Fluconazole resistance in *Candida parapsilosis* in India and analysis of sterol biosynthesis gene (*ERG11*)

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Background

- *Candida parapsilosis*, is a significant species in bloodstream infections (BSIs) world wide.
- This species is responsible for about 12 to 17% of cases of candidemia in the United States and is the most common cause of BSIs in Latin America.
- The present study aimed to explore azole resistance among Indian clinical *C. parapsilosis* isolates and investigated the azole resistance mechanism using sterol biosynthesis gene (*ERG11*) sequencing.

Materials and Methods

- ❑ **Fungal isolates:** A total of 199 *C. parapsilosis* isolates collected from eight tertiary care hospitals in Delhi, and the adjoining National Capital Region, during a 3-year surveillance study of candidiasis from 2015 to 2017 were analyzed. All isolates were screened on CHROMagar™ Candida medium.
- ❑ **Identification by MALDI-TOF MS:** Isolates were identified by MALDI-TOF MS using Flex Control 3.1 software (Bruker Daltonics) and MALDI Biotyper OC version 3.1.
- ❑ **Antifungal Susceptibility Testing (AFST):** AFST was carried out using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method, following the M27-A3 guidelines. The antifungal drugs tested included azoles, AMB and 5-FC.
- ❑ ***ERG11* gene sequencing:** A total of 45 isolates categorized as fluconazole susceptible (n=5, MICs ≤ 2 µg/ml), non susceptible (susceptible dose dependent, SDD, n=5, MICs 4 mg/L) and resistant (n=35, MIC ≥ 8 µg/ml) according to clinical break points CLSIM27/S4 2012 were subjected to sequencing of *ERG11* gene sequencing and mutation analysis.

Results

- ✓ All isolates were *C. parapsilosis sensu stricto* and blood stream isolates accounted for 48% (n=95) isolates.
- ✓ 38% (n=75) of isolates were non-susceptible (NS) to fluconazole including 29 % (n=58) resistant (MIC ≥ 8 µg/ml) and 9% (n=17) were SDD (MIC 4 µg/ml).

Results

- ✓ Among 58 FLU resistant isolates, 57% (n=33) were also NS to VRC (SDD MIC 0.25-0.5 µg/ml) and 17% (n=10) exhibited SDD MICs (0.25-0.5 µg/ml) to ITC.
- ✓ However, only 3 FLU resistant isolates were co-resistant to ITC (MIC 1 µg/ml) and a single FLU resistant isolate was co resistant to VRC (MIC 1 µg/ml).
- ✓ Among FLU NS isolates, novel amino acid K143R in Erg11 gene were observed in 67.5% of isolates whereas amino acid Y132F were found in 32.5% of isolates.

Table 1. *In-vitro* antifungal susceptibility profile of *Candida parapsilosis* isolates (n=199) against medical azoles, amphotericin B. and 5 flucytosine.

Parameter*	^a Drugs (µg/ml)					
	AMB	ITC	VRC	POS	FLU	5-FC
GM	0.287	0.115	0.048	0.035	1.503	0.128
MIC ₅₀	0.25	0.125	0.03	0.03	1	0.125
MIC ₉₀	0.5	0.25	0.125	0.125	16	0.125
Range	0.025-1	0.03-1	0.03-1	0.015-0.25	0.05-32	0.125-8

^aAMB, Amphotericin B; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; FLC, fluconazole; 5-FC, 5 flucytosine; *GM, Geometric mean of MICs; MIC₅₀, at which 50% of test isolates inhibited; MIC₉₀, at which 90% of test isolates inhibited

Table 2. Amino acid substitutions in the *ERG11* gene *Candida parapsilosis* (n=45) strains

FLU MICs	Number of isolates	Number of isolates NS or resistance to ITC/VRC	Mutation in <i>ERG11</i> (number of isolates)
16 µg/ml	15	2 (NS to VRC), 2 (NS to ITC)	K143R (12), Y132F (3)
8 µg/ml	20	6(NS to ITC) and 1(NS to VRC)	K143R (12), Y132F (8)
4 µg/ml	5	3 (NS to ITC)	K143R (3), Y132F (2)
2 µg/ml	3	All susceptible to ITC and VRC	Wild Type
1 µg/ml	2	All susceptible to ITC and VRC	Wild type

Conclusion

- The study reports first molecular analysis of azole resistant *C. parapsilosis* isolates from India and a high FLU resistance of 29% among Indian isolates.
- Amino acid substitution, K143R is reported for the first time in FLU resistant *C. parapsilosis* in this study, which is previously strongly associated with FLU resistance in *C. albicans*.
- Further, studies to elucidate the role of multidrug transporters and expression of *ERG11* isolates is warranted to gain insight in azole resistance.