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

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Objectives: *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 centers. 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 Spacer, ITS1. Antifungal susceptibility testing was performed by CLSI broth microdilution in accordance with standard operating procedure described in M27-A3 document. For amphotericin B, itraconazole, and posaconazole, the interpretation of the susceptibility data was done using epidemiological cut-off values provided in CLSI M59 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 termination ready-reaction kit, 3.1. The sequences were assembled and proofread using Seqman software (Laser DNA, Applied Biosystems). The nucleotide sequences were translated into ERG11 protein using nucleotide translation tool, https://web.expasy.org/translate. Multiple sequence alignment with *C. parapsilosis* reference sequence was performed using BioEdit Sequence Alignment 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 457 (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 $\geq$8 μg/ml) while 17 isolates exhibited susceptible dose-dependent phenotype (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% (23/466), respectively. For amphotericin B, the proportion of non-wildtype isolates was 17% (70/411), 25% (9/36), and 0% in *C. parapsilosis sensu stricto*, *C. metapsilosis*, and *C.* orthopsilosis, respectively. For itraconazole, the non-wildtype percentage was 1.7% (7/407), 1.35% (1/74), and 0% in *C. parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. metapsilosis*, respectively. For posaconazole, the non-wildtype percentage was 3.8% (16/413), 2.7% (2/74), and 0% in *parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*, respectively. Sequencing analysis of ERG11 gene revealed two homozygous mutations, Y132F mutation, and R398I in fluconazole-resistant isolates.

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

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

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Objectives: 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 HiCrome^{TM} *Candida* Differential Agar (HiMedia Laboratories Pvt. Limited, 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, caspofungin, amphotericin B, flucytosine, and micafungin were the antifungals tested for resistance. 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 1 year from February 1, 2021 to January 31, 2022.

Out of the 171 fungal isolates, 162 were *Candida* spp. *Trichosporon* spp. Saccharomyces cerevisiae and *Fusarium* spp. contributed 6, 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 isolate from patients admitted to the ICU/HDU was *C. auris* (31%). Whereas in wards *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 highest for Micafungin followed by amphotericin B. Micafungin was mostly sensitive for *C. auris* (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 = 135), antimicrobial therapy (n = 126), and diabetes (n = 46). In neonates also the most common risk factor was the presence of an IV line (n = 23) followed by outborn status (babies delivered outside in other hospitals and transferred subsequently) (n = 20).

A total of 30 days hospital mortality was observed to be 54.05% in patients with C *auris* isolates.

Conclusion: *Candida* BSI etiologies are shifting away from *C. albicans* and towards species that have a higher propensity for developing resistance, such as the multidrug-resistant *C. auris*, which is rapidly spreading throughout the world. This highlights the importance of stepping up hospital infection control practices and antimicrobial stewardship initiatives in order to counteract the rapidly increasing antifungal resistance threat.