolate/ DD (µg/mL)	*NA without DD*	*1,6*	*4*	*8*	*16*
796	4,5	5	5,5	5	NFG
905	4,5	5,5	5,5	6	NFG
1124	4,5	5,5	6	7	NFG
1278	4,5	5,5	6,5	NFG	NFG
1446	4,5	6	6	NFG	NFG
1195	6	7	7	NFG	NFG
1225	6	7	NFG	NFG	NFG
1530	5,5	7	7	NFG	NFG
1768	5,5	7	7	NFG	NFG
1882	5,5	7	7	NFG	NFG
Mean	5,1	6,25	6,4	6	NFG

NFG: No fungal growth

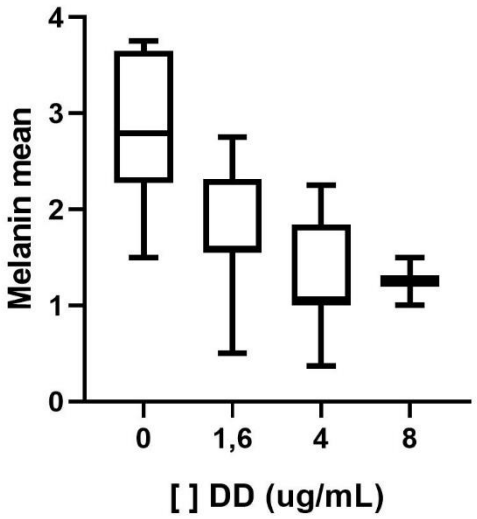

Figure 1. Mean melanization in three days in distinct concentrations of DD.

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Synergistic compounds with azoles kill drug-resistant Candida albicans by accumulation of eburicol resulted from inhibition on Erg251

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Synergistic compounds with azoles kill drug-resistant *Candida albicans* by accumulation of eburicol resulted from inhibition on Erg251

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Objectives:

Resistance to azoles in *Candida albicans* has long been a clinical concern. To solve this problem, we performed a series of modifications to berberine derivatives to obtain compound G42, which synergized with azoles against the drug-resistant *C. albicans* with fungicidal activity and low toxicity. This study aimed to elucidate the mechanism and the potential target of such synergistic fungicidal effect.

Materials & Methods:

In vitro antifungal activities of compound G42 and azoles against drug-sensitive and drug-resistant *C. albicans* strains were performed by using of microbroth dilution method and E-test. Their inhibition on hyphae growth and biofilm formation was further investigated. In vivo antifungal activity was performed in a *Galleria mellonella* model with systemic fungal infection. The expression levels of genes in the ergosterol synthesis pathway were investigated by real-time RT-PCR. The sterol components in cell membranes were analyzed by GC-MS upon the treatment of chemicals. Several genes including *ERG3*, *ERG6*, *ERG11*, and *ERG251* were further disrupted to propose the potential mechanisms.

Results:

G42 eliminated the tolerance and reversed the resistance of *C. albicans* to azoles. *In vitro*, the minimum inhibitory concentration (MIC_{80}) of fluconazole (FCZ) against the azole-resistant *C. albicans* decreased from >64.0 μg/ml to 0.5- 8.0 μg/ml when synergized with G42 with the FICI value of 0.016. *C. albicans* treated with G42 combined with FCZ failed to grow on YPD plates, and the same phenomenon was observed around FCZ susceptibility strips on G42-containing YPD medium. *C. albicans* treated with two agents under electron microscopy lost their normal cell structure, and showed the wrinkled and ruptured cell membrane. G42 enhanced the inhibitory effect of azoles on *C. albicans* hyphae growth and biofilm formation. Compared with FCZ group, G42 in combination with FCZ can significantly prolong the survival rate of *Galleria mellonella* with systemic fungal infection and reduce fungal burden. FCZ alone or in combination with G42 upregulated *ERG1*, *ERG3*, *ERG6*, *ERG7*, *ERG11*, *ERG24*, *ERG25*, *ERG26* and *ERG27* in *C. albicans*, among which the expression of *ERG6* was most significantly increased in the combination group. G42 combined with FCZ caused the

abnormal accumulation of eburicol in *C. albicans*, while G42 lost its synergistic effect with FCZ when the *ERG6* gene, responsible for converting lanosterol to eburicol, was disrupted. G42 alone against the *erg11Δ/Δ* strain, or FCZ alone against the *erg251Δ/Δ* strain, either led to the accumulation of eburicol and further resulted in fungal death.

Conclusions:
Compound G42 can overcome the resistance of *C. albicans* to azoles by accumulating large amounts of eburicol resulted from inhibition on Erg251, thus exerts fungicidal effect when it is synergistic with azoles.

P420

Antifungal drugs against drug-resistant fungi: new targets, new mechanisms and novel compounds

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Objectives:
Antifungal azoles are facing more and more serious drug resistance. We have focused on the strategies including: 1) new targets, novel mechanisms and structure optimization of several kinds of sensitizers which can synergize antifungal azoles to kill or inhibit drug-resistant fungi; 2)research on novel azole antifungals against drug-resistant fungi.

Materials & Methods:
Based on our previous study on the lead compounds including baicalein, berberine, and curcumin, which can significantly enhance the susceptibility of fluconazole against fluconazole-resistant *Candida albicans*, a series of novel derivatives were designed, synthesized, and evaluated for their in vitro and in vivo synergistic activity in combination with fluconazole. Photo affinity probes and chemical genetic techniques have been employed to investigate their targets.
Based on the genetic recombinant *Saccharomyces cerevisiae* with overexpression of drug-resistant elements inluding MDR1, CDR1 or ERG11 mutations, novel azole antifungal agents have been designed, synthesized and evaluated for their antifungal activity against fungi, especailly drug-resistant fungi.

Results:
A series of novel sensitizers have been obtainded to promote antifungal azoles to kill drug-resistant fungi. Compound SPH8740, at a concentration of 0.5 μg/mL, can enhance the susceptibility of fluconazole against fluconazole-resistant *C. albicans* from >64.0 μg/mL to 0.125-0.5 μg/mL. Moreover, SPH8740 in combination with fluconazole can also exibit synergistic antifungal activity against *Cryptococcus neoformans*, *Candida glabrata*, and *Candida tropicalis*. Their synergistic fungicidal activity has been well investigated both *in vitro* and *in vivo*.
Eno1 in *C. albicans* has been identified as a potential target of baicalin based on photo affinity probes.
Based on the chemical genetic techniques, Erg251 has been identified to be the target of CZ66 which can promote antifungal azoles to kill drug-resistant fungi.
A series novel azoles have been obtained to have potent and broad-spectrum antifungal activity.

Conclusions:
Erg251 and Eno1 have been identified to be new targets to exert their antifungal synergistic effect, which deserve further resarech for new approches to combat fungal drug resistance.
Two azole antifungal candidates are under pre-clinical research and development.

P421

Mechanistic insight on the cell membrane and virulence property of thymoquinone against Candida tropicalis

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Mechanistic insight on the cell membrane and virulence property of thymoquinone against *Candida tropicalis*

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Introduction: Candidiasis has become a significant public health concern in recent years. The fungistatic nature of conventional azole drugs is responsible for drug resistance in *candida* strains. thymoquinone exhibits immense therapeutic potential including antimicrobial activity. Although its antifungal activity has been reported, not much is known about its mode of action. **Objectives:** The present study aims to explore the antifungal activity of thymoquinone and its mode of action at the cell membrane level and virulence property in *Candida tropicalis*. **Methods:** Antifungal susceptibility of thymol was done in terms of Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), disc diffusion, growth curves ergosterol quantification assay and confocal microscopy and biofilm inhibition assay. This work presents antifungal efficacy and mode of action of thymoquinone on *C. tropicalis* ATCC 750. **Result:** The MIC and MFC values were 16 µg/ml and 24 µg/ml, respectively. Zones of inhibition formed after disc diffusion were 6mm, 11 mm and 15mm at MIC, 2MIC and 3MIC of thymoquinone. With increasing concentration, the *Candida* growth pattern changes with increased lag and reduced log phases. Ergosterol level was significantly reduced. The uptake of propidium iodide and DAPI by *Candida* cells confirmed the membrane disruption property of thymoquinone against Candida cells, biofilm was significantly reduced in *Candida tropicalis.* **Conclusion:** Thymoquinone has great potential as an antifungal and can be explored in future therapies. It inhibits growth and significantly reduced the ergosterol level which is crucial for survival suggesting that its antifungal properties originate from the inhibition of ergosterol level and disruption of membrane integrity.

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Peer Communitiy In (PCI), PCI Infections, and Peer Community Journal: diamond open access to publish research on fungal infections

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Peer Community In (PCI) (https://peercommunityin.org) is a non-profit organization consisting of communities of researchers, called recommenders, who handle the evaluation, peer review and recommendation of preprints in their scientific field. Following submission by the authors, the thematic PCIs (currently 17) evaluate preprints in their scientific fields based on rigorous peer-review. After evaluation, the PCIs may recommend those preprints, to make them complete, reliable and citable articles (with a DOI). When a recommender decides to recommend a preprint, she or he writes a recommendation text that is published along with all the editorial correspondence (reviews, recommender's decisions, and authors' replies) on the PCI website. The PCI initiative won the 2020 LIBER award for library innovation of the European League of Research Libraries

Authors who need to publish their article in a journal can submit to a "traditional" journal, or publish for free in the *Peer Community Journal*" (PCJ) (https://peercommunityjournal.org/, launched in Autumn 2021), which directly accepts all articles recommended by any of the existing PCIs. PCJ is the first generalist diamond open access journal to date, being totally free for authors and readers. The PCJ already exhibits very good citation statistics (722 citations, i.e.2.4 citations/year/paper).

PCI Infections (https://infections.peercommunityin.org/) was launched in August 2021. It welcomes all manuscripts dealing with host-pathogen-vector systems. We hope that the fungal infection research community decides to join the initiative.

P423

Evolutionary drivers in the Chaetothyriales (black yeasts and relatives)

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Introduction

The order *Chaetothyriales* contains a large number of species that are potentially able to cause infections in humans and other vertebrates. Black fungi are opportunists rather than pathogens, because the infections are obtained from fungi with environmental life cycles and which lack abilities of transmission to subsequent hosts. There is still no clear evidence concerning the infection mechanism of these fungi. The assimilation of alkylbenzene hydrocarbons as a potential virulence factor has aroused a lot of scientific interest. This hypothesis is based on the fact that human neurotransmitters have a structure similar to that of aromatic hydrocarbons.

Objectives

We aim to describe the evidence proving a relationship between toxin degradation and pathogenicity in black fungi of the order *Chaetothyriales*.

Materials & Methods

In the present study, 13 genes were selected as a query to search from 45 genomes of *Chaetothyriales*. The copies of each gene per genome were calculated. The result was plotted on a phylogenomic tree to analyze the distribution and evolution of the toluene pathway in *Chaetothyriales*.

Results

A significant difference in the copies of genes related to a toluene degradation pathway was observed between members of the derived family *Herpotrichiellaceae* (n = 12–23, av. 17.4) and those of the ancestral families lower down in the tree (n = 9–14, av. 11.4). The total number of these genes showed expansion, and toluene catabolism in the derived family was relatively complete, while in the lower families the pathway remained incomplete due to missing genes. Most of the investigated species in *Herpotrichiellaceae* were lacking only one or two genes, while the strains in the ancestral families missed four or five genes, indicating that their toluene pathway was far from mature.

Conclusion

Toxin management plays a role in the entire evolution of *Chaetothyriale*s. It can be surmised that the ability to decompose toxic substances has evolved over time.

P424

Neutralisation of the Candida albicans toxin, candidalysin, blocks epithelial damage and dampens inflammatory responses associated with vulvovaginal candidiasis immunopathogenesis

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Objectives:
Candida albicans is normally a harmless commensal of the human body, but can cause mucosal infections such as oropharyngeal candidiasis (OPC) and vulvovaginal candidiasis (VVC). The secreted toxin candidalysin, an integral virulence factor of *C. albicans*, is responsible for host cell damage and triggering immune responses. Unlike for OPC, the immune response during VVC is not protective and recruited neutrophils contribute to hyperinflammation that causes symptoms. VVC treatment is complicated by the idiopathic nature of infection, recurrence, and antifungal resistance. We therefore investigated nanobody-mediated neutralisation of candidalysin as a therapeutic strategy to prevent epithelial damage and activation of inflammatory responses.
Materials & Methods:
Vaginal epithelial cells were infected with *C. albicans* or treated with candidalysin in the absence and presence of anti-candidalysin nanobodies, whereafter host damage and cytokine release were measured. To determine if the nanobodies act by binding and neutralising candidalysin, permeabilisation of candidalysin-treated lipid bilayers was measured in the absence and presence of nanobody. The effect of nanobodies on neutrophil-driven inflammation was evaluated by stimulating primary human neutrophils with vaginal epithelial cell supernatants and quantifying IL-8 release and expression of neutrophil activation markers.
Results:
Addition of anti-candidalysin nanobodies during vaginal epithelial cell infection blocked host damage. Even when added 3 h post-infection, nanobodies prevented *C. albicans*-mediated damage of epithelial cells. In the presence of synthetic candidalysin, nanobodies delayed permeabilisation of lipid bilayers, indicating that the nanobodies act by directly neutralising candidalysin. Infected epithelial cells treated with nanobodies secreted less pro-inflammatory cytokines, such as IL-8, that are associated with neutrophil recruitment and immunopathology. Consequently, neutrophils stimulated with supernatants of infected vaginal epithelial cells secreted less IL-8 when nanobodies were present during infection. These neutrophils also expressed less surface activation markers linked to degranulation and migration.
Conclusions:

Anti-candidalysin nanobodies can neutralise the fungal toxin, candidalysin, and thereby block epithelial cell damage and resulting immune responses, including neutrophil activation and recruitment. The nanobody therefore has the potential to dampen the detrimental host inflammatory responses associated with symptoms during VVC.

P425

Candida albicans translocation through the intestinal barrier is promoted by fungal zinc acquisition and limited by host NFκB-mediated barrier protection

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Objectives:
The opportunistic fungal pathogen *Candida albicans* thrives on human mucosal surfaces as a harmless commensal. Translocation across the intestinal barrier into the bloodstream by intestinal-colonizing *C. albicans* cells serves as the main source of disseminated candidiasis, however, the host and microbial mechanisms behind this process remain unclear.

Materials & Methods:
We previously showed that the *C. albicans* peptide toxin, candidalysin (CaL), encoded by the gene *ECE1* is essential for damage of enterocytes and subsequent fungal translocation using an *in vitro* transwell model based on intestinal epithelial cells (IECs). We proposed that CaL-mediated damage is associated with nutrient acquisition during invasion. Furthermore, since invasion and low-level translocation also occurred in a CaL-independent manner, we suggested that additional mechanisms mediate translocation. Therefore, we performed dual-RNA sequencing during *C. albicans* infection of IECs to further investigate which fungal and host processes are involved during intestinal translocation.

Results:
We observed multiple transcriptional changes in wild-type *C. albicans* cells during infection of IECs compared to fungus-only samples over a 24 h time course. Importantly, we found a pronounced transcriptional zinc starvation response in the absence of host cells, suggesting that *C. albicans* acquires zinc from host IECs during infection. We used strains lacking genes involved in zinc transport and storage to show that zinc acquisition during IEC infection is necessary for the full virulence potential of *C. albicans* by supporting fungal growth. Acquisition of host zinc from IECs relies on *C. albicans*-mediated host cell damage as an *ece1*Δ/Δ mutant strain that is unable to produce CaL shows a stronger transcriptional zinc starvation response than wild-type *C. albicans* during infection. We visualized this phenomenon on a cellular basis *via* staining zinc-specific storage compartments, termed zincosomes, formed during zinc starvation at later stages of IEC infection. We confirmed microscopically that formation of zincosomes at these later time points was *ECE1*-dependent.
Among the host processes up-regulated upon infection were inflammatory pathways involving both MAPK and NFκB signaling. By inhibiting NFκB activation during *C. albicans* infection, we found that NFκB activation not only limits host cell damage, but also breakdown of the epithelial barrier and subsequent fungal translocation. While the decreased barrier integrity and increased translocation during NFκB inhibition were dependent on hypha formation, the increased host cell damage also required *ECE1*. In fact, IECs show an *ECE1*-dependent induction of c-FOS and production of IL-8 during *C. albicans* infection.

Conclusions:

We show that candidalysin fosters zinc acquisition during infection of IECs. We also found that *ECE1*-mediated host damage and subsequent fungal translocation are limited by NFκB-mediated maintenance of the epithelial barrier.

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An Alpha-Glucan from Lomentospora prolificans Mediates Fungal–Host Interaction Signaling through Dectin-1 and Mincle

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Objectives: Structural characterization of an α-glucan from *Lomentospora prolificans* and evaluation of its role in the phagocytosis, induction of oxidative burst and TNF-α release by peritoneal macrophages. Recognition of α-glucan from *L. prolificans* by Toll-like and C-type lectin receptors.

