P036

Epidemiology and antifungal susceptibility profile of infections caused by *Fusarium* species

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Aim: This study was performed to evaluate the clinical, epidemiological, and antifungal susceptibility profile of *Fusarium* species from clinical cases.

Methods: This study was conducted over a period of 14 years in a tertiary hospital in North India, 84 clinical isolates of *Fusarium* species isolated from various clinical samples like corneal scrapings, nail, tissue, and blood. The isolates were characterized phenotypically, and antifungal susceptibility testing was performed by broth microdilution method as per document CLSI M38-A3.

Results: On phenotypic identification, 69.04% were *Fusarium solani sensu stricto*, followed by *Fusarium oxysporum* (22.61%), *Fusarium dimerum* (8.33%) and *Fusarium incarnatum* (1.19%). The infection spectrum of *Fusarium* spp. was onychomycosis (54.76%), keratomycosis (19.04%), fusariosis (15.47%), white grain mycetoma (3.57%), burn wound infection (3.57%), hyalohyphomycosis (3.57%). In all 92.85% isolates were susceptible to amphotericin B (0.125-1 μg/ml). For voriconazole, 70.23% strains had MIC ranging between 0.5-1 μg/ml, while 29.76% had MIC >4 μg/ml. High MICs were found to itraconazole (>16 μg/ml), caspofungin (>16 μg/ml) and fluconazole (>64 μg/ml).

Conclusion: *Fusarium solani* is the most common species isolated. *Fusarium* spp. causes a broad spectrum of infections in humans including superficial, locally invasive, and disseminated infections. The clinical form of *Fusarium* species infections depends largely on the immune status of the host and the portal of entry of pathogen. Antifungal susceptibility testing is recommended owing to the variable susceptibility pattern of *Fusarium* spp. Large-scale studies are required to know the exact epidemiological, clinical factors, and antifungal susceptibility patterns of *Fusarium* infections.

P037

Study of magnitude and risk factors in patients with candidemia at a tertiary care hospital with speciation and antifungal susceptibility of pathogenic *Candida* isolates.

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Objectives: Nosocomial candidiasis is associated with a mortality rate of over 60% while the attributable mortality rate is 49%. The present study was to determine the magnitude and risk factors in patients with candidemia at a tertiary care hospital with speciation and antifungal susceptibility of pathogenic *Candida* isolates.

Methods: The present study was a prospective, cross-sectional, observational study, conducted at a tertiary care hospital for a period of 1 year after approval from Institutional ethics committee. It included a total of 150 patients of all age groups, admitted to hospital for >48 h and diagnosed as proven Candidemia with isolation of *Candida* species from at least two blood culture samples or from a clinically significant single blood culture sample. A thorough history and clinical characteristics of each patient was noted. Blood was collected and processed as per standard protocol. Pathogenic *Candida* species were identified and their antifungal susceptibility testing was performed by disk diffusion method as per the standard method. The antifungal discs used were fluconazole (25 μg), itraconazole (10 μg), voriconazole (1 μg), and amphotericin B (100 units). Results were analyzed statistically using SPSS statistics 20.

Results: *Candida* species was isolated as the pathogen in 24/150 (16%) of clinically suspected cases of candidemia. *Candida* species isolated were non-albican *Candida* (NAC) species, mainly *C. glabrata* 11/24 (45.83%) followed by *C. parapsilosis* 8/24 (33.33%), and *C. tropicalis* 5/24 (20.83%). *Candida* species was isolated as the pathogen, predominantly in patients of age group 0-10 years [15/24 (62.5%)]. Majority of *Candida* species were isolated from patients who had prolonged ICU stays. Among 24 patients of proven candidemia, 2 (8.33%) patients were from NICU, 10 (41.6%) from PICU, and 3 (12.5%) from MICU. Other important risk factors observed in the present study were, recent major abdominal surgery, malignancy, and mechanical ventilation, each accounting for 2/24 (8.33%) cases. The resistance pattern of isolates of *Candida* species to antifungals showed that *C. glabrata* showed 100% resistance to fluconazole, 63.6% to itraconazole, and 45.4% to voriconazole. *C. tropicalis* showed 80% resistance to fluconazole, 60% to itraconazole, and 40% to voriconazole. *Candida parapsilosis* showed 87.5% resistance to fluconazole, 62.5% to itraconazole, and 37.5% to voriconazole. All three isolated pathogenic *Candida* spp. showed 100% susceptibility to amphotericin B. Mortality observed in present study was 7/24 (29.7%). A total of 5/7 patients were from ICU.

