P019
A 5-year study on prevalence and molecular determinants of fluconazole -resistance in C. parapsilosis spp. complex

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Objective: Candida parapsilosis species complex is among the leading agents of invasive candidiasis, notably in neonates and transplant recipients. It exhibits intrinsically reduced susceptibility to echinocandins with increasing reports of acquired resistance to fluconazole from many centres. We evaluated antifungal susceptibility and molecular mechanisms of azole resistance in C. parapsilosis species complex, isolated in the last 5 years.

Methods: The isolates of C. parapsilosis species complex causing infections over a 5-year period (2017-2021) were included in the present study. Species identification of the isolates was performed using MALDI-TOF MS and sequencing of Internal Transcribed Spacing ITS3. Antifungal susceptibility testing was performed by CLSI broth microdilution according with standard operating procedures described in M27-A1 document. For amphotericin B, triazoles, and posaconazole, the interpretation of the susceptibility data was done using epidemiological cut-off values provided in CLSI M27 document. The entire coding sequence of ERG11 gene in fluconazole-resistant isolates was PCR-amplified in four overlapping fragments. The sequencing of each fragment was performed by Sanger's bidirectional sequencing method using BigDye terminator reagents reaction kit, 5.1. The sequences were assembled and proofread using Seqman software (DNAStar, Applied Biosystems). The nucleotide sequences were translated into ERG11 protein using nucleotide translation tool, (http://web.expasy.org/translate). Multiple sequence alignment with C. parapsilosis reference sequence was performed using Needle Sequence Aligment editor.

Results: A total of 580 C. parapsilosis complex clinical isolates were evaluated for antifungal susceptibility. C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis accounted for 447 (78.8%), 86 (14.8%), and 37 (6.3%), respectively. The isolation distribution revealed an increasing temporal trend over the years. A total of 40 (6.9%) isolates of the species complex exhibited reduced susceptibility to fluconazole, of which 23 were resistant (MIC ≥ 8 μg/ml) while 17 isolates exhibited susceptible dose-dependent phenotypes (MIC, 4 μg/ml). Only two of the fluconazole-resistant isolates were cross-resistant to voriconazole. The resistant isolates were predominantly from adult patients (Median age, IQR; 47 [16-70] years). The crude mortality rate in resistant cases was 56.5% (13/23). Interestingly, all the fluconazole-resistant isolates were C. parapsilosis sensu stricto, while C. orthopsilosis and C. metapsilosis isolates were susceptible. The fluconazole-resistance rate in the species complex and within the C. parapsilosis sensu stricto was 3.9% (23/599), 4.9% (21/466), respectively. For amphotericin B, the proportion of non-wildtype isolates was 1.7% (59/411), 2.1% (53/2566), respectively, and 0% in C. parapsilosis sensu stricto, C. metapsilosis, and C. orthopsilosis, respectively. For triazoles, the non-wildtype percentage was 1.7% (740/47, 3.5% (157/4544), and 0% in C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis, respectively. For posaconazole, the non-wildtype percentage was 5.8% (154/2615), 2.7% (274/10,000), and 0% in C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis, respectively. Sequencing analysis of ERG11 gene revealed two homogenous mutations, V12I, and R379A in fluconazole-resistant isolates.

Conclusions: C. parapsilosis sensu stricto complex infections are on the rise. The increasing azole resistance in C. parapsilosis with higher mortality is a great concern in clinical settings.

P020
Risk factors, speciation , and antifungal susceptibility in candidemia patients: An observational study

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Objective: To assess species distribution, antifungal susceptibility pattern, and associated risk factors in cases of candidemia among admitted patients in a tertiary care hospital in New Delhi, India.

Methods: Any positive blood culture bottle which revealed budding yeast cells on gram stain was included in the study. The samples were subcultured onto blood agar and HiConC. Candida Differential Agar (HiMedia Laboratories Pvt. Ltd, Mumbai), after which the colonies were subjected to identification with VITEK MS (bioMerieux, France). The antifungal susceptibility of each isolate was assessed with the help of Vitek 2 AST (bioMerieux, France). Fluconazole, voriconazole, and posaconazole were the antifungals tested for susceptibility. Antifungal susceptibility by broth microdilution was performed for C. auris and for fluconazole in the case of C. glabrata. Patient demographics, as well as risk factors associated with Candida infections, were collected from case files and by interviewing patients and bystanders.

Results: We isolated 171 fungal isolates from 160 patients admitted in the study during a period of 3 years from February 1, 2018, to January 31, 2021. Out of the 171 fungal isolates, 142 were Candida spp. Trushuppon spp. Saccharomyces cerevisiae and Pseudallescheria contributed 2, 2, and 1 isolate respectively. Among the Candida isolates, the commonest were C. auris (n = 37) followed by C. tropicalis (n = 34), C. albicans (n = 22), and C. glabrata (n = 22) (Fig. 1). The most common species from patients admitted to the ICU/PICU was C. auris (39%). Whereas in ward C. tropicalis (22%) and C. parapsilosis (22%) contributed the maximum number of isolates. Candida pelliculosa (n = 8) and C. tropicalis (n = 7) were the most common isolates among neonates.

Antifungal susceptibility results were interpreted as per Clinical Laboratory Standards Institute M27-A2 document. Overall sensitivity was higher for Micafungin followed by amphotericin B. Micafungin was mostly susceptible for C. aureus (94.11%), whereas in case of amphotericin B it was 47.22% (Table 1).

The most common risk factor observed was the presence of IV line (n = 155), antimicrobial therapy (n = 124), and diabetes (n = 46). In neonates also the most common risk factor was the presence of an IV line (n = 25) followed by authors status (values delivered outside in other hospitals and transferred subsequently) (n = 20). A total of 30 days hospital mortality was observed to be 34.01% in patients with C. auris isolates.

Conclusions: Candida Blastographics are shifting away from C. albicans and non-albicans species that have a higher propensity for developing resistance, such as the multiresistant C. auris, which is rapidly spreading throughout the world. This highlights the importance of stepping up hospital infection control practices and antifungal stewardship initiatives in order to counter the rapidly increasing antifungal resistance threat.