Materials & Methods: *Lomentospora prolificans* (FRM3569) mycelia were extracted with 2% KOH solution and the crude polysaccharide was purified and fractioned by gel filtration over a Superdex 200 column. Nuclear magnetic resonance (NMR, 1D, and 2D spectra) was performed for purified polysaccharide fractions (20 mg/0.5 mL D_2O). The *L. prolificans* α-glucan immunogenicity was tested by ELISA assay and its presence on conidia surface by flow cytometry (FACS). Both analyses were performed using the rabbit immune serum against whole *L. prolificans* cells. To investigate whether α-glucan is involved in phagocytosis of *L. prolificans*, peritoneal macrophages were incubated with *L. prolificans* conidia (5:1 ratio) and kept for 2h. In order to evaluate whether α-glucan could inhibit conidia phagocytosis, macrophages were incubated with either 50 or 100 µg/mL of α-glucan for 30 min prior to their interaction with *L. prolificans* cells. After staining with Giemsa, the phagocytic index was determined using an optical microscope. For Nitric Oxide and Superoxide quantification, macrophages were incubated with 200 µg/mL of α-glucan treated or not with α-amyloglucosidase and *L. prolificans* conidia also treated or not with α-amyloglucosidase at 37°C with 5% CO_2. Zymosan was used as a positive control. After 2h and 18h of incubation, the supernatant was collected from the wells for the measurement of superoxide and nitric oxide, respectively. For induction of TNF-α release, peritoneal macrophages were stimulated with α-glucan from *L. prolificans* (50, 100, and 200 µg/mL) or *S. boydii* (200 µg/mL) and *L. prolificans* conidia (5:1 ratio), treated or not with α-amyloglucosidase and incubated for 18h. TNF-α concentration was determined by using cytokines kit (BD OptEIA ELISA Set-BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. For the Reporter Assay, BWZ and B3Z reporter cells expressing Dectin-1, Dectin-2, Dectin-3, or Mincle, and HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells, were stimulated with 0.5 µg/mL *L. prolificans* or *S. boydii* α-glucan for 18h at 37°C.

Results: The glucan from *L. prolificans* consists of a predominantly linear polymer with little branching formed essentially by →4)-α-Glc(1→. This α-glucan is exposed on the fungal surface and it is recognized by peritoneal macrophages been important for conidia phagocytosis. Moreover, the α-glucan from *L. prolificans* induces oxidative burst by peritoneal macrophages but it is not able to induce TNF-α release, differently by an α-glucan from

Scedosporium boydii. Its recognition by macrophages is mediated by receptors that include Dectin-1 and Mincle, but not TLR2 and TLR4.
Conclusions: We described an α-(1→4)-glucan involved in *L. prolificans* phagocytosis that is recognized by Dectin-1 and Mincle. We attempted to show that, probably due to the absence of branching, α-glucan from *L. prolificans* and that from the related species, *S. boydii*, are differently sensed by PRRs.

P427

Neutrophil Recruitment Failure and Tendency Toward Maturation as Critical Factors for Sustained Low Immune Response in Candida glabrata Vaginal Infection

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Objectives:
Vulvovaginal candidiasis (VVC) is a prevalent infection of the external genitalia and vaginal mucosa caused by *Candida* spp. Among the *Candida* species, *Candida glabrata* is a most commonly occurring pathogen causing VVC besides *Candida albicans*, which presents a challenge due to its recalcitrance and high recurrence rate. Our studys aim to investigate the host's response to *C. glabrata* infection in the vagina, and elucidate the underlying mechanisms involved.
Materials & Methods:
VVC mouse models induced by *C. glabrata* and *C. albicans* infections were established, respectively. Vaginal tissues were analysed through H&E and PAS staining, in addition to detection of *Candida* spp. in the vaginal douched samples after infection. The vaginal immune regulatory mechanisms after *Candida* infection were investigated by analyzing the transcriptional and immune cells distribution changes in vaginal tissues collected before and after infection.
Results:
After *Candida* infection, the number of *C. glabrata* and *C. albicans* detected in the vaginal douched samples of mice was similar, while results from H&E and PAS staining suggest that infiltration of inflammatory cells in vaginal tissues from *C. glabrata* infected mice was milder and lasted longer than that from *C. albicans* infected mice. Differences in the transcriptional profiles between *C. glabrata* and *C. albicans* groups increased gradually with prolonged duration of infection, and fewer differently expressed genes were identified in the *C. glabrata* group than in the *C. albicans* group. Notably, the expression of the NF-κB signaling pathway was significantly down-regulated in *C. glabrata* infection but up-regulated in *C. albicans* infection, indicating the critical role of NF-κB in regulating immune response in *C. glabrata* infection. The neutrophil population was the largest subgroup of immune cells in vaginal tissues after *C. glabrata* infection, but no significant changes were observed between pre-infection and post-infection, demonstrating a recruitment failure of neutrophil. This failure may arise from the down-regulation of NF-κB signaling pathway, which result in the delayed clearence of *C. glabrata* and hypoinflammatory response. Moreover, neutrophils were further divided into three major subgroups according to CD11b and Ly6G expression levels. $CD11b^{hi}Ly6G^{hi}$ and $CD11b^{hi}Ly6G^{int}$ neutrophils showed a significant increase post-infection, indicating neutrophil maturation and stronger phagocytic function after *C. glabrata* infection. In addition, T cells significantly increased, and more myeloid cells infiltrated the tissues after infection. This may be the reason why *C. glabrata* infection could cause the persistent tissue damage.
Conclusions:

The immune response to *C. glabrata* vaginal infection is lower and more sustained than that of *C. albicans*, possibly due to the failed recruitment of immigrated neutrophil and the maturation of intrinsic neutrophils regulated by NF-κB signaling pathway. This study is valuable in exploring new therapeutic directions and drug targets, which is of great clinical and research significance in improving the cure rate and quality of life of patiens with VVC.

P428

Physiological and transcriptional changes exerted by exogenous homoserine-lactone exposure in Candida auris

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Objectives: *Candida auris* and *Pseudomonas aeruginosa* are two well-known opportunistic pathogens, which may coexist within the host in given clinical situations. Previous studies reported that *P. aeruginosa* affects the morphological state of *C. albicans* due to the release of *P. aeruginosa* quorum-sensing molecule 3-oxo-C12 homoserine-lactone (3OC12HSL). Nevertheless, the effects of this molecule to *C. auris* remained unknown so far.
Materials & Methods: The effect of 3OC12HSL was assess in growth-related experiments in the presence of 200 µM 3OC12HSL concentration. Growth was examined continuously at 1-hour intervals by the determination of cell density measuring the absorbance (at 640 nm). To assess whether *C. auris* can cross the epithelial cells *in vitro* in the presence of 3OC12HSL, Caco-2 human epithelium model was used in transmigration assay. An immunocompromised systemic mouse model was used to evaluate the effect of 3OC12HSL to *in vivo* virulence of *C. auris*. To reveal the molecular events induced by 3OC12HSL treatment, a genome-wide transcript profiling was performed with *C. auris* planktonic culture following 200 µM 3OC12HSL exposure using total transcriptome sequencing (RNA-Seq).
Results: Growth, measured by the change in the cell density, was significantly inhibited within 2 hours of the addition of 200 µM 3OC12HSL (1.15±0.005 *vs.* 0.89±0.015 for untreated control and 3OC12HSL -exposed cells, respectively) (p<0.001). Despite the observed growth inhibition, 200 µM 3OC12HSL treatment significantly enhanced the invasion ability of *C. auris* cells through Caco-2 cell layer at 48 hour post-inoculation (p<0.001), whereas the invasion was comparable at 12, 24 and 72 hours post-inoculation compared to control (p>0.05). Regarding *in vivo* experiments, 200 µM daily 3OC12HSL treatment resulted statisticaly comparable kidney fungal burden compared to untreated control (p>0.05). The 3OC12HSL exposure resulted in 1,911 differentially expressed genes (p<0.05). Of these genes, 359 and 299 genes with at least 1.5-fold increase or decrease in transcription, respectively, were selected for further investigation. Genes involved in transmembrane transport (50 genes) and alcohol biosynthetic process (11 genes) showed down-regulation, whereas those related to mitochondrion (84 genes), ATP biosynthetic process (11 genes), mitochondrial proton-transporting ATP synthase complex (10 genes), cellular respiration (27 genes), tricarboxylic acid cycle (11 genes), oxidative phosphorylation (10 genes), mitochondrial electron transport- ubiquinol to cytochrome c (7 genes), as well as nitrogen utilization (5 genes) processes were up-regulated.
Conclusions: The interaction between *C. auris* and *P. aeruginosa* reported here may have implications for clinical significance. In addition, the ability of 3OC12HSL to increase and decrease certain *C. auris* related characterisitics – either molecular or physiological level – could have useful clinical applications.

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Analysis of melanin content of Fonsecae pedrosoi isolates using Fourier transform infrared spectroscopy (FTIR) and chemometric methods

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Melanin is present in all forms of life, it is a heterogeneous, amorphous and highly resistant pigment substance. Despite its importance and presence, there are several unanswered questions about the pigment, including the details of its chemical structure. Melanin produced by fungi contributes to the virulence of human pathogens and food crops, increasing fungal resistance to environmental damage.

Objectives: Predict the melanin content of *Fonsecaea pedrosoi* isolates using Fourier transform infrared spectroscopy (FTIR) and chemometric analyses.

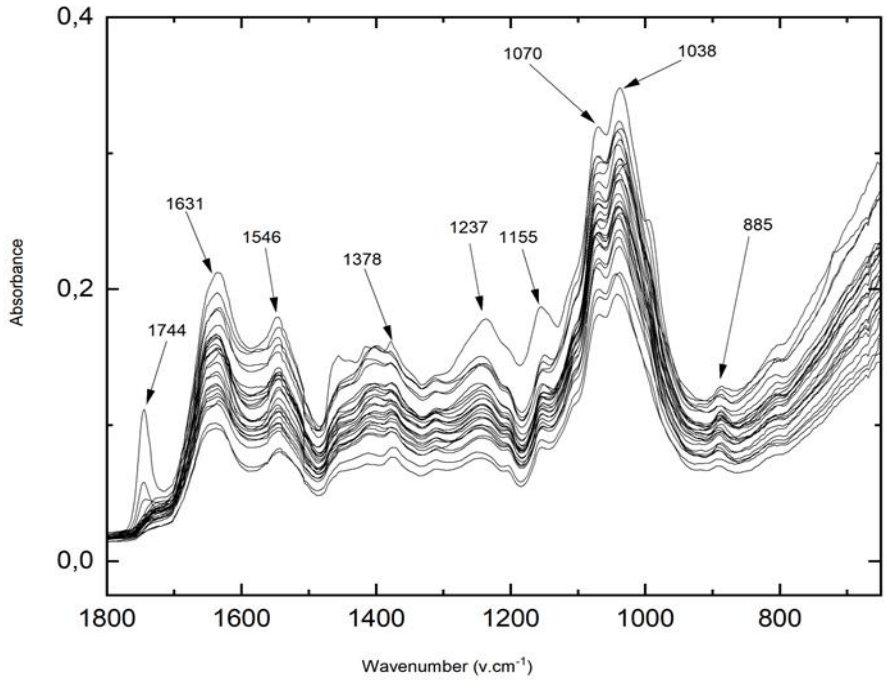
Materials & Methods: Twenty-six clinical isolates of *Fonsecaea pedrosoi* were analyzed. The melanin content, in percentage, was previously determined by gravimetry in relation to the ratio of extracted melanin per fungal mass. Quintuplicate spectra of each fungal sample were acquired by attenuated total reflection (ATR). The acquisition range was from 4000 to 650 cm^{-1} using a spectral resolution of 4 cm^{-1}, force gauge 70 and 4 scans. All chemometric analyses were performed using MATLAB R2012b (MahWorks, Natick, Massachusetts, USA). The preprocessing used was smoothing by the Savitzy-Golay method, normalization by amplitude (0-1) and 1st derivative (13 points). For melanin content prediction PLS modeling, the spectral range from 1800 to 750 cm^{-1} was used, as this is the region that presents the greatest contributions to the *F. pedrosoi* spectra, also including the melanin-DHN absorption regions (1647–1531 cm^{-1}, 1470–1450 cm^{-1} and 1380–1370 cm^{-1}). The models tested were full-spectrum partial least squares (PLS), interval partial least squares (iPLS), and successive projections algorithms for interval selection in partial least squares (iSPA-PLS). The samples were divided into a calibration set (CS) and a prediction set (PS) using the Kennard-Stone method, with a proportion of 70% CS and 30% PS. In all models, an orthogonal signal correction component (OSC) was used.

Results: The main bands in the mean spectra of *F. pedrosoi* were 1631 cm^{-1}, which is related to C = O molecular binding of amide I (proteins), and 1070 and 1038 cm^{-1}, which are associated with C-O-C, C-O elongation and C-C (carbohydrates) (Figure 1). The best modeling was obtained with iSPA-PLS with one factor, with an r val = 0.99712, R^2 = 0.99425, RMSEC = 0.10002, RMSECV= 0.11, r pred =

0.99622, and $R^2 = 0.9599$ with full cross validation. These results confirm that the choice of a significant region of the full spectrum allows obtaining an adequate result for modeling and predicting the melanin content of *F. pedrosoi*.

Conclusions: Analysis using (FTIR) and chemometric methods allows predicting the melanin content, contributing as an alternative analysis technique, due to the complexity of the standard methods used for this quantification.

Figure 1 - Set of mean FTIR-ATR spectra of 26 samples of *F. pedrosoi*, in the region of 1800 to 750 cm^{-1}.



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Potential drug-drug interactions of antifungal prophylaxis and midostaurin in FLT3-mutated acute myeloid leukemia – clinical implications of therapeutic drug monitoring

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Objectives: Midostaurin is a FLT3-inhibitor for the treatment of acute myeloid leukaemia (AML) that is metabolized via CYP3A4 and therefore has potential for drug-drug interactions (DDI) with triazole antifungals routinely administered for prophylaxis of invasive fungal disease (IFD). We determined midostaurin and posaconazole plasma concentrations and investigated adverse events (AE) resembling DDI when both drugs were administered concomitantly.

Materials & Methods: Twice weekly trough levels were performed for both posaconazole and midostaurin with validated LC-MS/MS methods. Demographic and clinical characteristics of patients were collected. Potential DDI of midostaurin were documented and independently reviewed by two physicians and attributed to DDI or otherwise in a five-level scale.

Results: Twenty-four patients were eligible for analysis (22 patients with AML, 2 patients with systemic mastocytosis with an associated hematological neoplasm). A high inter- and intra-individual variability of midostaurin and posaconazole plasma exposure was observed. Inter-individual concentrations ranged from <0.01 mg/l to 24.5 mg/l for midostaurin (Figure 1) and from <30 µg/l to 2571 µg/l for posaconazole (Figure 2).

In the DDI analysis exanthema (1/24; 4,2%) was evaluated as possibly related to increased midostaurin exposure. Prolonged QTc interval on electrocardiogram (2/24; 8,3%), perimyocarditis, hyperbilirubinemia/liver cell necrosis, diarrhea and nausea (each 1/24; 4,2%) were classified as probably related. Midostaurin dose was reduced in one patient by 50% and discontinued in three patients due to severe AE. Six patients (25%) developed IFD (two *Aspergillus* spp., one coinfection with *Aspergillus* spp. and *Rhizomucor* spp. and three possible IFD according to the revised 2019 EORTC/MSG criteria). Five patients had excessively high midostaurin plasma levels over 15 mg/l, however, clinical significance is hard to determine without larger studies.

Conclusions: DDI of posaconazole and midostaurin are clinically meaningful. TDM may serve for decision-making when DDI with strong CYP3A4 inhibitors are suspected clinically and could help to individually adapt the doses of both medications to reduce toxicity.

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Pharmacodynamic and pharmacokinetic consideration of cinnamaldehyde as an anticandidal agent

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Objectives: *Candida albicans* is a common commensal that resides in the human body and causes invasive fungal infections, especially in immunocompromised hosts. *Candida* co-infections are reported with alarming rate and can be associated with different health hazards Treatments for candidiasis are limited due to drug resistance, drug-drug interactions, side effects, and toxicity. Cinnamaldehyde (CIN) is a natural compound that possesses significant antifungal activity. Assessment of the anticandidal activity of CIN and evaluation of its absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters are important to propose CIN as a new anticandidal drug candidate for the treatment of candidiasis.

In this study, we investigated the *in silico* and *in vitro* anticandidal activity of CIN against *Candida albicans* (3017) and ADMET profiling of CIN to reduce the likelihood of treatment failures and drug resistance.

Methods and Materials: Interactions between CIN and potential antifungal cell-wall targets of *C. albicans* was elucidated using *in silico* approach. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of CIN against *Candida albicans* (3017) using broth microdilution method recommended by CLSI for yeast (M27-A2) were determined. The effect of different concentration of CIN (MIC/2, MIC and MICx2) on micromorphology of *C. albicans* was studied. Time-kill assay was performed using cell viability testing. *In silico* ADMET properties were evaluated using preADMET and SwissADME webservers. *In vitro* protocols were established for the elucidation of stability in simulated gastric and intestinal fluids, hepatic microsome stability, cytotoxicity, and plasma protein binding of CIN by using a spectrophotometer, high performance liquid chromatography (HPLC), and high resolution mass spectrometry (HRMS).

Results: CIN showed good binding affinity with ergosterol, N-myristoyl transferase and secreted aspartic proteinase 5, the potential targets of *C. albicans* in the *in silico* studies. MIC and MFC of CIN were found to be 8.2 mg/mL and 16.4 mg/mL respectively. In the morphological interference assays, it was observed that the CIN inhibited pseudohyphae, blastospores and chlamydospores formation. The time-kill curve of cinnamaldehyde showed

that it required only few hours of exposure to effectively kill greater than 90% of the inoculum. CIN showed favored range of drug likeness and ADMET properties.

Conclusion: Cinnamaldehyde showed good *in vitro* anticandidal potential against *C. albicans*. The resulting ADMET profiling of CIN will provide a better option for the management of *Candida* infection. The proposed study elucidated the ADMET profiles of CIN using *in silico* and *in vitro* approaches and will open the door for further clinical studies that could be a new anticandidal drug lead against candidiasis.