Conclusion: Non-albican *Candida* (NAC) species, mainly *C. glabrata*, *C. tropicalis* and *C. parapsilosis* were the causative agent of candidemia, seen to predominantly affect 0-10 year age group. Infections caused by *Candida* species remain a significant problem in ICU. An increase in resistance to azoles is a challenge to its empirical and prophylactic use. This necessitates the usage of antifungals, only on the basis of antifungal susceptibility patterns of the pathogenic isolates.

P039

Cross-resistance to clinical and agricultural azoles among *Aspergillus fumigatus* strains isolated from humans and environment in Italy

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Objectives: In Italy, a prevalence of 16.9% of resistance to clinical azoles was observed among *Aspergillus fumigatus* isolates from an agricultural environment. This spread of azole-resistance is attributed to the widespread use of 14a-demethylase inhibitors (DMIs).

The aims of the present study were to investigate: the DMIs resistance in Italian *A. fumigatus* strains of clinical and environmental origin, both susceptible and resistant to clinical azoles; the molecular mechanism of resistance in strains susceptible to clinical azoles but resistant to at least one of the tested DMIs; the *in vitro* DMI resistance induced by prolonged exposure to DMIs in susceptible clinical and environmental strains, and the molecular mechanism of resistance.

Methods: A total of 54 *A. fumigatus* strains were selected: 23 susceptible to clinical azoles (CAS) and 31 resistant (CAR) with and without mutations in the CYP51A gene (TR34/L98H, F219I, G54R, G54E, D269Y, M220I, or F46Y/M172V/N248T/D255E/E427K). Antifungal susceptibility testing was performed for 8 DMIs (tebuconazole, epoxiconazole, difenoconazole, propiconazole, tetraconazole, flusilazole, fenbuconazole, and prochloraz) using broth microdilution method according to EUCAST and CLSI methods. Mutations in CYP51A, CYP51B, and HMG1 genes were investigated in CAS with DMI high MIC values. In vitro induction of resistance was performed using the 8 DMIs on 11 (6 clinical and 5 environmental) *A. fumigatus* strains susceptible both to clinical azoles and DMIs. A suspension of 106 conidia was inoculated on glucose-yeast extract-peptone agar plates containing different DMIs at different concentrations and incubated at 37°C for 72 h for six repeated passages.

Results: Comparable results were obtained using EUCAST and CLSI methods.

Resistance (MIC ≥16) to tetraconazole and fenbuconazole was observed in 100% of isolates, both CAR and CAS. On the contrary, a statistically significant difference in tebuconazole, epoxiconazole, difenoconazole, propiconazole, and flusilazole MICs between CAR strains and CAS strains was observed with higher geometric means (GM) in CAR (range 4.9-9.3 mg/L) than in CAS (1.5-2.7 mg/L) strains. Prochloraz showed the lowest GMs: 0.6 and 0.25 mg/L in CAR and CAS strains, respectively.

A significant difference of the GMs for all the DMIs tested, except prochloraz, was observed between the isolates harboring a TR34/L98H or a M220I mutation (GM range 10.4-16 mg/L) and those with other CYP51A mutations (GM range 1-4.6 mg/L).

In the CAS showing high DMI MICs, the absence of CYP51A mutations was confirmed, while a synonymous mutation P394P, was identified in CYP51B. No mutations in HMG1 gene were found.

