

## P400

A study of the ecology, evolution and resistance mechanism of *Candida auris* at a tertiary care center in North India

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**Aim:** To study the ecology, evolution, and resistance mechanism of *Candida auris*, using samples from patients, healthcare workers, hospital and environmental niches, using amplified fragment length polymorphism (AFLP) and antifungal susceptibility testing (AFST).

**Methods:** A total of 720 samples were screened for *C. auris*, including clinical samples from patients (tissue, body fluids), surveillance samples from patients (axillar/groin swabs), swabs and water samples from environmental locations and objects, surface swabs from hospital locations, and screening samples from healthcare personnel for hand carriage of *C. auris*. Samples were cultured on Sabouraud Dextrose agar (SDA) and CHROMagar. Colonies morphologically suggestive of *C. auris* were identified by Matrix Assisted Laser Desorption-Time of Flight (MALDI-TOF) and isolates were subjected to antifungal susceptibility testing (AFST) by broth micro-dilution method. DNA was extracted for analysis by amplified fragment length polymorphism (AFLP) and cluster analysis. The amplicons were subjected to capillary electrophoresis and fluorescent amplified length polymorphism (FALP) for the generation of a heat map and dendrogram to evaluate single nucleotide polymorphisms and single nucleotide variations (SNPs and SNVs).

**Results:** Out of 720 samples, *C. auris* was isolated and identified by MALDI-TOF from 50, including 37 from routine patient samples, 12/674 axillar/groin surveillance swabs, and 1/66 samples from hands of healthcare workers. *C. auris* was not isolated from any environmental samples or hospital surfaces.

AFST revealed high overall rates of resistance to three important antifungal drugs—93.22%, 38.98%, and 52.54% of isolates were resistant to fluconazole, voriconazole, and amphotericin B respectively. Resistance to echinocandins was lower—1.81% of isolates were resistant to caspofungin, and micafungin. Additionally, 18 isolates showed only intermediate sensitivity to both voriconazole and caspofungin.

The highest rates of resistance to amphotericin B, and azoles were observed in isolates from blood (62.5% of isolates) and axillar/groin swabs (44.5% of isolates) respectively. Resistance to caspofungin was seen in 14.28% of isolates from both groups.

AFLP and capillary electrophoresis of extracted DNA revealed 188 variations in the range of 300-662 nucleotides. A total of 10 samples had no change in the nucleotides and were labeled as 'constant'. The dendrograms generated by bioinformatic analysis of FALP results yielded two different clusters provisionally designated as cluster I and cluster II. Cluster I could be further distinguished into sub-cluster Ia and sub-cluster Ib, indicating further variations.

**Conclusions:** *Candida auris* is a pathogen of emerging importance in our center, with significant levels of resistance to several important antifungal drugs. Incidence of both the pathogen and antifungal drug resistance was observed in samples collected from patients, but not from the hospital or environment, and minimally from healthcare personnel. This suggests that the source of most *C. auris* infections is colonizers from the patient rather than environmental sources or healthcare workers, and infection control measures should be tailored accordingly.

## P401

Identification and characterization of cryptic species of *Aspergillus* isolated from clinical samples

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**Background and Objective:** Molds are emerging as a major cause of life-threatening infections in immunocompromised patients. There is an increasing recognition of the cryptic *Aspergillus* species, which are organisms that are morphologically indistinguishable yet can be differentiated by molecular methods. These organisms have been known to show a higher minimal inhibitory concentration for the majority of the antifungal agents *in vitro*. Therefore, correct identification of these cryptic species is very important to administer a proper antifungal agent.

In this study, we wish to identify and characterize the cryptic species of *Aspergillus* from all clinical samples.

**Methods:** Patient routine samples like broncho alveolar lavage, endo-tracheal aspirate, sputum, pus, tissue, and CSF from various wards, OPDs, and ICUs of All India Institute of Medical Sciences, New Delhi which were processed in the Mycology laboratory, department of Microbiology showing growth of *Aspergillus* 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 the analysis of cryptic species, the isolates underwent PCR and then sequencing of the  $\beta$ -tubulin gene was done. Antifungal susceptibility testing was done using micro broth dilution as per the CLSI method.

**Results:** Of the 92 isolates, using morphological methods, 53 were identified as *A. fumigatus*, 33 were identified as *A. flavus*, 3 as *A. nidulans*, 2 as *A. terreus*, and 1 as *A. niger*. MALDI-TOF (Vitek MS database) misidentified 2 isolates of *A. nidulans* and 1 isolate of *A. stellatus* as *A. fumigatus* and 1 isolate of *A. tubingensis* as *A. niger*. The  $\beta$ -tubulin sequence analysis for the identification of cryptic species revealed that 2 isolates (2.08%) were cryptic, one was *A. stellatus* morphologically identified as *A. nidulans* and another one was *A. tubingensis* morphologically identified as *A. niger*.