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Physiological-based pharmacokinetic analysis of drug–drug interactions between isavuconazole and vincristine in pediatric subjects

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Objectives: To predict the potential change in vincristine exposure in a pediatric cancer population following coadministration with isavuconazonium sulfate using physiological-based pharmacokinetic analysis (PBPK). Invasive fungal diseases (IFD), including invasive aspergillosis (IA) and invasive mucormycosis (IM), occur primarily in immunocompromised and/or hospitalized pediatric patients and are associated with morbidity and mortality. Pediatric patients being treated for leukemia with vincristine frequently experience secondary fungal infections, requiring treatment. Isavuconazole (ISAV) is approved for the treatment of IA/IM in adults; but there is limited data for its use in pediatric patients. Isavuconazonium sulfate is the prodrug of the active triazole ISAV, an antifungal agent that inhibits sterol 14α-demethylase. Isavuconazonium is rapidly converted by plasma esterases to ISAV, which is subsequently metabolized by the liver through CYP3A. ISAV was found in clinical drug–drug interaction (DDI) studies to be a moderate inhibitor of CYP3A, an inducer of CYP2B6, and to have inhibitory potential for P-gp and OCT-2 transporters. Because vincristine is eliminated hepatically via CYP3A and biliary excretion through P-gp, there is a potential for interaction with triazoles.
Materials & Methods: PBPK models were built for ISAV and vincristine in a pediatric cancer population using Simcyp® simulator software with in vitro and in vivo data. The final verified models were used to predict change in vincristine exposure following coadministration with ISAV.
Results: The predicted geometric mean (GM) area under the curve (AUC) ratio of vincristine co-administered with ISAV ranged from 1.54-fold to 1.69-fold depending on the dosing scheme used for the prediction. The largest magnitude of drug-drug interaction (DDI) was predicted when the dose of isavuconazonium sulfate was 372 mg, the first maintenance dose was administered 12 hours following the last loading dose, and vincristine was co-administered on Day 5. Co-administration of vincristine when ISAV was at steady state (14 days) did not result in a significant difference in the magnitude of DDI from other dosing schemes. No effect was predicted on the GM Cmax ratio of vincristine regardless of the ISAV dosing scheme.
Conclusions: ISAV is predicted to be a weak inhibitor of vincristine elimination in pediatric cancer participants, and DDI modelling predicts a slight increase in vincristine exposure when vincristine is co-administered with ISAV.

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Posaconazole-Induced Excess Mineralocorticoid Syndrome with Hypertension, Hypokalemia, and Inhibition of 11-beta-hydroxylase in Pediatric Patients

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Objectives: Posaconazole is a potent broad spectrum mould-active triazole that is increasingly used in children for treatment of aspergillosis, mucormycosis, and endemic mycoses. Although posaconazole has a favorable safety profile in pediatric patients, we recently observed an excess mineralocorticoid syndrome characterized by hypertension and hypokalemia within three weeks of treatment initiation in two patients. Both showed endocrinological evidence of posaconazole inhibition of 11-β-hydroxylase. As this condition is seldom reported in children, we conducted a systematic review of the literature for reports of this condition in pediatric cases.

Methods: A systematic review of the literature (https://pubmed.ncbi.nlm.nih.gov/ and https://scholar.google.com/) was performed using key phrases of pediatrics (<18 years) plus posaconazole plus hypertension, hypokalemia, mineralocorticoid excess, or 11-β-hydroxylase. Variables included age, sex, underlying condition, indication for posaconazole, blood pressure > 95th% for age and height, time from exposure to posaconazole to onset of hypertension, hypokalemia (≤3mEq/L), plasma aldosterone, serum 11- deoxycorticosterone, and serum 11-deoxycortisol.

Results: The systematic literature review identified three reported cases. Clinical characteristics and laboratory data from all five cases are summarized in the table. Median age was 7yrs (range: 5-13 yrs). Four of 5 patients were male. Patients were treated for mucormycosis, histoplasmosis or ABPA. All patients developed hypertension within three weeks of starting posaconazole. Hypokalemia occurred in 4 out of 5 cases. Consistent with posaconazole inhibition of 11-β-hydroxylase, patients with available data had elevated serum 11- deoxycorticosterone, and/or elevated serum 11-deoxycortisol, and/or decreased or undetectable plasma aldosterone. All patients were managed with antihypertensive therapy.

Conclusion: Children who develop hypertension and hypokalemia within 3 weeks of receiving posaconazole should be evaluated further for an excess mineralocorticoid syndrome and 11-β-hydroxylase inhibition. Management may consist of discontinuation of posaconazole and/or initiation of antihypertensive therapy.

Table. Systematic Review of Clinical and Laboratory Parameters of Posaconazole-Induced Excess Mineralocorticoid Syndrome and Inhibition of 11-β-hydroxylase in Pediatric Patients

Case (reference)	Case 1 (new)	Case 2 (new)	Case 3 (Agarwal *et al.*, 2020	Case 4 (Marpole *et al.*, 2021)	Case 5 (Marpole *et al.*, 2021)
Age (years)	5	13	6	9	7
Sex	M	M	M	M	F
Primary underlying condition	High-risk ALL	Previously healthy	Cystic fibrosis	Cystic fibrosis	Cystic fibrosis
Indication for posaconazole	Sino-orbital cerebral mucormycosis	Disseminated histoplasmosis	ABPA	ABPA	ABPA
Hypertension (BP > 95th %tile for age and height)	+	+	+	+	+
Time from exposure to posaconazole to onset of hypertension	1 week	3 weeks	2 weeks	2 weeks	2 weeks
Hypokalemia (≤3mEq/L)	+	+	-	+	+
Plasma aldosterone level	Undetected	Normal 11.5 ng/dL	Decreased 45 pmol/L (80-970 pmol/L)	NP	NP
Serum 11-deoxycorticosterone	Elevated 42.70 ng/dL (normal ≤19 ng/dL for age)	Elevated 212 ng/dL (normal ≤19 ng/dL for age)	Elevated 41 nmol/L (Post SST normal <1.4nmol/L)	NP	NP
Serum 11-deoxycortisol	Normal 61.1 ng/dL (normal ≤ 105 ng/dL)	Elevated 2670 ng/dL (normal ≤ 111 ng/dL)	Elevated 102 nmol/L (Post-SST normal <2.7nmol/L)	NP	NP
Antihypertensive therapy	Clonidine Enalapril Isradipine Amiloride Labetalol	Labetalol Spironolactone	Amlodipine	Amlodipine	Clonidine
Median (range) serum posaconazole trough levels (mcg/mL)	2.6 (1.6-5.6)	4.6 (1.31-5.5)	6 (single value)	3.8 (single value)	6 (4-8)

Abbreviations: ABPA: allergic bronchopulmonary aspergillosis; BP: blood pressure; NP: not performed; SST: Standard synacthen test

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A Phase I, Single-dose, Parallel Group Study to Assess the Pharmacokinetics of Olorofim in Subjects with Hepatic Impairment

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Objectives: Olorofim is a novel antifungal active vs. *Aspergillus* (including azole-resistant strains), resistant moulds (e.g., *Lomentospora prolificans*), and dimorphic moulds. As olorofim is predominantly subject to metabolic clearance, it was necessary to determine the impact of hepatic impairment upon the clearance and systemic exposure of olorofim, in order to provide appropriate guidance on dosing.

Materials & Methods: Male and female subjects with mild hepatic impairment (Group A; Child Pugh score of 5 and 6; N=8), moderate hepatic impairment (Group B; Child Pugh score of 7 to 9; N=8) and matched healthy control subjects (Group C; gender, age and BMI matched to 1 mild and/or 1 moderately impaired subject; N=12) participated in this open label, non-randomized, single oral dose study. Eligible subjects received a single oral administration of 120 mg olorofim on Day 1; PK sampling and safety evaluations were performed for 96 h post-dose with a final follow-up visit on Day 10.
Plasma samples were analysed by validated LC-MS/MS assays for olorofim and 2 key metabolites: F902412 and H26C.

Results: Systemic exposure of olorofim in subjects with mild or moderate hepatic impairment was similar to exposures in matched heathy controls (Table). Across the 3 groups of subjects, geometric mean olorofim C_{max} ranged from 1.001 µg/mL to 1.139 µg/mL and geometric mean olorofim AUC_{0-t} ranged from 11.05 µg·h/mL to 12.00 µg·h/mL.
Geometric mean olorofim $t_{1/2}$ was 30.9 hours in the control group with geometric mean $t_{1/2}$ of 26.3 hours and 31.9 hours in subjects with mild and moderate hepatic impairment, respectively. Geometric mean olorofim CL/F ranged from 9.1 L/h to 9.9 L/h across the mild and moderate hepatic impairment groups and their matched controls.

Statistical Analysis of Olorofim PK parameters by Population

Population	C_{max}		AUC_{0-t}	
	Geo Mean Ratio (%)	90% CI	Geo Mean Ratio (%)	90% CI
Mildly impaired vs healthy	102.88	67.95, 155.78	83.10	56.43, 122.36
Moderately impaired vs healthy	113.41	85.89, 149.75	97.80	70.36, 135.95

Systemic exposure of the metabolite F902412 followed a similar pattern to that seen for olorofim, with no clinically relevant difference in C_{max} and AUC_{0-t} between mild and moderate hepatically-impaired subjects and matched healthy controls. Metabolite ratios (MR) for AUC_{0-t} and C_{max} were similar for all groups, with geometric mean values ranging from 0.18 to 0.23 for $MRAUC_{0-t}$ and 0.13 to 0.16 for MRC_{max}.
The metabolite H26C was slowly formed and slowly eliminated: median T_{max} of 24 and 30 hours in subjects with moderate hepatic impairment and healthy controls respectively and corresponding mean $t_{1/2}$ of 50 and 47 hours. Mean AUC_{0-t} of H26C was similar to olorofim, with mean $MRAUC_{0-t}$ ratios of 0.75 and 0.94 in hepatically-impaired and healthy subjects, respectively. In contrast, peak levels for H26C were notably lower than olorofim, with corresponding mean MRC_{max} of 0.10 and 0.16.

A single dose of 120 mg olorofim was safe and well-tolerated by all subjects.

Conclusions: No dose adjustment is needed when administering olorofim to patients with mild or moderate hepatic impairment.

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A new PK/PD target for micafungin and Candida parapsilosis supports current clinical breakpoint

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Objectives: *Candida parapsilosis* is a major cause of candidemia, ranking second to fourth most common species causative of candidemia, depending on geographic region and patient age. Echinocandins are considered first-line agents for patients with invasive candidiasis including infections by *C. parapsilosis* despite its inherently higher MICs. Conventional PK/PD targets for stasis and 1log kill failed to support current clinical breakpoints as the probability of target attainment (PTA) is low for most wild-type isolates. We there investigated the pharmacodynamics of micafungin against *C. parapsilosis* isolates using a recently optimized *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model for studying echinocandins pharmacodynamics in the presence of 10% human serum.

Materials & Methods: The *in vitro* antifungal activity of micafungin against two clinical *C. parapsilosis* isolates (CLSI MICs of 2 and 1 mg/L) was tested. Different micafungin exposures with tC_{max} 2, 8 and 32 mg/L in 10% human serum and half-life of 15h were simulated in a previously validated *in vitro* PK/PD model (Beredaki JAC2023). The relationship between PK/PD indices $tAUC_{0-24}$/MIC and the 72h log_{10}CFU/mL was analyzed with the Emax model and different targets (stasis and 1log kill compared to initial inoculum, 5%, 10% and 50% reduction from drug-free control) were determined. Monte Carlo analysis of 5000 patients was then performed in order to determine the PTA for *C. parapsilosis* isolates with MICs 0.015-8 mg/L and the standard dose of 100 mg 24h of micafungin attaining a steady state mean±SD AUC_{0-24} of 97.11±28.97 mg.h/L (Mycamine SPC) taking into account the 99.8% protein binding. The PK/PD target which supported the current clinical breakpoints of 2 mg/L was defined.

Results: Fungal burden increased from 3 log_{10}CFU/mL (t=0) to 8.4 log_{10} CFU/mL (t=72h) in drug free controls. A fungistatic effect was found at micafungin tCmax 32 mg/L against both isolates. No antifungal activity (1.5-4.9 log_{10}CFU/mL increase from initial inoculum) was found with tCmax 2 and 8 mg/L. The *in vitro* PK/PD relationship followed a sigmoid curve (R^2=0.95) with a $tAUC_{0-24}$/MIC associated with stasis of 204 (128-326, 95%CI) which is comparable with the clinical PK/PD target of 285 previously determined (Andes AAC2011) validating the *in vitro* PK/PD model. However, the PTA for this target was >95% only for isolates with CLSI MICs

≤0.25 mg/L which is lower than the ECV and clinical breakpoint of 2 mg/L. Among the different PK/PD targets investigated, the 5% log_{10}CFU/mL reduction from drug-free control (24 tAUC/MIC) resulted in PTAs≥95% for isolates with CLSI MICs ≤ 2 mg/L thus covering most clinical isolates.

Conclusions: A new PK/PD target was found for micafungin and *C. parapsilosis* that supports current clinical breakpoint. The low pharmacodynamic effect needed for micafungin efficacy may be attributed to the low virulence of *C. parapsilosis* and immunomodulatory effect of micafungin.

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A Phase I, Single-dose, Parallel Group Study to Assess the Pharmacokinetics of Olorofim in Subjects with Renal Impairment

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Objectives: Olorofim is a novel antifungal active vs. *Aspergillus* (including azole-resistant strains), resistant moulds (e.g., *Lomentospora prolificans*), and dimorphic moulds. Although olorofim undergoes minimal renal clearance, a risk of increased exposure due to renal impairment cannot be completely excluded. As a consequence, determination of the impact of severe renal impairment upon the disposition of olorofim was required in order to provide appropriate guidance on dosing.

Materials & Methods: Eight male and female subjects with severe renal impairment or end stage renal disease who were not on dialysis and had an estimated glomerular filtration rate <30 mL/min and eight matched healthy control subjects (matched by gender, age and BMI) particpated in this Phase I, parallel group study. Each subject received a single oral dose of 120 mg olorofim on Day 1; PK sampling and safety evaluations were performed for 96 h post-dose with a final follow-up visit on Day 10.

Plasma samples were analysed for total olorofim by a validated LC-MS/MS assay. In addition, plasma levels of total and free olorofim were analysed by a qualified LC-MS/MS assay, using plasma samples pooled within a subject over 1-6 and 7-12 h postdose. Free olorofim levels were determined after 4-hours of equilibrium dialysis.

Results: In subjects with severe renal impairment, olorofim C_{max} (total drug) was approximately 27% lower compared to subjects with normal renal function. In addition, AUC_{0-t} (total drug) was approximately 46% lower in renally impaired subjects compared to healthy subjects (Table 1).

Table 1: Statistical Analysis of Olorofim (total drug) PK parameters by Population

Population	Total drug C_{max}		Total drug AUC_{0-t}	
	Geo Mean Ratio (%)	90% CI	Geo Mean Ratio (%)	90% CI
Severe renal impaired vs healthy	73.10	53.95, 99.03	54.05	31.83, 91.78

Olorofim was highly bound to plasma proteins (>99% bound), although the degree of binding was slightly less in patients with renal dysfunction compared to normal subjects (mean % free fraction values of 0.9% and 0.6% respectively). As a result, even though plasma levels of total olorofim were lower in renally impared subjects, concentrations of free olorofim were similar between the two populations (Table 2).

Table 2: Geometric Mean Plasma Levels of Total and Free Olorofim and Mean Fraction Unbound

Timepoint pool	Renal function group	Total Plasma concentration (ng/mL)	Unbound plasma concentration (ng/mL)	Fraction unbound (fu) (%)
1-6 hours	Impaired	262	2.00	0.84
	Normal	448	2.23	0.55

7-12 hours	Impaired	107	0.794	0.81
	Normal	162	0.967	0.65

A single dose of 120 mg olorofim was safe and well-tolerated by the population of severe renal impairment and healthy matched control subjects.

Conclusions: Although mean systemic exposure to total olorofim was reduced by up to 50% in subjects with severe renal impairment compared to matched healthy subjects, there was minimal impact upon the plasma levels of unbound olorofim. Taken together with olorofim's high volume of distribution (> 3L/kg), any change in tissue levels of unbound olorofim is considered slight and a dose adjustment is not needed when administering olorofim to patients with renal impairment.