In the induction tests, the prolonged exposure to DMIs showed an induced phenotypic resistance of 100% (11/11 isolates) for epoxiconazole, of 72.7% (8/11) for propiconazole, of 54% (6/11) for tebuconazole and difenoconazole, and of 9.1% (1/11) for prochloraz.

Molecular analysis to understand if the phenotypic resistance corresponds to induced mutations in CYP51A, CYP51B, and HMG1 genes is in progress.

Conclusions: Preliminary results confirm cross-resistance between clinical azoles and DMIs, with MIC differences between CAR and CAS and between strains with different mutations in the CYP51A gene. Furthermore, the ability of DMIs to induce resistance *in vitro* was highlighted.

P041

Preliminary evaluation of gradient concentration strips for detection of terbinafine resistance in *Trichophyton* spp.

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Objectives: Dermatophytosis is the most common superficial fungal infection. *Trichophyton rubrum* and *T. mentagrophytes* are the most frequently isolated species, but their incidence varies according to geographical regions. Terbinafine is the main molecule used to treat this type of infection. In recent years, a high incidence of chronic infections, reinfections, and treatment failures due to a newly described specie, *T. indotineae*, have been reported in India and recently described in Europe. It is currently a public health problem for the management of these infections in this country.

Until now, the monitoring of dermatophyte susceptibility to antifungals was rarely performed due to the lack of standardized *in vitro* tests. Since then, an *in vitro* technique has been standardized by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) to test terbinafine and other antifungals. Recently, a gradient concentration strip method has been marketed.

The aim of this study was to compare terbinafine susceptibility testing by the gradient concentration strip (GCS) method and the EUCAST standardized method.

Methods: A panel of 47 molecularly identified isolates of *T. interdigitale*, *T. mentagrophytes*, and *T. indotineae* was used. The panel included 39 terbinafine- susceptible isolates and 8 terbinafine resistant isolates for which the squalene epoxidase gene was sequenced.

Minimum inhibitory concentration (MIC) of terbinafine was determined using EUCAST microdilution broth method for dermatophytes. Inoculum was supplemented with cycloheximide and chloramphenicol. Final drug concentrations ranged from 0.008 to 8 μg/ml and microtiter plates were incubated at 25°C for 5 days. The MIC was determined spectrophotometrically with a 90% growth inhibition endpoint.

MIC of terbinafine was also determined using GCS (Terbinafine Ezy MIC™ Strip, HiMedia, India) on RPMI agar. The plates were incubated for 5 days at 25°C. After incubation, MIC was read by using a complete inhibition endpoint. Isolates were considered wild-type when MIC was ≤ 0.125 μg/ml.

Results: EUCAST MIC values ranged from 0.008 to 0.0625 μg/mL and from 0.25 to 16 μg/ml for susceptible and resistant isolates, respectively.

GCS MIC values ranged from 0.002 to 0.03 μg/ml and 0.125 to >32 for susceptible and resistant isolates, respectively.

The categorical agreement (percentage of strains found in the same category) by the two techniques was 98%.

Conclusion: These preliminary results show that GCS can detect resistance to terbinafine and could be used as a screening method. These results must be confirmed on a larger panel of isolates.

