Molecular mechanisms and clinical features of fluconazole-resistant Candida spp.

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Background: Azole resistance in Candida spp. is slowly emerging and has been associated with various different mechanisms. We aimed to investigate the resistance mechanisms and clinical features of fluconazole non-susceptible (FNS) Candida spp. recovered from a major regional tertiary referral hospital in Singapore.

Material/methods: All non-duplicate FNS Candida blood isolates collected during a candidemia surveillance study conducted from 2012 to 2015 were included for analyses. A fluconazole-susceptible (FS) control isolate (fluconazole MIC ≤2mg/L for Candida albicans and Candida tropicalis, MIC ≤32mg/L for Candida glabrata) was included for each FNS isolate. RT-PCR was performed for the evaluation of CDR1/CDR2/MDR1/ERG11 expression. Gene over-expression was defined as >3x the value of the wild-type ATCC strain. ERG11 gene mutation analyses were performed using PCR and sequencing. Clinical information was collected retrospectively and included demographics, antifungal therapy, and outcomes.

Results: Forty-eight (24 FNS, 24 FS) isolates were included (8 C. albicans, 32 C. tropicalis, 8 C. glabrata). Cross-resistance to other azoles was detected in all FNS isolates except 2 C. albicans. In 7 out of 8 C. albicans isolates (3 FS and 4 FNS), amino acid substitutions in Erg11p were detected (FS – D116E, D153E, A383C, S536L; both FS and FNS – E266D). A114S and Y257H were detected together in 3 FNS isolates. No amino acid substitutions were detected in FS C. tropicalis, while 3 amino acid substitutions (Y132F, F145L, S145F) were detected in 8 out of 16 FNS C. tropicalis. Y132F and S145F were detected together in the 8 FNS C. tropicalis. Two amino acid substitutions (I166S, L172P) were detected together in the 8 FNS C. tropicalis. Two amino acid substitutions (I166S, L172P) were detected together in 3 C. glabrata isolates (2 FS and 1 FNS). All Erg11p substitutions have been previously reported except S536L, I166S and L172P. Figure 1 illustrates the mean relative expression levels of ERG11/CDR1/CDR2/MDR. The rates of overexpression of the various genes were similar among FS and FNS Candida spp., with the exception of CDR2 in C. albicans (0/4 FS vs. 3/4 in FNS). Eight FNS C. tropicalis did not exhibit any of the studied mechanisms. FNS isolates were more commonly isolated from patients with prior azole exposure.
(40% vs. 8%, p=0.008). Echinocandins were the most common definitive treatment prescribed (88%). High mortality was observed in patients with both FNS and FS *Candida* infections (64% vs. 48%, p=0.25).

**Conclusions:** Different mechanisms of azole resistance are implicated in different *Candida* spp. A114S and Y257H Erg11p substitutions and CDR2 overexpression were predominant mechanisms in FNS *C. albicans*, while the role of gene overexpression was less significant in *C. tropicalis* and *C. glabrata*. Azole resistance was more commonly observed in patients with prior antifungal exposure. Knowledge of these resistance mechanisms may allow rapid detection of these gene targets to guide antifungal therapy.

![Expression levels of CDR1, MDR1 and ERG11 in FS and FNS (a) C. albicans (b) C. tropicalis (C) C. glabrata. Quantification of each target gene was determined by the 2ΔΔCT method using the housekeeping gene ACT as a control. Error bars show the standard deviations.](image)