P440

Cerebrospinal Fluid Concentrations of Posaconazole in Pediatric Leukemia Patients

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Objectives: Invasive fungal diseases are important causes for morbidity and mortality in pediatric patients with leukemia, and current guidelines recommend predominantly azole-based antifungal prophylaxis. While the disposition of certain other azoles into the central nervous system (CNS) has been relatively well characterized, little is known about the distribution of posaconazole in brain tissue and cerebrospinal fluid (CSF). We therefore studied trough concentrations of posaconazole in pediatric leukemia patients in non-inflamed CSF.
Materials & Methods: The study included pediatric patients < 18 years of age with acute leukemia in remission who received posaconazole tablets for prophylaxis or treatment and who underwent repeat therapeutic lumbar punctures as part of their anti-leukemia treatment. A cohort of similar patients not receiving posaconazole served as controls. CSF and blood were obtained at 20-24 hours after dosing. Blood was immediately centrifuged at 1000 g/10 minutes, and both plasma and CSf were stored at until assay. Posaconazole in plasma was measured with a validated liquid chromatography tandem mass spectrometry method (Müller et al, Ther Drug Monit 2017).
Results: A total of 6 patients (median age: 10; range, 6-14 years) with acute lymphatic (3) or acute myeloid (3) leukemia undergoing intensive chemotherapy with repeat intrathecal treatments consisting of methotrexate or the combination of methotrexate, cytarabin, and prednisolone were included. Five patients received posaconazole gastroresistant tablets at weight-banded doses (Tragiannidis et al, J Antimicrob Chemother 2019), and one patient the oral solution at a dose of 6 mg/kg TID. Median protein content of the 11 analyzed CSF samples was 18.7 mg/dL (range, 14.6 to 23.3), and median cell count was 0/3 cells (range, 0-3). In contrast to 14 control samples of patients not receiving posaconazole, posaconazole was detectable in all samples of treated patients. CSF concentrations in the 11 samples ranged from 8.3 to 42 ng/mL with a median CSF concentration of 13.6 ng/mL. Concurrent plasma concentrations were within the dosing target for prophylaxis and treatment and ranged from 965 ng/mL to 5177 ng/mL with a median of 1716 ng/mL.
Conclusions: In this study in children with leukemia in remission undergoing intensive chemotherapy with repeat intrathecal treatments but no signs of meningeal inflammation, trough concentrations of posaconazole in the CSF after systemic administration were low but detectable in all subjects. Concurrent plasma concentrations were in the target range for prophylaxis (>700 ng/mL) and treatment (>1000 ng/mL) in the majority of samples (100 and 90 %, respectively).

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Quality of compounded itraconazole capsules and its possible impact on the treatment of cat-transmitted sporotrichosis (CTE): a Brazilian pilot study

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Objectives: To evaluate the quality of compounded itraconazole capsules and compare them with the reference drug and a generic version, in order to identify possible divergences between formulations.
Materials & Methods: Itraconazole 100mg capsules were purchased from four different magistral pharmacies in the city of Curitiba, Brazil; two of human specification and two veterinary, plus a generic version from a comercial pharmacy. Samples were represented as A1, A2, A3, A4 and A5 and were properly blinded by a researcher not involved in carrying out the subsequent tests, ensuring the absence of bias and the preservation of sample' identities. The Reference Chemical Substance (SQR) was identified by the letter R. Of an experimental nature, several pharmacotechnical, physical-chemical and analytical tests according to the Pharmacopeia-National Formulary were carried out in a specialized laboratory to attest capsules quality.
Results: Similarities and divergences in patterns between itraconazole samples when compared to the reference drug were identified. In view of the tests, sample A3 had the lowest average weight, the lowest pellet count and the lowest drug concentration. Sample A4 showed the highest concentration and the presence of a deviation in the chromatographic profile. Sample A5 was quite discrepant due to the presence of pellets of different diameters and colors, higher average weight and higher chromatographic deviation (Figures 1 and 2). Through the detailed counting of the pellets, three samples obtained variation of up to 30.06% (A1, A4, A5) and two of up to 35.60% (A2 and A3) when compared to the reference drug (R). In another intragroup analysis, it was observed that all the capsules broke in approximately one hour with a variation of 8'24' (A3) at a pH lower than 1 and 60'12'' (A5) at a pH of 1.2, indicating that the more acidic the environment is, the faster the capsule disintegrates, a factor that justifies the orientation of doctors and pharmacists to take the medicine with citrus juices and preferably with meals. In less acidic solutions, such as pH 4.5 and 6.8, none of the capsules broke within an hour.
Conclusions: Itraconazole 100mg capsules is the treatment of choice for humans and other animals with sporothicosis. When not accessible or available in its reference or generic formulations, the compounded itraconazole formulation is used as an alternative. Nonetheless, there are reports of therapeutic failure in this modality and its prescription has been discouraged by specialists. The average weight is considered the most important test in the quality control of magistral pharmacies. However, its singular value may not be considered satisfactory to qualify a sample. Variations in the quality of itraconazole capsules found can possibly interfere and negatively impact the clinical treatment of the disease. Therefore, there is a need to standardize additional tests that validate the critical points found, in order to guarantee that compounding itraconazole is considered an effective and safe therapeutic option to treat CTE.

Figure 1. Diameter analysis of Itraconazole 100mg pellets.

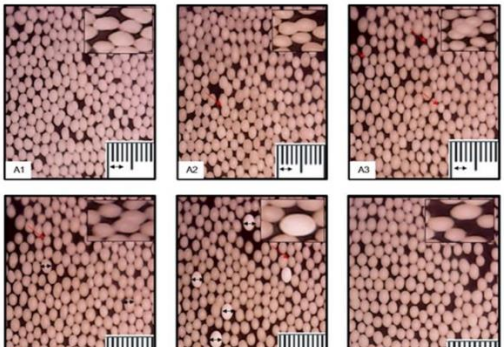
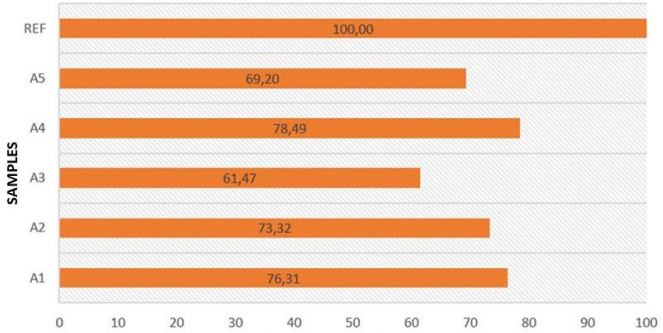


Figure 2. Variation of Itraconazol 100mg concentration based on absorbances.



P445

Two cases of superficial fungal infection caused by non-albicans Candida species manifest greenish-black discoloration

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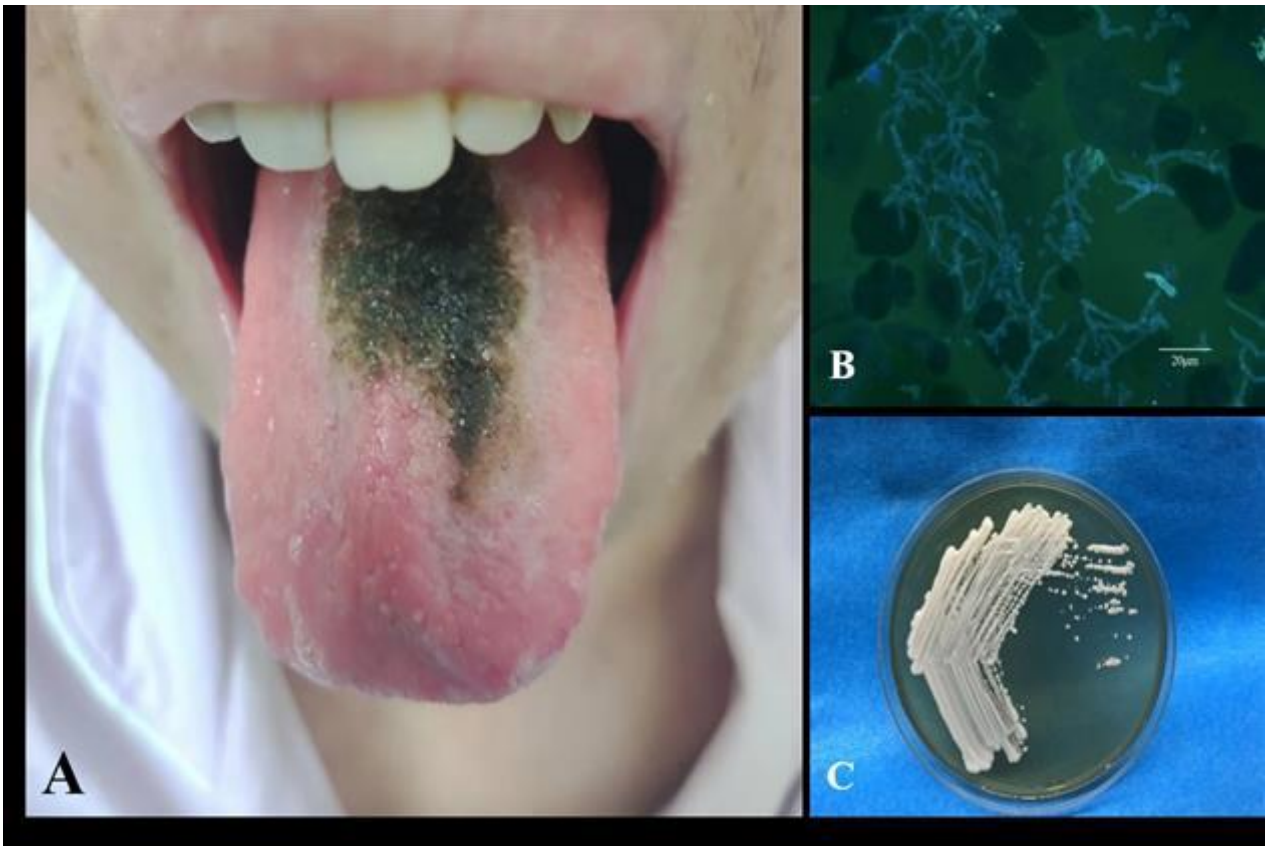
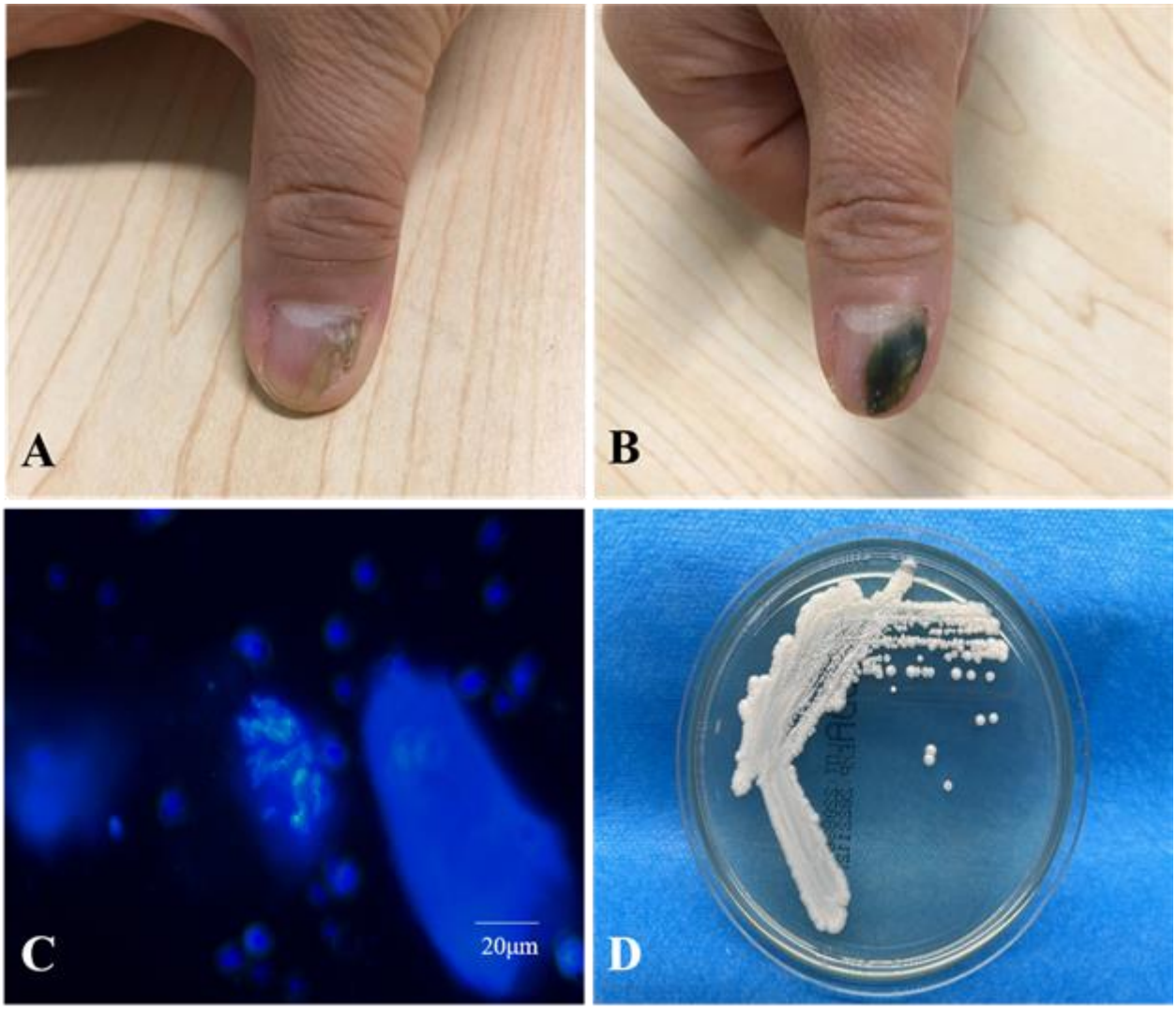
Objectives: The clinical manifestation of superficial candidiasis varies depending on the infectious sites and causative *Candida* species that brings a great challenge to diagnose or treat without mycological or pathological evidence in clinical settings. Oral mucosal candidiasis and onychomycosis are most common types of superficial candidiasis. Typically, oral mucosal candidiasis manifests as white or erythematous thrush coated on the tongue and other interior oral cavity; and onychomycosis caused by *Candida* spp. presents with thick, fragile, or cracked fingernails or toenails in yellow or white discoloration.
Materials & Methods: Both fungi in this case report were identified by fluorescence microscopy and morphological and molecular sequencing. And the cases of superficial candidiasis with the same discolored lesions were searched in literature and compared with our cases in clinical manifestation, causative pathogen and treatment.
Results: Here, we report one case of patient with a black hairy tongue caused by *Candida tropicalis* (case 1) and one case of greenish discolored onychomycosis caused by *Candida parapsilosis* (case 2).
Conclusions: These cases highlight the importance of mycological diagnosis for identifying non-*Candida albicans Candida* species (NCAC) in superficial infections to guide an effective therapy.




A
B
C
20µm
D

P446

Significance of molecular diagnosis in toenail onychomycosis

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Objectives:
Superficial fungal infections are common cutaneous infections worldwide, affecting about 20% to 25% of the world's population. These infections are mostly caused by dermatophytes, and the most common type are tinea pedis and tinea unguium. It is estimated that about 5% of the world's population has some form of onychomycosis which makes it a significant health problem. The aim of this study was to identify causative agents of toenail onychomycosis and to asses significance of application of molecular techniques in laboratory diagnosis of toenail onychomycosis.

Materials & Methods:
Nail samples were collected from patients with suspected toenail onychomycosis according to clinical type of onychomycosis. Every sample was subjected for conventional diagnosis and PCR. Conventional microscopy included direct microscopy with potassium hydroxide and culture on Sabouraud dextrose agar and Dermatophyte test medium. For molecular testing was used Dermatophyte PCR kit which enables detection of dermatophytes in general (panderm marker), as well as specific detection of *Trichophyton rubrum* (specific marker for *Trichophyton rubrum*). Patients were found positive if either conventional or PCR was positive. In statistical analyses Fisher's exact test was used to compare categorical variable.

Results:
A total of 163 patient were confirmed with toenail onychomycosis either by conventional methods or by PCR. The causative agents of toenail onychomycosis were dermatophytes in 95.1% of patients. The most frequent was *Trichophyton rubrum* confirmed in 141/163 (86.6%), followed by *Trichophyton interdigitale* in 6.8%. Among the non-dermatophyte molds that were isolated in 7/163 (4.29%), *Scopulariopsis brevicaulis* (1.2%) and *Aspergillus flavus* (1.2%) were the most common, while the other species were extremely rarely isolated. Yeasts were isolated in only one patient (0.6%) in which *Candida krusei* was confirmed as causative agent. By conventional diagnosis onychomycosis was confirmed in 134/163 (82.21%) patients, while PCR confirmed diagnosis in 155/163 (95.1%) patients. In 155 patients with dermatophyte onychomycosis conventional diagnosis was positive in 126/155 (81.29%), while PCR was positive in all patients with toenail onychomycosis. In 29/155 (18.71) patients conventional diagnosis was negative, and diagnosis was only be possible to obtain by PCR which found to be highly significant ($p<0.01$).
Conclusions:
Identification of the most common causative agents of onychomycosis revealded the predominance of dermatophytes which is consistent with other studies and may be useful in discerning the epidemiological situation and planning strategies in prevention of toenail onychomycosis. The results of this study could help in determining the optimal laboratory protocols for reliably identification of causative agents of toenail onychomycosis suitable to local epidemiology and the economic situation.

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Prevalence of mold-related onychomycosis: feedback from 15 years in a French University hospital

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Objectives: Onychomycoses are frequent and ubiquitous fungal infections due to yeasts, dermatophytes or molds, and their epidemiology depends on the geographical region. At Henri Mondor University Hospital in Créteil, a Dermatology-Mycology consultation has been receiving over 1,000 patients a year for the diagnosis and/or follow-up of fungal infections for some twenty years. The aim of this study was to evaluate the epidemiological profile of onychomycoses detected in our center, especially those caused by molds.
Materials & Methods: Over a 15-year period (from January 2008 to December 2022), 11,004 nail samples from 9,084 patients with clinically suspected onychomycosis were examined by direct examination with chlorazole black and cultured on sabouraud medium with and without actidione. Fungi were identified according to macroscopic and microscopic morphological characteristics and by Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using the online MSI-2 fungal database, if necessery.
Results: Toenails accounted for 87% of samples (n=9,578) and fingernails for 13% (n=1426). Of these cases, 13% (63 patients) of positive samples showed mold in pure culture. The diagnosis of onychomycosis was confirmed in 11 out of 63 patients by a second culture-positive sample with molds of the genus *Fusarium* (n=10) and *Aspergillus* (n=1). In toenails, 17% (600 patients) of positive samples were culture positive for mold with 14% of patients (n=82) had confirmed onychomycosis (2 pure culture samples) to *Fusarium* (n=22), *Aspergillus* (n=18), *Scytalidium* (n=16), *Scopulariopsis* (n=14), *Acremonium* (n=8), *Penicillium* (n=2) or *Onychocola cadanensis* (n=1).
Of 663 patients, 82% (544) were not reinvestigated for confirmation of mold pathogenicity.

