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

Sonakshi Gupta1, Immaculata Xeesa1, Gagandeep Singh2, Sauriya CS3, Adia Iram4, Manish Soman2
1Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India
2Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India
3Department of Medicine, All India Institute of Medical Sciences, New Delhi, 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 (AST).

Methods: A total of 720 samples were screened for C. auris, including clinical samples from patients (tissue, body fluids), surveillance samples from patients (arthroconidic crusts), seabs 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 (SDM) and CHROMagar. Colonies morphologically suggestive of C. auris were identified by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) and isolates were subjected to antifungal susceptibility testing (AST) 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 analyze 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 15 from routine patient samples, 124/94 arthroconidic surveillance swabs, and 146 samples from hands of healthcare workers. C. auris was not isolated from any environmental samples or hospital surfaces.

FALP revealed high overall rates of resistance to most 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 less—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 arthroconidic smears (64.5% of isolates) respectively. Resistance to caspofungin was seen in 14.28% of isolates from both groups.

AFLP and capillary electrophoresis of extracted DNA revealed 180 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 A and sub-cluster B, 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, not just from the hospital or environment, and minimally from healthcare personnel. This suggests that the source of most C. auris infections is from the patient rather than environmental sources or healthcare workers, and infection control measures should be tailored accordingly.

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

Srutiji Jani R1, Gagandeep Singh1, Immaculata Xeesa1, Bimal Kumar Das2
1AIIMS, New Delhi, India
2Department of Microbiology, AIIMS, New Delhi, India

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Background and Objectives: 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 unidentifiable yet can be differentiated by molecular methods. These organisms have been known to show a higher minimal inhibitory concentrations for the majority of the antifungal agents in vitro. Therefore, current identification of these cryptic species is very important to administer the proper antifungal agent.

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

Methods: Patient routine samples like bronchial alveolar lavage, endotracheal aspirate, sputum, pus, tissues, and CAF 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 β-tubulin gene was done. Antifungal susceptibility testing was done using micro broth dilution as per the CLSI method.

Results: Out of 97 isolates, using morphological methods, 55 were identified as A. fumigatus, 35 were identified as A. flavus, 5 as A. niger, 5 as A. alutum, 2 as A. terreus, and 1 as A. niger MALDI-TOF Vitek MS database misidentified 2 isolates of A. niger and 1 isolates of A. niger A. fumigatus and 1 isolates of A. fumigatus were not identified. The β-tubulin sequence analysis for the identification of cryptic species revealed that 2 isolates (2.06%) were cryptic, one was A. steineke morphologically identified as A. alutum and another one was A. niger morphologically identified as A. niger.

Conclusions: 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.

Comparison of PCR-RFLP with 23-plex PCR and -rDNA: sequencing for identification of clinical yeast isolates

Sadegh Khodavand1, Mohammad Karfi2, Ahmad Eimilghadr1, Seyed Jamal Hashemi2, Saeed Rezaei1, Rostamak Dae Shavazi3, Mohammadreza Salehi2, Ali Ahmad3, Iman Booshkool3
1Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2Department of Infectious Diseases and Tropical Medicine, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
3Department of Pathology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
4Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

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Objective: Non-allelic 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 171 yeast isolates from clinical sources were selected and identified with sequence analysis of the D1/D2 domains of -rDNA (D1/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 93.6% of common pathogenic Candida species (A. albicans, C. glabrata, C. parapsilosis, C. tropicalis, and P. anomala) and was able to identify 41.5% 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.

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

Tiansi Liang1,2, Cunwei Cao2,3
1The First Affiliated Hospital of Guangxi Medical University, Nanning, China
2Guangxi Health Commission Key Lab of Fungi and Mycose Research and Prevention, Nanning, China
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Objective: The diagnosis of Talaromyces marneffei (T. marneffei) 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. marneffei 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. marneffei infection and 28 were non-T. marneffei infected patients. Compared with the final diagnosis, mNGS showed a sensitivity of 97.22%, which was higher than that of conventional culture (81.1%). In addition, mNGS provided greater diversity in sample selection compared to culture.

Conclusions: Our study demonstrated that mNGS can be considered a promising tool for rapid and accurate pathogenic diagnosis in HIV-negative patients with suspected T. marneffei infection.
Biofilm formation by Cryptococcus species isolated from Cerebrospinal fluid (CSF)

Priyolakshmi Ningthoujam, Robertson Sawian, Ranjana Devi Khuraijam
RIMS, Imphal, India

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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 biofilms 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), 15 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 methods and by using an automated VITEK-2 (Biomerieux) identification system. Biofilm production was estimated by XTT reduction assay. Statistical analysis was done in SPSS version 21 (IBM) and Fisher’s exact was used to find an association between biofilm formation and relapse.

Results: Out of 15 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