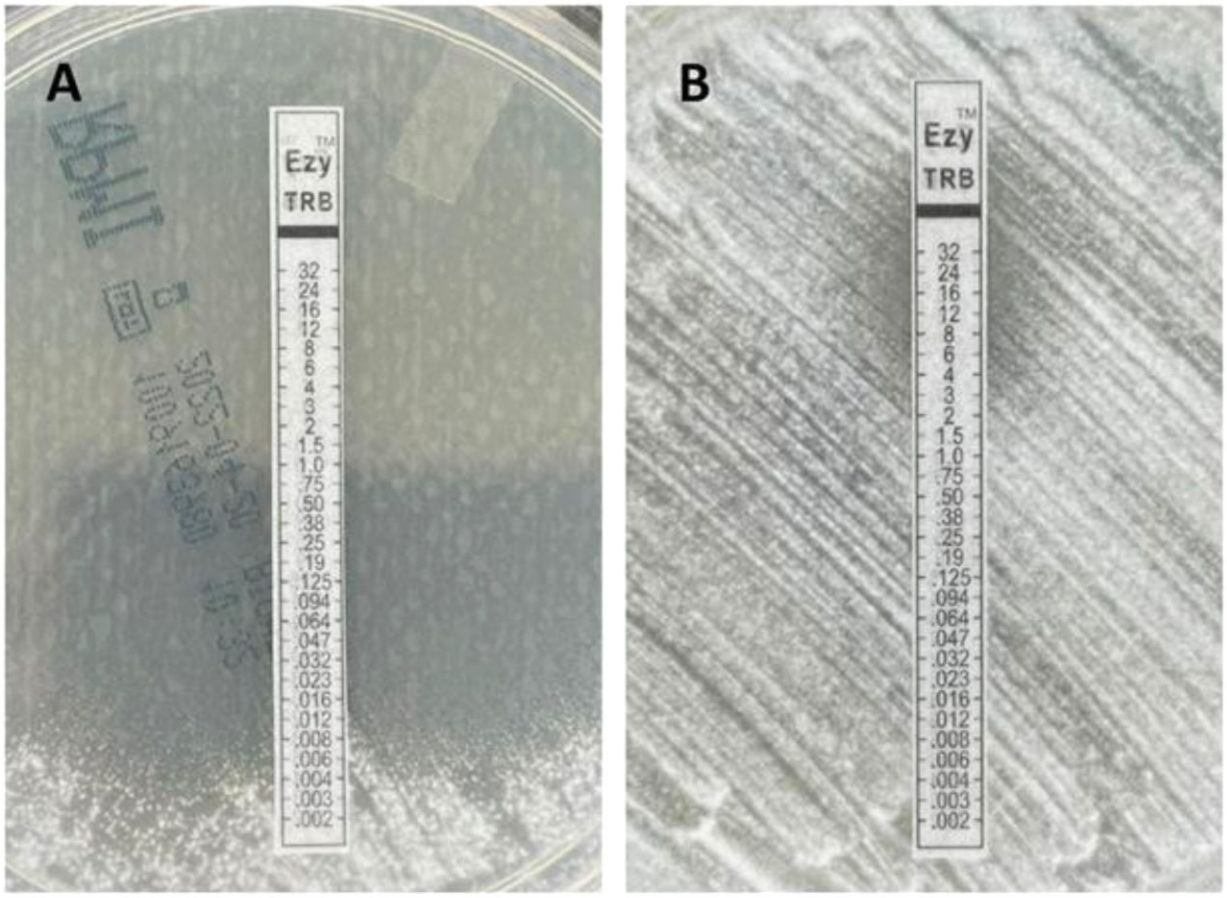

Figure: Example of two isolates tested for terbinafine with GCS method : one susceptible with MIC of 0.008 µg/ml (A) and one resistant (B) with MIC of 3 µg/ml.

P042

Identification, clinical profile, antifungal susceptibility pattern of *candida auris* from a tertiary care center in india

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Objectives:

To identify the phenotypic characteristics of *Candida auris*.

To analyze the clinical profile of *Candida auris* infection.

To describe the antifungal susceptibility pattern of *Candida auris*.

Methods: The study was conducted in the Department of Microbiology in Mycology division at Sri Ramachandra Institute of Higher Education and Research from December 2019 to November 2021. The study protocol was approved by Institutional Ethics Committee.

Candida species isolated from various specimens sent to the laboratory were identified by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF). The growth characteristics of *C. auris* were investigated on various media including Selective Auris Medium (SAM), HiChrome agar *Candida* and Tetrazolium reduction agar.