**Conclusion:** Currently the available data on cryptic *Aspergillus* species is very limited. Because of its varied susceptibility pattern, it is important to identify the *Aspergillus* isolates to its species level. There is also a need for expansion of the number of strains for each species in MALDI-TOF MS databases for convenient, faster, and correct identification.

## P402

## Comparison of PCR-RFLP with 21-plex PCR and rDNA: sequencing for identification of clinical yeast isolates

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**Objectives:** Non-*albicans* *Candida* species and other rare yeasts have emerged as major opportunistic pathogens in fungal infections. Identification of opportunistic yeasts in developing countries is mainly performed by phenotypic assay, which is time-consuming and prone to errors. The aim of the present study was to evaluate PCR-RFLP as a routinely used identification technique for the most clinically important *Candida* species in Iran and make a comparison with a novel multiplex PCR, called 21-plex PCR.

**Methods:** A total of 173 yeast isolates from clinical sources were selected and identified with sequence analysis of the D1/D2 domains of rDNA (LSU rDNA) sequencing as the gold standard method.

**Results:** The results were compared with those obtained by PCR-RFLP using MspI restriction enzyme and the 21-plex PCR. PCR-RFLP correctly identified 93.4% of common pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *P. kudriavzevii* (= *C. krusei*)) and was able to identify 45.5% of isolates of the uncommon yeast species compared to the D1/D2 rDNA sequencing. Compared with PCR RFLP, all common *Candida* species and 72.7% of uncommon yeast species were correctly identified by the 21-plex PCR.

**Conclusion:** The application of the 21-plex PCR assay as a non-sequence-based molecular method for the identification of common and rare yeasts can reduce turnaround time and costs for the identification of clinically important yeasts and can be applied in resource-limited settings.

## P403

## Metagenomic sequencing as an effective diagnostic tool for Talaromycosis in HIV-negative patients: A retrospective study

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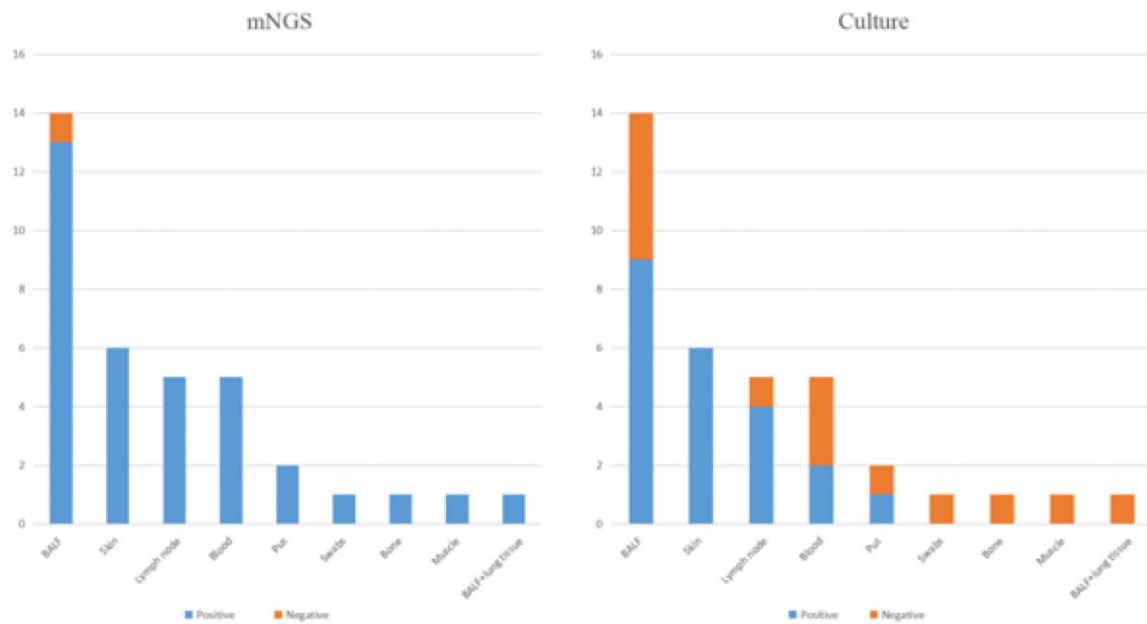
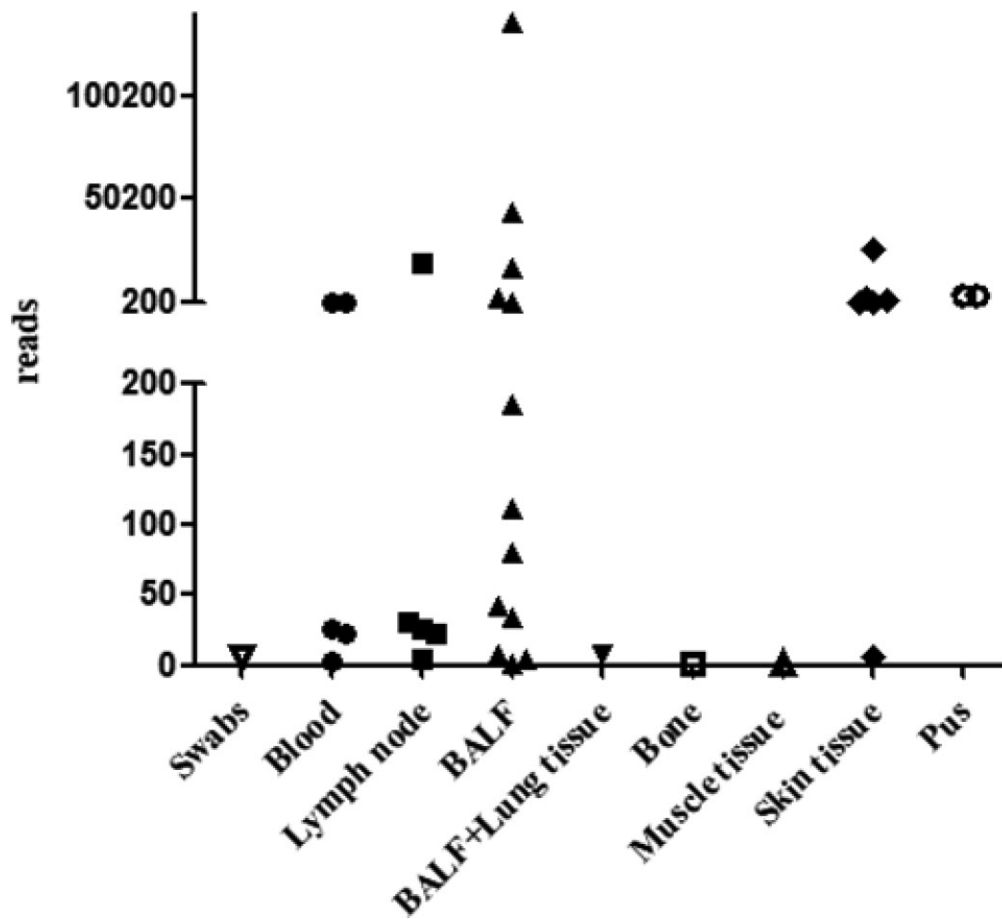
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**Objective:** The diagnosis of *Talaromyces marneffe* (*T. marneffe*) infection in HIV-negative patients is still challenging. The aim of this study was to evaluate the effectiveness and efficiency of metagenomic next-generation sequencing (mNGS) for *T. marneffe* infection in non-HIV-infected patients by comparing the diagnostic value of mNGS and traditional microbiological culture on different specimens.