Conclusions: Although less frequent than dermatophytic infections, mold onychomycoses are generally more difficult to diagnose and treat, and are responsible for therapeutic failures. New therapeutic strategies seem indispensable to treat molds.

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Epidemiology of Candida africana isolates from vagina in French Guyana

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Objectives:
Candida africana, now recognized as a phylogenetic clade of *C. albicans* species, is mostly responsible for vulvovaginitis. Cases have been reported from Africa or African women in other countries but data from South America, which has been subject to the slave trade, are very scarce. In this study, we investigated the epidemiology of *C. africana* in vaginal specimens from women seen at Cayenne Hospital (French Guiana). For this, we set up a specific qPCR to identify *C. africana*. Finally, the genetic diversity of *C. africana* strains isolated in this study was assessed by means of an MLST consensus scheme.

Materials & Methods:
This study was conducted from May to August 2019 at the Centre Hospitalier Andrée Rosemont, Cayenne, French Guiana. Epidemiological and clinical characteristics of patients were recorded. All strains isolated from vaginal swabs identified as *C. albicans* were tested with a SybrGreen qPCR able to identify *C. africana* thanks to a specific dissociation temperature. Molecular typing was done using a previously published protocol for MLST (pubmlst.org).

Results:
From May to August 2019, 212 *C. albicans* isolates were collected from 176 women. 8 isolates (3.8%) collected from 6 women were identified as *C. africana*. No particular epidemiological or clinical trait was observed for women harbouring *C. africana*. 2 new alleles for the MPIb locus were detected. Diploid Sequence Type (DST) 182 was the predominant genotype of those strains. We found a clear clusterisation of Guyanese strains suggesting a local genetic evolution.

Conclusions:
For the first time, we evaluated the epidemiology of *C. africana* in vaginal samples of women living in French Guiana. Our results showing the predominance of DTS182 in French Guiana are in accordance with those of previous studies indicating this genotype is the most common and geographically dispersed in South America. However, one other DST was identified. Our results support the hypothesis that the distribution of *C. africana* genotype may be based partially on geographical variation.

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LABORATORY CAPACITIES TO DIAGNOSE AND TREAT INVASIVE FUNGAL INFECTIONS IN AUSTRIA, GERMANY AND SWITZERLAND

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Objectives:

Invasive fungal infections (IFI) are a major threat for patients under immunosuppression or with viral infections. Access to appropriate tools is vital for early diagnosis and management. The European Confederation of Medical Mycology (ECMM) survey on laboratory capacities aims to decipher the diagnostic capacity and availability of treatments for IFIs to guide health professionals, patients, and policymakers.

Materials & Methods:
The ECMM IFI diagnostic capacity survey is accessible at clinicalsurveys.net/uc/IFI_management_capacity. The survey was disseminated to all mycologists affiliated with the ECMM and via social media (i.e., LinkedIn or Twitter) and email. Collected dimensions were a) institution profile, b) self-perceptions on IFI, c) microscopy, d) culture and fungal identification, e) serology, f) antigen detection, g) molecular tests and h) therapeutic drug monitoring.

Results:

50 centers have participated from Germany (n=30), Switzerland (n=6) and Austria (n=4). IFI incidence was reported as very low or low in 50% of the institutions. Aspergillus spp. (94%) and Candida spp. (92%) were considered the most relevant fungi. Most of the institutions (96%) had access to cultures, 92% perform susceptibility. Regarding other diagnostic tests, 94% utilize microscopy, 92% antigen detection assays, with 92% Aspergillus galactomannan assays, and 88% molecular tests and antibody tests, each. There was no statistically significant defence in the access to any technique between countries. At least one triazole was available for prescribing in 92% of the institutions, same as for echinocandins; liposomal amphotericin B could be used in 84%, with no statistically significant differences between countries.

Conclusions:

Austria, Germany and Switzerland are generally well prepared to diagnose and treat invasive fungal infections. However, some small size institutions miss access to certain diagnostic tools and antifungal drugs.

P450

Personalized approach to prediction of recurrence of foot onychomycosis due to the formation of risk classes

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Objectives

To develop a method for predicting the recurrence of onychomycosis of the feet in immunocompetent and immunocompromised patients.

Methods

Four clinical observation groups of their patients suffering from feet onychomycosis were formed: 112 immunocompetent patients, 127 patients with *diabetes mellitus*, 150 patients with autoimmune diseases and 35 patients with HIV infection. To solve the problem of express prediction of long-term onychomycosis treatment results, the method of decision trees was used. To assess the predictive quality of the constructed decision tree, such characteristics as AuROC, sensitivity and specificity were used.

Results

Of the 210 binary indicators, three factors were selected that predominantly affect the development of recurrence of feet onychomycosis in immunocompetent patients. In general, the risk of developing this condition was 11.6%. Using the decision tree, 4 risk classes were identified. The highest risk of recurrence - 60% (group size = 15) was observed in patients with the following combination of factors: "Place of work - Worker" and "Not treated with terbinafine". AuROC of 0.95 indicates a high predictive quality of the decision tree.

Of the 218 binary indicators, four factors were selected that predominantly affect the development of recurrence of feet onychomycosis in patients with *diabetes mellitus*. In general, the risk of recurrence was 21.3%. Using the decision tree, 5 risk classes were identified. The highest risk of developing this condition 66.7% (group size = 6) was observed in patients with a combination of factors: "Weakened temperature sensitivity and reduced pulsation on the posterior tibial artery". AuROC 0.80 indicates the average predictive quality of the decision tree.

Of the 227 binary indicators in patients with autoimmune diseases, four factors were selected that predominantly affect the development of onychomycosis recurrence. In general, the risk of developing this condition was 17.3%. Five risk classes were identified using the decision tree. The highest risk of recurrence was found in patients aged 65 years or more who were taking three or more immunosuppressants simultaneously (risk = 100.0%, group size = 6). AuROC 0.79 indicates the average predictive quality of the decision tree.

Of the 231 binary indicators in patients with HIV infection, three factors that predominantly affect the development of onychomycosis recurrence were selected. In general, the risk of developing this condition in the HIV group was 48.6%. There are 4 different risk classes. The highest risk (risk = 100%, group size = 5) of recurrence occurs in patients with the following combination of factors: CD4 level less than 400 and cardiovascular disease. AuROC 0.84 indicates the average predictive quality of the simulated decision tree.

Conclusions

The risk classes for the development of recurrence of feet onychomycosis have been identified, characterizing immunocompetent and immunocompromised patients.

The risk of recurrence of onychomycosis of the feet is higher among all groups of immunocompromised patients in comparison with immunocompetent individuals.

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mycetoma in Turkana County - North-western Kenya

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Objectives:
Mycetoma is one of the six Neglected Tropical Diseases that are prevalent in Turkana County (northwest Kenya). The aim of the study was to estimate the prevalence of mycetoma in the county, as well as to describe the main causative agents involved in the disease using methods that can be affordable locally.

Materials & Methods:
Based on the data collected by the team of cooperative medicine Cirugia en Turkana (Surgery in Turkana), a specific study for mycetoma was started during the 16th humanitarian medicine campaign in February 2019. Patients with suspected mycetoma were studied at the Lodwar County Referral Hospital (LCRH). After informing the patient and getting their consent, the lesions were examined and sampled (mainly by biopsy) and clinical data were recorded. Samples were washed in sterile saline solution and cut in fragments. Some of these were inoculated on Sabouraud Dextrose Agar, Malt Extract Agar, and diluted Nutrient Agar plates. One fragment of each sample was used for DNA extraction. The DNA and the rest of the fragments of samples were kept at -20ºC. All cultures were incubated at room temperature at the LCRH laboratory. The DNA obtained from clinical samples was submitted to PCR amplification of the ITS-5.8S and the V4-V5 16S rRNA gene region, for the detection and identification of fungi and bacteria respectively.

Results:
From February 2019 till February 2022, 60 patients were studied. Most of them were men (43, 74,1%) between 13 and 78 y.o. (mean age 37) and half of them were herdsmen. Lesions were mainly located at the feet (87.9%) and most patients (54; 93.1%) reported discharge of grains in the exudate, being 27 (46.6%) yellow or pale coloured and 19 (32.8%) of them black or dark grains.

Culture of samples yield 35 fungal and bacterial putative causative agents. Phenotypic and molecular methods identified 21 causative agents of mycetoma (39.6% of cases studied). Most of them (17) corresponded to fungi (80.9%) being the most prevalent the genus *Madurella* (7; 41.2%), followed by *Aspergillus* (2; 11.8%). Other minority genera were *Cladosporium*, *Fusarium*, *Acremonium*, *Penicillium*, and *Trichophyton* (5.9% each of them) Actinobacteria were identified in 19.1%. Some other species detected such as *Cellulosimicrobium cellulans* (actinobacteria), *Subramaniula,* and *Macroventuria* (fungi), are reported here as putative new agents that would need more evidence to consolidate their involvement in the disease.

Conclusions:
The estimated prevalence of mycetoma in Turkana is 1.33 cases/100,000 inhabitants/year, which is considered among the highest reported so far.

Clinical characteristics of the disease as well as the species involved are very similar to what has been reported in the area of East-Africa being *M. mycetomatis* the prevalent species involved.

Difficulties in providing appropriate treatment and inconveniences in the follow up of the patients, are important aspects that need special attention to improve the situation of the management of the disease in Turkana County.

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Fifteen cases of Trichophyton mentagrophytes genotype VII among men who have sex with men

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Objectives: Since 2014, *Trichophyton mentagrophytes* genotype VII (TMVII) has been implicated in cases of sexually transmitted dermatophytes diagnosed in Europe. We report 15 cases of TMVII infections diagnosed between January 2021 and January 2023 in three hospital centers in Paris in men who have sex with men (MSM).

Materials & Methods: In 2 hospital centers, for all isolates likely to morphologically correspond to *T. mentagrophytes*, species and genotype were determined by sequencing of the ITS region. In the third center, only isolates responsible for extensive dermatophytosis were sequenced. For TMVII isolates, patient-related clinical information was obtained from medical softwares.

Results: Of 78 isolates, 15 corresponded to TMVII and 58 to *T. indotineae*. TMVII infections were diagnosed in men (14/15 being MSM), with a median age of 35 years [19-59]. Seven patients were living with HIV and 7 were taking HIV pre-exposure prophylaxis (PrEP). Skin lesions were multiple in 9 patients and single in 6. One patient had nodular lesions suggestive of Majocchi granulomas, 2 had facial kerion and the others had circular erythemato-squamous lesions. The buttocks (n=7), face (n=7) and genital region (n=5) were particularly affected by the lesions. Two patients had concomitant Mpox virus infection. At least 9 of the patients had had no contact with animals that might explain the infection with *T. mentagrophytes*. The median time from lesion onset to mycological sampling was 17 days [7-102 days]. Six patients had already received antibiotic, antifungal or topical steroid treatment. Systemic antifungal treatment was prescribed for eleven patients. One patient with beard kerion required hospitalization because of a bacterial superinfection.

Conclusions: These 15 cases of infection are suspected of being linked to sexual transmission (location of lesions, history of STIs, multiple sexual partners, absence of contact with animals suggesting human-to-human contamination, co-infections with Mpox virus, involvement of TMVII). This series indicates an active transmission of this genotype among MSM patients, which had not been described until now, and confirms its circulation in Europe.

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Genotypic characterization of Trichophyton mentagrophytes isolates from companion animals in France

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Objectives: During the past few years, circulating genotypes of *Trichophyton mentagrophytes* complex have been characterized in various human populations. These investigations highlighted first the emergence of *T. indotineae* in India and its spread in other countries through migration and tourism. Geographic clustering was also identified for some *T. mentagrophytes* ITS genotypes while other genotypes were cosmopolite. Moreover, clinical manifestations and hosts (children or adults) may differ also according ITS genotypes. Especially, *T. mentagrophytes* genotype VII was associated with sexual transmission in adults. However, no large study has been performed until now in the veterinary field to characterize the diversity of *T. mentagrophytes* ITS genotypes. The objective of our study was to genotype a large number of isolates of *T. mentagrophytes* collected from several animal populations in France.

Materials & Methods: : In collaboration with Laboniris, the diagnostic platform of the veterinary school of Nantes France, we retrospectively cultured samples collected in 2020 from companion animals with cutaneous lesions compatible with dermatophytosis. The samples were pieces (3 x 3 cm) of carpet initially positive for *T. mentagrophytes*. They were sub-cultured on Sabouraud chloramphenicol gentamycin for the entire fungal flora and in parallel on Sabouraud supplemented with actidione for dermatophyte detection. Identification of the grown isolates was performed through MALDI ToF mass spectrometry and the MSI-2 database, and genotyping was done through ITS sequencing.

Results: Of the 632 samples, approximately 56% remained positive for dermatophytes after 2 years of storage at 4°C. Identification by MALDI ToF mass spectrometry confirmed *T. mentagrophytes* species complex for 92% of the dermatophyte-positive cultures. Other dermatophytes included *Nannizzia persicolor*, *Paraphyton cookei*, *Microsporum canis, Arthroderma crocatum, T. benhamiae* and *T. erinacei*. An alignment of the ITS sequences and a comparison with the reference sequences proposed by Taghipour et *al*. (2019) and Klinger et *al*. (2021) identified several ITS genotypes whose distribution was different depending on the animal population: genotypes II*, III and III* were identified in dogs and cats while genotype XXIV was identified only in chinchillas. New genotypes were also obtained, one of which was identified in several samples. No genotype corresponding to *T. interdigitale*, *T. mentagrophytes* genotype VII or *T. indotineae* was found.

Conclusions: Mainly three ITS genotypes were identified in both cats and dogs, with an equivalent proportion of the three in both populations. One ITS genotype (XXIV) seems to be only associated with chinchillas. The lack of identification of genotypes corresponding to *T. interdigitale* in animals is in favor of a low transmission rate of dermatophytes from humans to companion animals. No animal reservoir was identified for *T. mentagrophytes* genotype VII in France, bringing a new argument for direct inter-human transmission of this emerging virulent genotype.

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Dermatophytes frequency and diversity in dermatomycoses within the area of Zagreb (Croatia)

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Objectives: Dermatophytes colonize the skin and its appendages asymptomatically for extended periods, but can later cause diseases due to environmental and host factors. Dermatomycosis, including onychomycosis, affects approximately 5% of the general population and can persist for years or even decades, leading to a reduced quality of life. Besides dermatophytes, non-dermatophyte molds and yeasts can also cause these fungal infections. However, there is a lack of epidemiological data on skin and skin appendage mycoses caused by dermatophytes and non-dermatophyte molds in Croatia. Therefore, our study aimed to investigate the involvement of dermatophytes and non-dermatophyte molds in dermatomycoses by analyzing skin, scalp, and nail samples from patients.
Materials & Methods: The study was taken in Croatian Institute of Public Health in the year 2022, on 719 participants: 523 patients (P) and 196 controls (C); females 471/719 (65.5%), males 248/719 (34.5%). Samples included 581/719 (80.8%) nails, 130/719 (18.08%) skins and 8/719 (1.11%) scalps, and were analysed by combining microscopy (20% KOH solution) and cultivation (Mycoline agar) with ITS sequence-based method.
Results: In the P group, 464/523 (88.71%) samples showed a positive result in wet mount analysis, while in the C group, only 31/196 (15.81%) samples were positive. Positive cultures were obtained in 240/523 (45.88%) P group subjects and 23/196 (11.73%) C group subjects. Among the 240 positive cultures in the P group, 45.41% were dermatophytes, 81.66% were non-dermatophyte molds (including 3.06% *Fusarium* spp, 12.24% *Aspergillus* spp., 9.69% black molds, and 75% unidentified saprophytes), and 37.91% were yeasts. However, colonization with dermatophytes was not confirmed in the control group. Among the dermatophytes, *Trichophyton rubrum* (TR) was the most dominant species (56.88%), followed by *T. interdigitale* (TI) (22.93%), *T. mentagrophytes* (TM) (14.67%), *T. tonsurans* (TT) (2.75%), *Nanizzia nana* (NN) (0.91%), *Microsporum canis* (MC) (0.91%), and *Epidermophyton floccosum* (EF) (0.91%). Notably, there were some discrepancies between morphological types and the ITS sequence-based method of identification;19 species identified as TI were confirmed as 8 TM/TI and 9 TM, 4 TM species were confirmed as 3 TM/*T. krajdenii*, *N. nana* was identified as *Aphanoascus fulvescens*, and *M. canis* was confirmed as *Arthroderma gypseum*. The infection rate of TI/TM was higher in the age group of 40-85 years (median 56). Among subjects with positive cultures, 14.58% engaged in sports, 14.16% had pets, and 8.75% reported health disorders requiring long-term therapy, all of which might have contributed to the development of dermatomycosis.
Conclusions: Among skin and skin appendage infections, onychomycosis was the most frequent. *Trichophyton* species were isolated in 97.24% of dermatomycoses, indicating their higher pathogenic potential compared to other dermatophytes. Among *Trichophyton* isolates, *T. rubrum* comprised more than half of the cases, whereas the incidence of infections caused by *T. interdigitale* and *T. mentagrophytes* was two to four times lower.While the ITS sequencing method offers a more efficient diagnostic approach that enables improved differentiation of related dermatophyte species compared to traditional mycological cultivation methods, it is necessary to develop new molecular markers to ensure even more accurate discrimination among closely related species.