Antifungal susceptibility testing was performed by using the Clinical and Laboratory Standards Institute broth microdilution method M27-A3. Antifungals tested were fluconazole, itraconazole, voriconazole, posaconazole, micafungin, anidulafungin, caspofungin and amphotericin B. *Candida albicans* American Type Culture Collection (ATCC) 22 019 was used as quality control strains.

Data were collected for demographics, risk factors for candidemia, treatment, and outcome from the respective wards and ICUs.

Results: A total of 37 *C. auris* isolates were collected. Both adult and pediatric cases were included. The majority (23.3%) of the *C. auris* cases were seen in the age group of 55-64. Median age was 54 years for the adults. Among the 7 children, 6 were neonates and 1 was an infant. The most common source of isolation is urine and blood.

A total of 35/37 isolates showed moderate to heavy growth on the SAM, while 2 isolates showed mild growth after 72 h. But all the other *Candida* species and other yeasts tested were inhibited on this medium. All the isolates of *C. auris* grew as cream to pinkish purple colonies on Hichrome agar *Candida*. On Tetrazolium reduction agar, all of them formed maroon colonies.

The average duration of hospital stay was 25 days (range 4-65). A total of 35 of the patients were admitted to ICU, 8 had undergone mechanical ventilation and intubation. Central venous catheter was inserted in 9 patients and post-operative catheter placed in 6 patients; 4 patients had undergone tracheostomy and 25 of them had undergone some other invasive procedure. Total parenteral nutrition was received by 3 patients, 16 were diabetics and 11 were hypertensives. Prior antifungal exposure was present in 9 patients and 26 had received broad-spectrum antibiotics.

The crude mortality rate with *C. auris* infection in patients was 32.43% and the attributable mortality rate, as considered by the treating physician was 10.81%.

Antifungal resistance was noted to be amphotericin B ($n = 15$, 40.5%), fluconazole ($n = 30$, 81.1%), voriconazole ($n = 4$, 10.81%), itraconazole ($n = 6$, 16.21%), posaconazole ($n = 5$, 13.51%), caspofungin ($n = 4$, 10.81%). Multidrug resistance was noted in 15 (40.54%) isolates and 3 isolates (5.4%) were resistant to a drug from all three groups.

Conclusion: *C. auris* poses a great threat to immunocompromised individuals and those admitted in ICUs for long term.

P043

Amphotericin B in pediatrics: analysis by age stratification suggests a greater chance of adverse events from 13-month of age onward

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Background. Deoxycholate amphotericin B (D-AMB) remains an antifungal of great therapeutic value in pediatrics. It is generally accepted that its use in neonates is safer than in older children. However, childhood presents different periods of development which deserves to be evaluated more precisely. Our goal was to assess the usage profile of D-AMB in stratified pediatric age groups, adapted according to the National Institute of Child Health and Human Development (NICHD) classification.

Methods. We conducted a retrospective cross-sectional observational study at a Brazilian tertiary children's hospital. Non-parametric tests were applied, such as the chi-square test to compare proportions and Fisher's exact test to assess the association between categorical variables or in contingency tables.

Results. A total of 127 medical records were stratified as preterm neonatal (birth <37 weeks postmenstrual age), term neonatal (birth 27 days), infants (28 days-12 months), toddler (13 months-2 years), early childhood (3-5 years), middle childhood (6-11 years) and early adolescence (12-18 years). Very few acute infusion-related side effects were observed during administration of D-AMB in pediatrics. We found an unfavorable impact of D-AMB from 13 months onward, suggesting this group as a turning point for a greater chance of adverse events, and not soon after the neonatal period as is conventionally known (Fig. 1).

Conclusions: Clinical or observational studies based on age stratification are essential to precisely elucidate whether drugs with toxicity potential can be used safely in the pediatric population. Searching for a turning point has been shown to contribute to the accuracy of the study, while providing more substantial information on the impact of D-AMB on different pediatric age groups.