**Methods:** A total of 57 HIV-negative patients with suspected infectious diseases were enrolled in the study from July 2018 to November 2021. mNGS and culture were performed on all enrolled samples. Data were collected on demographic characteristics, clinical symptoms, laboratory findings, and antifungal results. The diagnostic value of mNGS and conventional microbiological culture was compared.

**Results:** A total of 66 samples from 57 patients were analyzed, 29 were diagnosed with *T. marneffe* infection and 28 were of non-*T. marneffe* infected patients. Compared with the final diagnosis, mNGS showed a sensitivity of 97.22%, which was higher than that of conventional culture (61.11%). In addition, mNGS provides greater diversity in sample selection compared to culture.

**Conclusion:** Our study demonstrated that mNGS can be considered a promising tool for rapid and accurate pathogenic diagnosis in HIV-negative patients with suspected *T. marneffe*.



**P404**  
**Biofilm formation by *Cryptococcus* species isolated from Cerebrospinal fluid (CSF)**

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**Introduction:** Cases of Cryptococcal meningitis have increased exponentially in the last 30 years due to the advent of AIDS, the use of immunosuppressive drugs, and chemotherapeutic agents. Among HIV-infected patients' relapse is seen often.

The ability of *Cryptococcus* to form biofilm may influence the clinical outcome of patients though poor adherence to ART and/or anti-fungal therapy is a known factor for relapse.

**Methods:** During a period of 2 years and 8 months (August 2019 to March 2022), 11 *Cryptococcus* spp. were isolated from 59 cerebrospinal fluids (CSF) collected from patients clinically suspected of meningitis. Samples were examined and processed by direct microscopy and fungal culture. Identification of cryptococcal isolates was carried out by conventional method and by using an automated VITEK-2 (Biomérieux) identification system. Biofilm production was estimated by XTT reduction assay. Statistical analysis was done in SPSS version 21 (IBM) and Fischer's exact was used to find an association between biofilm formation and relapse.

**Results:** Out of 11 *Cryptococcus* isolated 10 were identified as *C. neoformans* and one was *C. gattii*. Three HIV-infected patients had recurrent cryptococcal meningitis. A total of 6 (42.8%) cases reported non-adherence to ART and/or anti-fungal