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P455

Species identification and in vitro anti-fungal susceptibility testing of Aspergillus section Nigri strains isolated from otomycosis patients

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Objectives: Aspergillus niger is the most commonly reported etiology of otomycosis based on morphological characteristics. This fungus is a member of Aspergillus section Nigri, a set of morphologically indistinguishable species that can harbor various antifungal susceptibility patterns. The aim of this study was to accurately identify and determine the susceptibility pattern of a set of black aspergilli isolated from otomycosis patients.

Materials & Methods: Forty-three black *Aspergillus* isolates from otomycosis patients were identified by using the PCR-sequencing of the *β-tubulin* gene. Furthermore, the susceptibility of isolates to three antifungal drugs, including fluconazole (FLU), clotrimazole (CLT) and nystatin (NS), were tested according to CLSI M38-A2. The data were analyzed using the SPSS software.

Result: Based on the analysis of the b-tubulin gene sequences, the majority of the isolates were identified as A. tubingensis (32/43, 74.42%) followed by A. niger (11/43, 25.58%). Therefore, the prevalence of A. tubingensis in this study was almost three times greater than A. niger. The evolutionary dendrogram of all the isolates is shown in (Fig. 1). According to the results of the antifungal susceptibility tests, the widest MIC ranges were observed for CLT among both A. tubingensis (MIC range: 4– > 16 mg/mL) and A. niger (MIC range: 2–16 mg/mL) isolates. FLU had no activity against A. niger (GM > 64 mg/mL) and A. tubingensis (GM > 64 mg/mL) isolates. CLT exhibited lower MICs against A. niger (GM: 5.15 mg/mL) in comparison to A. tubingensis (GM: 9.72 mg/mL). According to the data analysis, this difference was statistically significant ($P < 0.05$). NS was almost similarly active against the A. tubingensis (GM: 4.65 mg/mL) and the A. niger (GM: 4.83 mg/mL) isolates. In general, the lowest MIC values in this study were observed for NS. The detailed results of the antifungal susceptibility-testing of the isolates are presented in Table 2.

Conclusion: Species other than A. niger can be more frequent as observed in our study. In addition, considering the low and variable activity of tested antifungal drugs, empirical treatment can result in treatment failure. Accurate identification and antifungal susceptibility testing of isolates is, however, recommended.

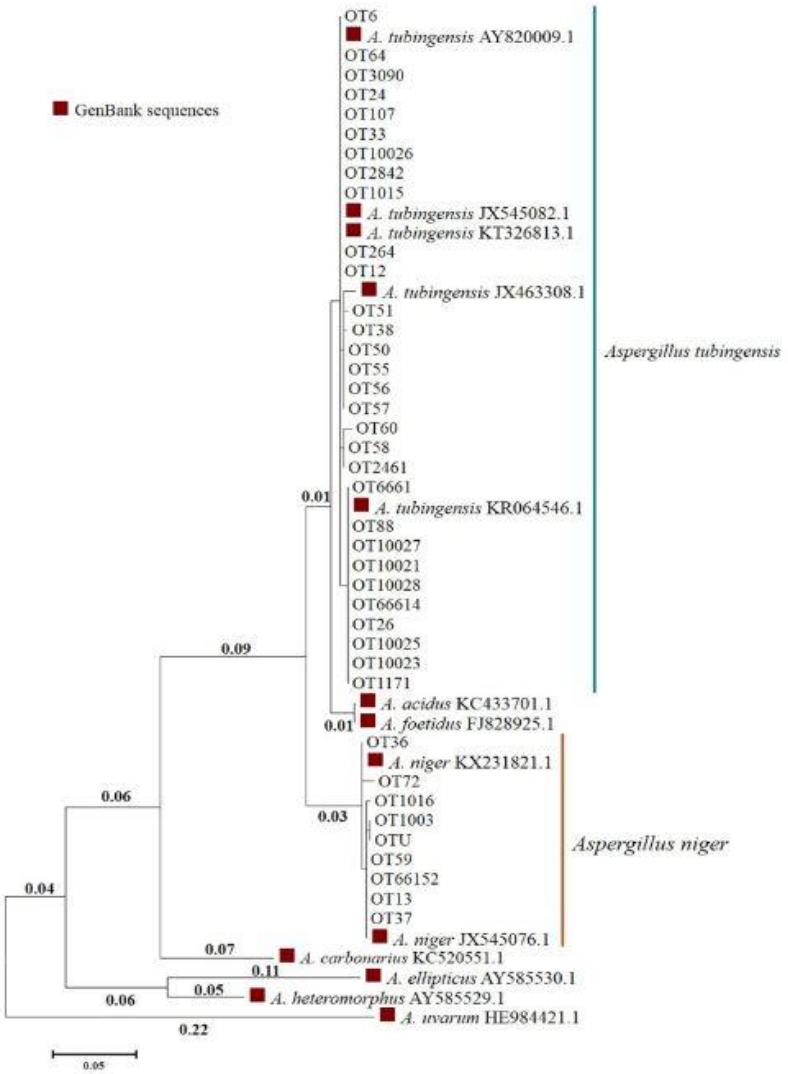

Fig. 1. The phylogenetic dendrogram of 39 strains of *Aspergillus* section *Nigri* isolated from otomycosis patients and GenBank sequences of some related species was constructed using the maximum likelihood method based on the Tamura-Nei model in MEGA 6.

Table 1

The minimum inhibitory concentration (MIC) values of three antifungal drugs against strains of *Aspergillus* section *Nigri* isolated from otomycosis patients.

Species (n=45)	Antifungal drugs	MIC values (μg/ml)								MIC Range	MIC50[a]	MIC90[b]	GM[c]
		2	4	8	16	>16	32	64	>64				
A. tubingensis (32)	Fluconazole							2	30	64–>64	>64	>64	>64
	Clotrimazole		5	14	12	1				4–>16	8	16	9.72
	Nystatin	1	23	8						2–8	4	8	4.65
A. niger (11)	Fluconazole								11	>64	>64	>64	>64
	Clotrimazole	1	6	3	1					2–16	4	8	5.15
	Nystatin		8	3						4–8	4	8	4.83

[a] The lowest concentration which inhibits 50% of isolates.
[b] The lowest concentration which inhibits 90% of isolates.
[c] Geometric mean.

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Human and Zoonotic Dermatophytes in Romania: An Updated on Species and Clinical Aspects

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Objectives: Dermatophytes are a group of closely related filamentous keratinophilic fungi that infect keratinized tissues such as skin, hair, nails. The incidence and prevalence of these superficial infections are extremely high, and it is estimated that over 20 to 25% of the global population is affected. In recent years, the widespread but uneven distribution of dermatophytes in the world warrants local epidemiological studies. In Romania, only few studies have provided data on epidemiology of this fungi due to its limited in identification. To our knowledge In this context, the main goal of this study was the identification of the species, for the first time in Romania by molecular methods.
Materials & Methods: The identification of all 81 isolates in the laboratory was performed by phenotypic and molecular methods. Thus, all samples were cultured on Sabouraud chloramphenicol actidione agar (Bio-Rad, France) in 90-mm culture plates at 24°C. Colony diameters and the colors of the obverse and reverse sides were recorded between 2-4 weeks. Strains grown on medium were examined by microscopy, and the presence of micro- and macroconidia and reflexive branches was recorded. The subsequent molecular identification was performed by sequencing the internal transcribed spacer (ITS). DNA extraction from pure colonies of fungal material was performed using the CTAB (cetyltrimethylammonium bromide) method. The ITS region was amplified using primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The sequences were edited and assembled with the SeqMan program (DNAStar, Madison, WI, USA), manually corrected, and aligned using the MAFFT server with default parameters. The sequences of newly sequenced strains were deposited in GenBank.
Results: *Trichophyton rubrum* (38% of tinea unguium and tinea pedis) was the most common species followed by *Microsporum canis* (28,5% of tinea corporis, tinea faciei and tinea barbae). Other prevalent dermatophytes were: *Microsporum gypseum* (7,4% of tinea corporis), *Trichophyton mentagrophytes* (6,2% of tinea unguium and tinea pedis), *Epidermophyton floccosum* (5% of tinea pedis and tinea corporis), *Trichophyton verrucosum* (5% of tinea corporis), *Trichophyton tonsurans* (3,7% of tinea capitis), *Microsporum audouinii* (2,5% of tinea capitis), *Trichophyton interdigitale* (2,5% of tinea pedis) and *Trichophyton erinacei* (1,2% of tinea manuum).
Conclusions: Dermatophytes are usually identified by phenotypic methods even though the methods sometimes remain difficult or unreliable. In this context, for routine diagnostics, ITS sequencing is recommended. The dominant species were anthropophilic and zoophilic dermatophytes, presumed to be the result of anthropo-zoonotic transmission.

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Human adaptation and diversification in the Microsporum canis complex

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Introduction

The *Microsporum canis* complex consists of one zoophilic species, *M. canis*, and two anthropophilic species, *M. audouinii* and *M. ferrugineum*. These species are the most widespread zoonotic pathogens causing dermatophytosis in cats and humans worldwide. Significant phenotypic and ecological variation exists between the three members of the *M. canis* complex, but evidence of sexual reproduction underlines a strong connection between the species.

Objectives

Whether the above anthropophilic and zoophilic species should be considered members of a single biological species is still an open question. To better delineate species identities in the *M. canis* complex and to understand the transition process from animal to human hosts.

Methods or Materials

This study combines multiple approaches, including phylogenetic analysis, population structure analysis, multispecies coalescent analyses, determination of *MAT* idiomorph distribution, sexual crosses, and macromorphology and physicochemical features.

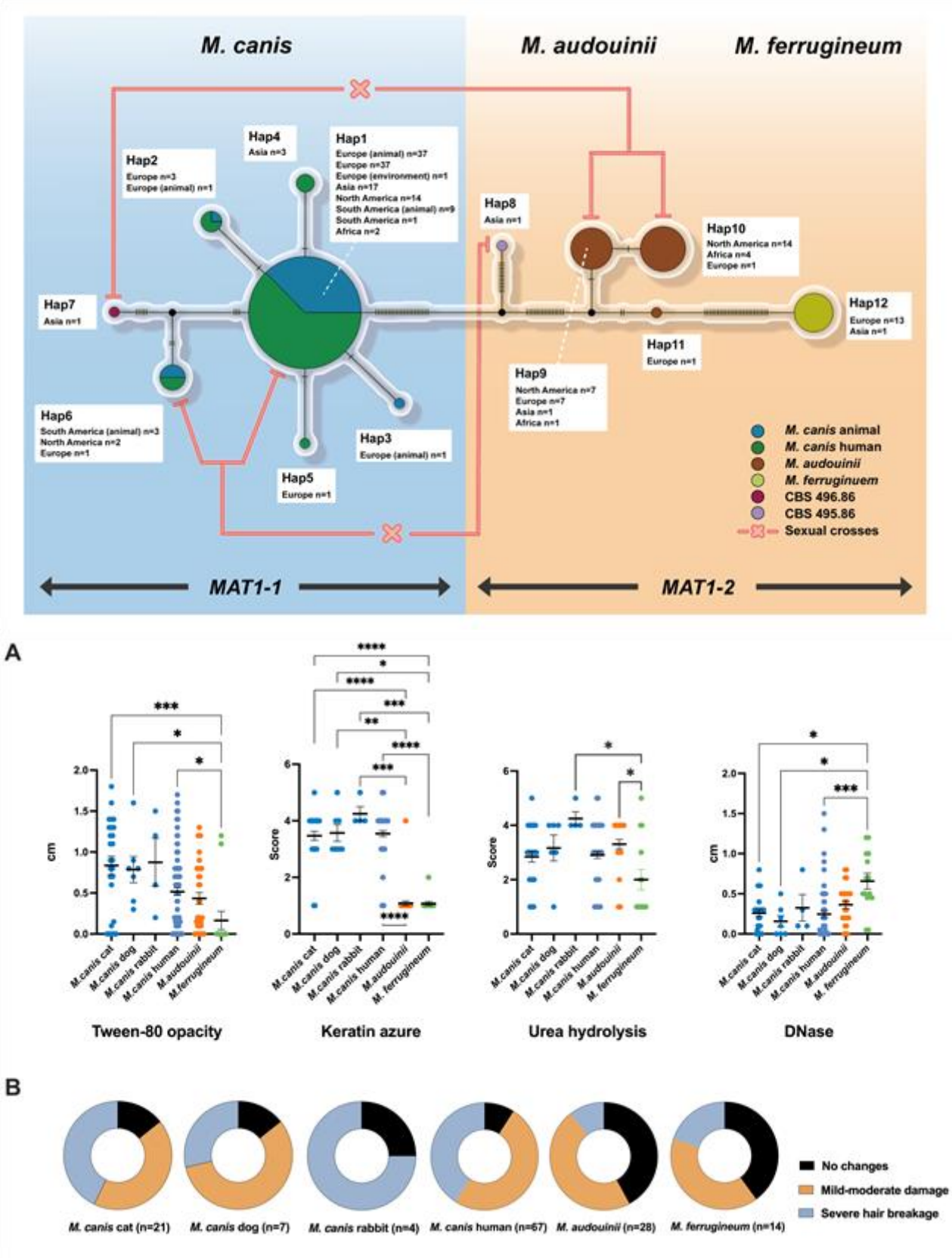
Results

The *M. canis* complex comprises 12 genotypes (Fig1). *MAT1-1* was present only in *M. canis*, while the anthropophilic entities contained *MAT1-2*. The pseudocleistothecia were yielded by the mating behaviour of *M. canis* and *M. audouinii*. Growth rates and lipase, keratinolysis and urea hydrolytic capacities of zoophilic *M. canis* isolates were all higher than those of anthropophilic strains; DNase activity of *M. ferrugineum* exceeded that of *M. canis* (Fig2). The optimum growth temperature was 28°C, but 22°C favoured the development of macroconidia. Molecular data, physicochemical properties and phenotypes suggest the adaptation of *M. canis* to *M. ferrugineum,* with *M. audouinii* in an intermediate position.

Conclusion

Phylogeny, population genetics and multispecies coalescent analyses clearly distinguished the three species, not only phenotypically but also genetically. Combined with physicochemical properties and

MAT idiomorph distribution analysis, we could reconstruct the host shift to the human within the *M. canis* complex.



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A new family of black yeast-like fungi

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Introduction The order *Chaetothyriales* comprises the black yeasts and relatives, of which numerous species are prevalent as opportunists on human hosts. **Objectives and Methods** In order to reconstruct the evolutionary trajectory towards pathogenicity on vertebrate hosts, molecular phylogeny of the order was analyzed. Phylogenetic placement of clades in the *Chaetothyriales* was based on a combined dataset of the internal transcribed spacers (ITS) and partial large subunit (LSU) of ribosomal DNA. To evaluate the validity of each taxon and to clarify the phylogenetic relationships of analyzed species, further analyses using sequences from transcription elongation factor 1-a (EFl), β-tubulin (BT2) and RNA polymerase II large subunit (RBP1), were also conducted, and genomes of voucher strains were analyzed. **Results** A new family of *Chaetothyriales* was introduced to accommodate new taxa from ant nests inside living plants (domatia). Monophyly of the family of shown and strong statistical support for each genus was yielded by analyses of a combined dataset of the four regions (ITS +BT2+ EFl+ RBP1). The consensus between five different species delimitation methods for the ITS and LSU marker suggests the existence of 16 new species, which were also characterized by phenotypic characters of colony morphology, micromorphology and physiological characters. **Discussion** Some of the new described species seem to be specifically enriched types of ant nests and this may be an important factor in their evolution. Ant nests are a remarkable habitat in that they contain hydrocarbons antiseptic to most microorganisms but which are tolerated by the fungi under study. As the family is ancestral to the *Herpotrichiellaceae* containing numerous opportunistic species hydrocarbon tolerance seems to play an essential role in black yeast evolution.

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Unveiling the Candida spp. burden: workload and cost considerations in a tertiary hospital.

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Objectives: Our objectives were to determine the workload for a tertiary hospital in processing and isolating *Candida* spp. in all types of samples and patients, its distribution by type of samples, origin and species, and the associated cost.

Materials & Methods: Descriptive and retrospective study from January 2021 to December 2022. Samples were classified into superficial (skin, mucosal, catheters and urine) and deep (ordinarily sterile tissues or fluids and blood). The origin of the samples was classified into in-hospital (medical, surgical, ICU and emergency) and out-of-hospital (community) services. Laboratory workload was reported as samples processed per day and samples and isolates expressed per 1,000 admissions/year and per 100,000 inhabitants/year. Samples with *Candida* spp. were processed and identified according to international standards. Patients were identified as inpatients, when they were admitted to the hospital for at least 24 hours, and outpatients. Cost estimation was calculated from hospital finance department data and microbiology laboratory cost records.

Results: HGUGM serves a population area of more than 800,000 inhabitants and during the study period there have been 95,192 hospital admissions. During these two years, the Microbiology department processed a total of 1,008,231 samples, of which 8,775 (0.87%) had one or more isolates of *Candida* spp. (9,683 *Candida* isolates). Overall, 5,167 patients had one or more positive samples with *Candida* spp. *Candida* spp. isolates were recovered in 515.3 samples/100,000 population/year and in 92 samples/1,000 admissions/year as shown in **Table 1**. The mean number of samples with *Candida* spp. identified per day was 12. Of the community samples, almost 100% were superficial samples, of which mainly mucosal (71%) and cutaneous (27%) samples. Regarding inpatient samples, the relationship between the main *Candida* spp. species and the different types of samples is shown in **Table**

2. *C. albicans* is the predominant species. In regard to non-*albicans* species, *C. parapsilosis* is the most common species in skin, catheter and blood samples. *C. glabrata* is mainly found in urine, followed by mucosal and sterile tissues samples. Both species share proportion in samples such as sterile liquids. *Candida* spp. was isolated in 0.9% of the samples processed in the laboratory, so we extrapolated the total costs of the microbiology laboratory and of the laboratory personnel. Accordingly, the average cost per sample for *Candida* spp. is estimated to be 25 € (approximately 109,687 €/year).

Conclusions: Investigating the workload and cost associated with processing and isolating *Candida* spp. in a Microbiology department highlight the substantial burden of *Candida*. The findings underscore the importance of efficient resource allocation and targeted interventions to mitigate the impact of Candida spp. on patient care and healthcare expenditures.

Table 1. Workload of *Candida* spp. over the total number of samples and isolates received and processed in the Department of Clinical Microbiology, expressed in absolute values, incidence of samples per 1,000 admissions/year and per 100,000 population/year.

	Total N (%)	Incidence/ 1,000 admissions/yr	Incidence/ 100,000 inhab/yr
Superficial samples	7969 (90.8)	83.7	468.2
Mucosal samples	5642 (70.8)	59.3	331.5
Skin samples	1313 (16.5)	13.8	77.1
Urine	867 (10.9)	9.1	50.9
Catheters, blood from catheter, hub sample*	144 (1.8)	1.5	8.5
Catheter tips only	83 (1)	0.9	4.9
Deep samples	796 (9.1)	8.4	46.8
Ordinarily sterile fluids	347 (43.6)	3.6	20.4
Ordinarily sterile tissues	255 (32.0)	2.7	15.0
Blood	194 (24.4)	2.0	11.4
Other samples (not classified)	10 (0.1)	0.1	0.6
Community samples	1812 (20.6)	19	106.5
In-hospital samples	6959 (79.3)	73	408.8
Medical wards	3499 (50.3)	36.8	205.6
ICU	1728 (24.8)	18.2	104.7
Surgical wards	1170 (16.8)	12.3	68.7
Emergency room	558 (8)	5.9	32.8
Not reported	4 (0.05)	0.04	0.2
Isolates of *Candida* spp.	9683	101.7	568.9
Candida albicans	5668 (58.5)	59.5	666.0
Candida parapsilosis, metapsilosis and *orthopsilosis*	1704 (17.6)	28.8	100.1
Candida glabrata	1068 (11.0)	11.2	62.7
Candida tropicalis	587 (6.1)	6.2	34.5
Candida krusei	232 (2.4)	2.4	13.6
*Candida auris***	1 (0.01)	0.01	0.06
Others	423 (4.4)	4.4	24.9
C. lusitaniae	119 (1.2)	1.3	7.0
C. guilliermondii	92 (1.0)	1.0	5.4
C. dubliniensis	77 (0.8)	0.8	4.5
C. kefyr	43 (0.4)	0.5	2.5
C. rugosa and pararugosa	24 (0.2)	0.3	1.4
C. lipolytica	19 (0.2)	0.2	1.1
C. famata	13 (0.1)	0.1	0.8
C. intermedia	11 (0.1)	0.1	0.6
Candida sp.	6 (0.1)	0.1	0.4
C. bracarensis	5 (0.1)	0.1	0.3
C. lambica	5 (0.1)	0.1	0.3
C. zeylanoides	3 (0.03)	0.03	0.2
C. inconspicua	2 (0.02)	0.02	0.1
C. boidinii	1 (0.01)	0.01	0.06
C. lactiscondensi	1 (0.01)	0.01	0.06
C. sake	1 (0.01)	0.01	0.06
C. haemulonii	1 (0.01)	0.01	0.06

Abbreviations: ICU, intensive care unit.
*Catheters: including catheter tips, catheter hubs cultures and blood from the catheter.
**From a quality control sent by the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC).

Table 2. Distribution of *Candida* spp. in the different samples from hospitalized patients.

***Candida* spp.**	**Superficial samples**				
	Cutaneous N (%)	**Mucosal samples N (%)**	**Catheter N (%)**	**Urine N (%)**	**TOTAL N**
Candida albicans	238 (61.5)	2333 (61.2)	50 (36.8)	358 (48.4)	2979
Candida parapsilosis, metapsilosis* and *orthopsilosis	87 (22.5)	436 (11.4)	58 (42.6)	63 (8.5)	644
Candida glabrata	26 (6.7)	441 (11.6)	7 (5.1)	184 (24.9)	658
Candida tropicalis	21 (5.4)	271 (7.1)	17 (12.5)	98 (13.2)	407
Candida krusei	5 (1.3)	139 (3.6)	1 (0.7)	8 (1.1)	153
Others	10 (2.6)	194 (5.1)	3 (2.2)	29 (3.9)	236
TOTAL	389	3814	136	740	5077
***Candida* spp.**	**Deep samples**				
	Ordinarily sterile tissues N (%)	**Ordinarily sterile fluids N (%)**	**Blood N (%)**	**TOTAL N**	
Candida albicans	129 (54.9)	154 (51.7)	89 (47.1)	372	
Candida parapsilosis, metapsilosis* and *orthopsilosis	34 (14.5)	40 (13.4)	49 (25.9)	123	
Candida glabrata	37 (15.7)	40 (13.4)	22 (11.6)	99	
Candida tropicalis	13 (5.5)	33 (11.1)	26 (13.8)	72	
Candida krusei	7 (3.0)	7 (2.3)	2 (1.1)	16	
Others	15 (6.4)	24 (8.1)	1 (0.5)	40	
TOTAL	235	298	189	722	

TIMM
Trends in Medical Mycology

P462

Global incidence and mortality of fungal disease

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Objectives

Current estimates of the incidence of fungal disease are imprecise and were last published in 2017. The number of deaths linked to fungal disease has been estimated between 1.5 and 2.0 million. Here these estimates are revised.

Methods

Where possible, population at risk denominators are used to estimate annual incidence, using the given year usually 2019-2021. Extensive literature searches using Pubmed were combined with searches of my personal literature archive on the topic collected over 13 years, references listed in the retrieved published country and global burden papers (https://gaffi.org/media/country-fungal-disease-burdens/). Crude and attributable mortality was estimated using a combination of the survival percentage in treated patients, the untreated mortality and the proportion who are treated, focussing on published real-life experiences. The ratio of treated to untreated cases is a personal reflection on the many factors that could alter this – awareness, guidelines, accessibility of diagnostics and therapies etc. Greater weight was given to more recent and larger studies. Estimates do not account for influenza or Covid-19 outbreaks. Over 250 published papers from >120 countries contributed data to these rounded estimates.

Results

The estimates are shown in the table below:

Fungal condition	Annual incidence (mean (range), 000's)	Treated mortality	Untreated mortality	Ratio of treated to untreated cases	Estimated deaths (mean (range), 000's)	Percentage of deaths attributable to fungal infection	Attributable deaths (000's)
Invasive aspergillosis in COPD	1,513 (753– 2,272)	43–72%	>95%	1:5	1325	~80%	1,060
Invasive aspergillosis in ICU	519 (208 – 1,038)	50%	>95%	1:3	416	~50%	208
Invasive aspergillosis in leukaemia and lymphoma	24	45% (30-57%)	>95%	10:1	12	~80%	10
Invasive aspergillosis (lung cancer)	57[9]	51%	>95%	1:4	49	~40%	19
Chronic pulmonary aspergillosis	2,484	8%	20%	1:12	460	60% (0-85.7%)	276
Candida bloodstream infection	575	35% (8.7-56.9%)	~90%	9:1	233	~65% (21-100%)	151
Invasive candidiasis without positive blood culture	858	35% (27-60%)	~90%	1:5	678	~65% (21-100%)	441
Pneumocystis pneumonia in AIDS	400	15% (0-71%)	>95%	4:1	140	90%	126
Pneumocystis pneumonia non-AIDS	105	40% (8-58%)	100%	1:1	74	35%	49
Cryptococcal meningitis	194	60% (20-70%)	100%	3:2	147	80%	118
Disseminated histoplasmosis in AIDS	71 (47- 95)	30%	100%	1:10	66	80%	53
Talaromycosis	19	28%	>95%	3:1	9	90%	8
Mucormycosis	211	25%	100%	4:1	84	70%	59
Coccidioidomycosis (USA, Mexico)	30	-	-	10:1	2	90%	2
Fungal asthma	11,690	-	-	1:20[a]	92	50%	46
Totals	**18,750**	-	-	-	**3,804**	-	**2,573**

Conclusions

These estimates are necessarily crude, and there is large variability in the underlying risk populations, major regional differences, significant differences in the proportions affected in different studies, and genuine difficulty and uncertainty in the mortality rate of undiagnosed and untreated patients and in the ratio of diagnosed to undiagnosed patients for a given disease. The fungal diseases with the most uncertainty (and likely variability) are invasive aspergillosis in COPD and ICU, the early mortality of chronic pulmonary aspergillosis, invasive candidiasis with a negative blood culture, disseminated histoplasmosis and fungal asthma. These estimates build upon the work of >300 collaborators and others' work over the last 13 years. Nonetheless these figures suggest ~3.8 million deaths from fungal infection annually of which ~2.6 million are directly attributable to that disease.

P463

Whole genome sequencing of Candida auris using nanopore technology. An analysis during the outbreak of the fungus in Northern Greece.

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Objectives
Candida auris is an emerging, multi-drug resistant yeast. Its identification and epidemiological monitoring are challenging, while there is need for deeper understanding of its genetic basis. The first case in Northern Greece was reported in Thessaloniki in October 2022. In this study, the process that was followed in order to achieve the whole genome sequencing (WGS) of clinical strains of *C. auris,* using MinION nanopore technology, is described.
Methods
Several approaches were tested in order to obtain high-quality fungal genomic DNA for library preparation. The extraction methods consisted of bead-beating on various time scales or heating under buffered conditions, followed by column-based purification with the NucleoSpin® Blood QuickPure method. The quality (purity and fragmentation) and quantity of the isolated DNA were assessed by spectrophotometry, Qubit™ fluorometry and electrophoresis. WGS was performed on the Oxford Nanopore MinION platform using the ligation sequencing native barcoding kit (SQK-NBD112.24) for library preparation on three selected strains. The samples were sequenced on the MK1B MinION (MIN-101B) device with a FLO-MIN106D flow cell using MinKNOW release 23.04.4. The duration, quality and depth of sequencing and the ability to sequence multiple isolates were evaluated. Raw nanopore reads were base-called using ONT Guppy version 6.5.7+ca6d6af. Quality checks were performed by NanoPlot 1.41.3 and adapters were trimmed off by Porechop 0.2.4. De novo assembly was performed by Flye 2.9.2-b1786 by setting genome size to 12.5m. fastANI 1.33 was used to calculate identity scores to the *C auris* reference sequences. The assemblies were evaluated against the closest reference genomes using QUAST v5.2.

Results
DNA of adequate quantity and purity was obtained by the bead-beating method followed by column purification. However, it resulted in significant fragmentation of gDNA. Overall, 2.9M, 1.5M and 1.1M reads were obtained from the three DNA extracts with mean read length of 648bp, 968bp and 728 bp, respectively. Q20 obtained for 73%, 70% and 66% of the total called bases. 19% had (on average) a Phred score of Q30. The genome constructs varied with respect to the number of assembled fragments. Specifically, the isolate with the highest average read length was less fragmented (7 contigs, N50=757,689 bp), while the other two genomes were assembled by 703 (N50=27,815 bp) and 2,129 (N50=7,708 bp) contigs. Genome-wide comparisons against representative genomes of the five *C. auris* clades identified B8441 (Clade I) as the most closely related clade to the three *C. auris* genomes, with an average of 99.9% genome identity. The average nucleotide identifications against the reference genomes of the remaining clades were 99.47% (B11221; Clade III), 99.25% (B11220; Clade II), 98.30 (B11243) and 97,40% (Clade V, GCA_016809505.1).
Conclusions

The described methodology for WGS of *Candida auris* is reported for the first time in Greece. It allowed successful phylogenetic analysis and it is anticipated to be able to provide useful information even for antifungal resistance genes, for the prompt diagnosis and targeted treatment of patients.

P464

Fungal and bacterial co-infections of the respiratory tract among patients with COVID-19 hospitalized in intensive care units

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Objectives: The pandemic of COVID-19 has created a global public health crisis. ICU patients with COVID-19 are prone to infections of bacterial and/or fungal origins due to several risk factors. Consequently, the current study was conducted to evaluate the frequency, demographic characteristics, underlying conditions, and etiologic agents of fungal and bacterial co-infections of the respiratory tract among ICU patients with COVID-19 in Iran.

Materials & Methods: This was a cross-sectional single-center study involving ICU hospitalized patients at Imam Khomeini Hospital Complex, a referral tertiary center in Tehran, Iran. During a period of 6 months (from May to October 2020), lung specimens were obtained from ICU hospitalized patients with a positive real-time PCR test for COVID-19 who also were suspected of bacterial and/or fungal co-infections. One respiratory sample was obtained from each patient. The patients have had at least two of the hereunder conditions: (1) patients receiving immunosuppressants, (2) having clinical symptoms of pulmonary fungal and/or bacterial infections reported by a specialist in fields of pulmonary diseases (dyspnea, cough, fever, chest pain, purulent sputum, weight loss, hemoptysis, and wheezing), (3) suspicious radiographic findings indicating a pulmonary fungal and/or bacterial infection according to a pulmonologist opinion. The etiologic agents of bacterial co-infections were identified using the Vitek 2 identification method. For fungal identification, all samples were analyzed by direct microscopy using KOH 10% and culture. Furthermore, all isolates were subjected to PCR-sequencing method.

Result: A total of 73 lung specimens were obtained from patients who met the inclusion criteria. Of these, in 15 cases (20.54%) fungal and/or bacterial co-infections were confirmed. Males were more infected (73.33%) and all of them were between 49 and 79 years. Candida albicans (n = 8, 61.53%) and Klebsiella pneumoniae (n = 5, 38.46%) were the most frequent etiologic agents related to fungal and bacterial co-infections, respectively. Pneumonia (n = 15, 100%) and diabetes mellitus (n = 8, 53.33%) were documented as the most prevalent underlying conditions. In the current study, 3 out of 15 patients (20%) died.

Conclusion: The frequency of bacterial co-infections of the respiratory tract in ICU patients hospitalized with COVID-19 was relatively high. According to the results, one of the causes of death of these patients could be a secondary infection.

P465

Candida colonization and candidemia in intensive care units: Mapping of the Candida invasion

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Introduction and objectives:
Invasive Candidiasis (IC), particularly candidemia, causes high mortality in critically ill patients and accounts for a significant proportion of infections in Intensive Care Units (ICUs). Host colonisation by Candida species is usually a precursor to IC. Therefore, understanding the colonization patterns of individual *Candida* species may facilitate the prediction of candidemia. The aim of this study was to investigate the colonization rates of *Candida* spp. during prolonged hospital stays and to analyse their relationship with the development of candidemia.

Materials & Methods:
Retrospective study conducted between May 2016 and December 2022, including patients admitted to intensive care units for at least one month at La Fe University and Polytechnic Hospital in Valencia, Spain.
Throat, urine, rectal, groin, axillary and oropharyngeal samples were collected weekly. All samples were cultured on CHROMagar® and CHROMagar supplemented with 32 mg/mL fluconazole medium (MAIM, Barcelona, Spain). The samples were incubated at 37ºC for 48 hours.

The colonisation index (CI) and corrected colonization index (CCI) were calculated to assess the degree of colonisation. These indices were calculated for each patient per week. Both indices were calculated on a weekly basis for all patients. Data were collected from the hospital computer systems GestLab and Orion. Statistical analysis was performed with R-Statistics 4.2.3.

Results:
A total of 12820 samples from 604 patients were analysed. Seventy-seven episodes of candidemia were recorded during the patients' admission.
C. auris, C. parapsilosis and *C. glabrata* showed a positive trend with increasing length of hospital stay, whereas *C. albicans, C. tropicalis* and *C. krusei* remained stable over time (Table1).

Table1 – Mean CI and CCI according to weeks of hospitalisation.

		Week				
Species	**Score**	**1**	**2**	**3**	**4**	**5**
C. auris	CI	0,004	0,035	0,067	0,095	0,12
	CCI	0,001	0,025	0,043	0,058	0,06
C. albicans	CI	0,156	0,195	0,217	0,213	0,204
	CCI	0,08	0,107	0,12	0,123	0,098
C. parapsilosis	CI	0,025	0,035	0,051	0,061	0,068
	CCI	0,01	0,017	0,026	0,031	0,038
C. glabrata	CI	0,07	0,097	0,126	0,135	0,152
	CCI	0,034	0,05	0,067	0,073	0,085

C. tropicalis	CI	0,015	0,018	0,014	0,013	0,014
	CCI	0,006	0,008	0,005	0,006	0,004
C. krusei	CI	0,006	0,009	0,013	0,011	0,008
	CCI	0,003	0,006	0,007	0,004	0,005

Regarding the development of candidaemia, *C. auris* colonisation was associated with the highest rate of candidaemia, followed by *C. parapsilosis* (Table 2).

Table2 – Candidemia and colonization rate.

	C. auris	***C. albicans***	***C. parapsilosis***	***C. glabrata***	***C. tropicalis***	***C. krusei***
Candidemia/ Colonization (%)	33/177(18.6%)	14/421(3.3%)	13/199(6.5%)	5/336(1.5%)	2/53(3.8%)	0/16(0%)
Candidemia without previous colonization (%)	5/427(1.8%)	1/183(0.54%)	3/405(0.74%)	1/268(0.37%)	0/551(0%)	0/588(0%)
Odds Ratio	19.1[7.741-56.1]	6.248[1.094-134.3]	9.33[2.814-41.32]	4.026[0.5533-96.3]	-	-
p-value	<0,001	0,0108	<0,001	0,09	0,007	-
Mean colonization days before candidemia	22.31	44.33	20.41	14	31.5	-

Conclusions

1. Colonisation by *C. parapsilosis*, *C. glabrata* and *C. auris* was associated with longer hospital stays.
2. *C. auris* and *C. parapsilosis* colonisation was associated with the highest risk of candidemia.

TIMM Trends in Medical Mycology

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The first warning sign in the Nasal and Oral for Initiating Preemptive Anti-fungal Therapy in Febrile Neutropenic Patients.

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Objectives: Invasive fungal infections (IFIs) are complications that lead to mortality and morbidity in hematologic malignancies. The time of starting antifungal therapy is vital. Preemptive antifungal therapy has appeared recently as a new policy for the management of IFIs based on noninvasive ways in neutropenic patients.
Materials & Methods: We enrolled leukemia patients with neutropenia after chemotherapy in Imam Khomeini Hospital Complex, Tehran, Iran. Patients who entered the neutropenic phase were divided into two categories (empirical and preemptive) for receiving antifungal agents. The patients were clinically examined in the preemptive group every day to find IFIs. As soon as clinical evidence of IFIs was observed, antifungal was prescribed. The empirical group of patients received antifungals based on the ward protocol. Based on the data in each group, the diagnostic and therapeutic results of cases are followed up to 3 months. To compare percentages between the two groups, the chi-squared test was used. And to compare two means between the two groups, the independent t-test was used. All the statistical analyses were done in the Statistical Package for the Social Sciences (SPSS) version 24 software (IBM Corporation, Armonk, New York, USA).
Results: We assessed 132 leukemic patients with inclusion and exclusion criteria. Eventually, 80 patients were enrolled. The mean age was 35.52 years. Demographic data and distribution of leukemia type show no significant differences between the two groups. Despite a higher percentage of IFIs discovered in the preemptive group than in the empirical group (25 vs. 18.75%, respectively), data show no significant differences (Table1, 2). The average days of IFI diagnosis since the beginning of neutropenia in the empirical group were 9.5 days while in the preemptive group, the average days were 5.4 days ($p < 0.05$). Totally, there were 15 patients with a proven IFI in each group (40% in the empirical group and 60% in the preemptive group) (Table 2). Results significantly show an increase in surgical sinus debridement in the empirical groups (83.3%) vs. the preemptive groups (55.5%), ($p < 0.05$). The mortality rate differed significantly among the two groups; it was 7.5% in the preemptive group and 25% in the empirical group ($p < 0.05$). Based on the results, nasal cavity symptoms were the dominant site (88.8%) of IFIs in this study, while 11.11% of patients had the symptoms of fungal infection in the oral cavity (change the color of the palate).In each group, two patients had positive serum galactomannan levels (Table 3).
Conclusions: Daily oral and nasal cavities examination to find the symptoms of IFIs and then start preemptive antifungal agents may be able to lead to accurate diagnosis, earlier treatment, and decreasing sinus surgery debridement in leukemia patients with neutropenia.

Table 1.

Type of malignancy, N (%)	Empirical	Preemptive	Total, N (%)
AML[a]	29 (36.25%)	30 (37.50%)	59 (73.75%)
ALL[b]	11 (13.75%)	10 (12.50%)	21 (26.25%)
Total (%)	40 (50.00%)	40 (50.00%)	80 (100.00%)

[a]*AML, acute myeloid leukemia;* [b]*ALL, acute lymphoblastic leukemia.*

Table 2.

		Empirical			Preemptive			Total (%)
Probable	IFIs positive, N (%)	9 (11.25%)			11 (13.75%)			
	IFIs negative, N (%)	31 (38.75%)			29 (36.25%)			
Proven	IFIs positive, N (%)	6 (7.50%)	Mucormycosis	3 (20.00%)	9 (11.25%)	Mucormycosis	5 (33.33%)	
			Aspergillosis	3 (20.00%)		Aspergillosis	4 (26.67%)	
	IFIs negative, N (%)	34 (42.50%)			31 (38.75%)			
Total (%)		40 (50.00%)			40 (50.00%)			80 (100%)

Table 3.

Galactomannan Test, N (%)	Empirical		N (%)	Preemptive		N (%)	Total (%)
	Mucormycosis	Positive	0 (0.00%)	Mucormycosis	Positive	0 (0.00%)	0 (0.00%)
		Negative	3 (21.43%)		Negative	5 (35.71%)	8 (57.14%)
	Aspergillosis	Positive	2 (14.29%)	Aspergillosis	Positive	2 (14.29%)	4 (28.58%)
		Negative	1 (7.14%)		Negative	1 (7.14%)	2 (14.28%)
Total (%)			6 (42.86%)			8 (57.14%)	14 (100%)

P468

A Two-Year Surveillance of Candida Blood-stream Infections in a Tertiary Cancer Center in Muscat; First Report from Sultanate of Oman

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BACKGROUND AND OBJECTIVES
Candidemia, the most common form of invasive cadidiasis, is one of the most frequent bloodstream infections. It is assosciated with high (40-60%) mortality, increased hospital stay and significant economic burden. In Oman, there are no studies about the epidemiology and outcomes of candidemia. Hence, our aim is to determine the characteristics and clinical outcomes of cancer patients with candidemia, identify the causative fungal species and understand the susceptibility to antifungal treatments in cancer patients of Muscat, Oman.
METHODS
In this study, we review the results of all positive blood cultures with Candida species from June 2021 till June 2023 in Sultan Qaboos Comprehensive Cancer Care Center, a tertiary center for solid malignancies in Muscat. Further confirmation of species was performed by MALDI-TOF and of fungal susceptibility by VITEK 2. We analyzed the characteristics, causative candida species, anti-fungal susceptibility and clinical outcomes in cancer patients with candidemia.
RESULTS
Throughout two years, we identified 14 cancer patients with candidiasis, composed of 11 females and 3 males. Mean age was 53.14 (range: 23-74) years. All patients had advanced solid malignancy, with the two most common being myxoma peritonei (28.5%) and gastric adenocarcinoma (28.5%). The majority of the patients had central line (85.7%) and were exposed to at least one antibiotic within 1 month (92.8%). Almost half of the patients were admitted to the ICU at the time of candidemia, and one-third had recent abdominal surgical procedure. Regarding speciation and anti-fungal susceptibility, Candida albicans was detected in 4 patients only (28.5%), and the rest were non-albicans (71.4%), with Candida glabrata being the most common causative agent. The other three detected Candida non-albicans were Candida krusei, Candida tropicalis and Candida dubliniensis. All species were susceptible to azoles and amphotericin B. Moreover, all isolates were susceptible to echinocandins tested (Micafungin and Caspofungin) except three; two of which were intermediate and one (C. glabrata) was resistant to Caspofungin. One patient deceased before starting empiric antifungal treatment and the rest were initiated on echinocandins empirically. Infectious diseases team were consulted in 92.3% (12 of 13 patients); in all of these patients, surveillance blood cultues was performed and in 10, central line was removed. Echocardiology was done only in 4 of 13 patients which were negative for endocarditis. The 30 day all-cause mortality was 50% (7 of 14 patients), with 6 of the patients having been already on DNR code.
CONCLUSIONS
In Oman, there are no epidemiological studies for invasive candida infection and the predominant causative species. We present the data in a tertiary cancer center in Muscat that showed the predominance of Candida non albicans (C. glabrata) as a causative agent for candidemia. The majority of the Candida isolates were pan-susceptible. As per International guidelines, all our patients were initiated on echinocandins pending susceptibility testing. Although most of our patients already have advanced disease with poor outcomes, the mortality rate was within that reported in the literature (40-60%).

P469

Pneumocystis jirovecii pneumonia in pediatric systemic lupus erythematosus patient: A challenge for diagnosis and prophylaxis

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Objectives: The occurrence of *Pneumocystis jirovecii* pneumonia (PCP) in patient with systemic lupus erythematosus (SLE) is not uncommon. Unlike HIV-infected patients, currently there are no consensus guidelines for prophylaxis against PCP in SLE patients. We report a case of the patient with SLE who were a highly suspected case of PCP. The aim is to emphasize the importance of high index of suspicious, early diagnosis and proper management.

Materials & Methods: We reviewed a patient's medical records, which included the patient's medical history, clinical presentations, laboratory findings, radiographic imaging, and culture results. The literature review was conducted, and the data are summarized as shown.

Results:

A case of 13-year-old boy with known history of SLE for 3 years. Two weeks prior to this admission, he was diagnosed with active disease SLE and the first-time renal involvement. Corticosteroid was increased to 60 mg/day, for combating with active SLE, and mycophenolate was also added.

This admission, he presented with active lesion on his right shoulder, suspected of fungal infection. On examination BT 36.6 °C, RR 22/min, SpO_2 98%, there was Itchy, circular red patch with a raised scaly edge, central hypopigmentation size 15 x 20 cm on right shoulder, other systems were unremarkable. KOH stain from the lesion showed septate hyphae with arthroconidia, compatible with tinea corporis. Laboratory results showed no lymphopenia, WBC was 15,400 cells/mm^3 with PMN 56% and lymphocyte 44% (lymphocyte count 6,776 cells/mm^3), platelets 118,000 cells/mm^3.

Two days after the admission, he developed fever and non-productive cough. CXR showed bilateral patchy infiltration both lower lungs zone (figure 1). Piperacillin/tazobactam was started for treatment of hospital acquired pneumonia. On the second day, he had significant hypoxia, SpO_2 was 90% so high flow nasal cannula (HFNC) of 30 liters, FiO2 0.4 was introduced. There was increased in patchy infiltration on both lungs and ground glass opacity predominantly at right middle lung. CT chest found ground glass opacity with minimal consolidation at both lungs, more severe on RML. Also, PCP was then suspected in this patient due to his immunocompromised state. Further investigation showed serum LDH 683 IU/L, arterial blood gas PaO_2 67 mmHg, $PaCO_2$ 25 mmHg (A-a gradient 216.95), serum galactomannan negative. Sputum gran stain, AFB stain, culture for bacteria yielded negative result, unfortunately sputum testing for PCP was unavailable. Trimethoprim/sulfamethoxazole and meropenem were administered 3 days after the onset of symptoms and prednisolone was prescribed as recommended in moderate to severe PCP. The patients's symptoms gradually subsided and HFNC was discontinued after 2 days. CXR after 7 days of Trimethoprim/sulfamethoxazole appeared resolved of all infiltration (figure 2).

Conclusions:
PCP is a common opportunistic infection in immunodeficiency population. Recently there is an increased incidence in non-HIV populations. The primary symptoms include fever, cough, dyspnea, and significant hypoxemia. Prognosis is influenced by the level of awareness and timely initiation of treatment. The implementation of prophylactic guidelines for immunocompromised patients with Systemic Lupus Erythematosus (SLE) could potentially decrease the incidence of PCP-related complications and mortality within this population.

P470

An unusual case of Cryptococcal Meningitis in a patient with Chronic Lymphocytic Leukemia

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Objectives:
Cryptococcosis is an important opportunistic fungal infection causing an estimated 1 million cases and 625,000 deaths every year among patients with human immunodeficiency virus (HIV) worldwide(1). The vast majority of cases globally is caused by *Cryptococcus Neoformans* and present either as pneumonia or meningitis .Although cryptococcosis is most often associated with HIV infection, in many centers of the developed world, the majority of cases occur among non–HIV-infected individuals including transplant recipients or patients with hematologic or immunosuppressive disorders (2).

Materials & Methods:
In this context, we present an unusual case of a 64 year old man who was hospitalized for neurological alteration. The patient complained mainly of severe headache, but progressively developed speech impairment and confusion, which led to a state of lethargy. Brain CT and MRI scans were unremarkable. His HIV status was negative. Lumbar puncture showed leukocytosis (WBC =291, of which 95% were lymphocytes) and elevated protein levels (= 194mg/dl). A complete blood count revealed pronounced lymphocytosis (70% of the overall WBC) and whole body CT scan showed abdominal lymph nodes >5cm and mediastinal lymphadenopathy. Immunophenotype of both blood and cerebrospinal fluid was sent, which was positive for Chronic Lymphocytic Leukemia (CLL). In the following days the patient's neurological status worsened and he was intubated for airway protection. A repeat LP was performed and *Cryptococcus* Ag was sent and came back positive, whereas cultures later revealed *Cryptococcus Neoformans*. The patient was promptly started on Liposomal Amphotericin B at 4mg/kg/per day and Fluconazole 100mg/kg/day (due to shortage of Flucytosine). The patient also underwent electroencephalogram (EEG) which revealed Non-convulsive status epilepticus (NCSE) and was treated with levetiracetam and lacosamide. During his hospitalization in the ICU the patient presented clinical signs of elevated intracranial pressure (ICP). Hydrocephalus and periventricular edema was confirmed via CT scan, and a ventricular drain was placed to alleviate the elevated ICP.

Results:
Despite the improvement, during the following two weeks, both in terms of EEG monitoring and in ICP values, the patient remained unresponsive, thus requiring tracheostomy and prolonged mechanical ventilation. A decision was reached to extend the induction antifungal therapy for at least 6 weeks. After that, consolidation therapy was administered with Fluconazole 800mg/day for 8 more weeks. Repeat LP showed negative *Cryptococcus* Ag and cultures. During the 7th week the patient regained limited consciousness, and was weaned off mechanical ventilation. After that he progressively showed vast improvement of his neurological status, as he was able to communicate easily and move all four extremities adequately. In the following days the patient was transferred to the ward and eventually discharged at home.

Conclusions:
Cryptococcosis is a fungal infection that can occur on an immunosuppressed patient on top of the established diagnosis. In our patient, that was the case, as cryptococcal meningitis emerged in the presence of CNS involvement in an established CLL diagnosis. An extended period of antifungal therapy is often needed when neurological symptoms persist. This case illustrates the diagnostic and therapeutic difficulties of Cryptococcosis, which may arise in non-HIV patients.

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P471

Clinical outcome in a US patient with A.ustus complex pulmonary invasive aspergillosis treated with olorofim in a Phase2b study (NCT03583164)

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Objectives: Cryptic *Aspergillus* species represent a serious threat in clinical practice, being frequently multi- resistant, challenging to identify, and often reported in immunosuppressed patients. Olorofim is a first in class oral antifungal, selectively inhibiting fungal dihydroorotate dehydrogenase (DHODH), a key enzyme in fungal pyrimidine biosynthesis, ultimately leading to cell death. Olorofim has been shown to be active *in vitro,* with very low MICs consistently reported across difficult-to-treat mould species including cryptic *Aspergillus* species. Here we report clinical outcomes in an immunosuppressed patient with invasive pulmonary aspergillosis due to *A. ustus* complex treated with olorofim in a Phase 2b study (NCT03583164, Study 32).

Methods and Materials: A phase 2b open-label study of patients with probable pulmonary invasive aspergillosis or proven mould infections and limited or no treatment options enrolled a number of patients with azole resistant or cryptic *Aspergillus* infections. Study design encompassed treatment with olorofim (150 mg load Day 1, followed by 90mg BID) up to 84 days +/- 6 days in the main phase, allowing extended therapy beyond day 90. Primary end point was Data Review Committee adjudicated response at day 42 with day 84 response and ACM at day 42 and 84 key secondary endpoints.

Results: A 60-year-old woman with history of acute myeloid leukaemia (AML) had a history of allogeneic stem cell transplant complicated by graft versus host disease (GVHD) of skin, eyes, mouth, and history of sinusitis; bacterial discitis osteomyelitis; bacteremia; and persistent CMV viremia. She was diagnosed (53 days before enrolment in Study 32) with proven pulmonary aspergillosis (EORTC-MSG criteria) based on fungal elements detection in pulmonary biopsy specimens compatible with *Aspergillus* species at direct microscopy, isolation of *A. ustus* complex, and consistent radiology changes that developed during posaconazole prophylaxis. Voriconazole was promptly started, however serum galactomannan increased over time (OD from 0.68 to 3.32), with pulmonary radiological findings of persistent bilateral nodules increasing in number in the left upper lobe, and new signs of pulmonary embolism in the left lower lobe pulmonary artery extending into the posterior basal segment artery. Susceptibility testing (CLSI methodology) showed resistance to all azoles (MICs: isavuconazole 4 mg/L; itraconazole 8 mg/L; posaconazole and voriconazole >16 mg/L) and AmB (MIC: 2mg/L), with olorofim MIC of 0.06 mg/L. Due to highly active, multi-resistant and uncontrolled IFI at baseline, not responding to prolonged antifungal treatment, patient was enrolled in the clinical trial.
DRC adjudicated responses at day 42 (primary end point) and day 84, using the EORTC-MSG criteria, were Stable and Partial, respectively. Prompt clinical (improved dyspnoea) and radiological (≥ 25% to < 50% improvement across all lesions) response seen from day 28.

Patient required extended treatment of 373 days during which time olorofim was well-tolerated, with Investigator assessment of Success Complete at End of treatment.

Conclusions: Invasive aspergillosis due to cryptic species causes serious morbidity and a high rate of mortality. This case highlights the need for susceptibility testing when treating breakthrough infections on azole prophylaxis. Olorofim offers a therapeutic option for these difficult-to-treat infections.