

**P0324 Investigation of ERG11 gene mutations in invasive *Candida albicans* isolates with resistant/reduced susceptibility (non-WT) to fluconazole by DNA sequencing**

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**Background:** The cytochrome P450 lanosterol 14 $\alpha$ -demethylase (Erg11p) encoded by ERG11 gene is the primary target for azole antifungals. Decrease in azole affinity of this enzyme caused by amino acid substitutions have been reported as a mechanism of azole antifungal resistance. This study aimed to investigate the relationship between fluconazole resistant/reduced susceptibility (non-WT) *C. albicans* isolates and amino acid substitutions in Erg11 gene

**Materials/methods:** *Candida albicans* isolates (n=88) obtained from blood cultures of invasive candidiasis patients from various intensive care units in Turkey between 2010-2014 and six reference strains (*C. albicans* ATCC90028, *C. albicans* ATCC64124-Darlington strain, *C. albicans* ATCC76615, *Candida dubliniensis* ATCC-MYA-583, *Candida krusei* ATCC6258, *Candida parapsilosis* ATCC22019) were included in the study. Isolates were identified by conventional methods. Susceptibility testing was performed for fluconazole with the broth microdilution method according to CLSI M27-A3, and interpreted according to CLSI M27-S4, as susceptible (S), susceptible-dose dependent (S-DD), resistant (R) and ECVs criteria, for non-WT (MIC > ECV) strains.

Twenty nine clinical isolates and four reference strains were genotyped. The *ERG11* genes of 12 clinical isolates of *C. albicans* (n=3 S; MIC  $\leq$ 2  $\mu$ g/mL, n=3 R; MIC,  $\geq$ 8  $\mu$ g/mL, n= 6 non-WT; MIC >0.5  $\mu$ g/mL) and three reference strains (n=1 S, n=2 R) were amplified and sequenced

**Results:** In the 12 isolates and three reference strains, 16 types of amino acid substitutions were found of which 5 substitutions [G510E, V497I (two non-WT, one S isolate), D153E (one non-WT isolate) E266N (one non-WT isolate), and R265E (one non-WT isolate)] have not been reported previously. Y132H and G510E (not previously reported) substitutions were detected in the Darlington strain E266D amino acid substitution was found in 7 fluconazole R / non-WT isolates and in 3 fluconazole S isolates.

**Conclusions:** Amino acid substitutions which are generally seen in fluconazole resistant strains were not determined in our isolates. Unique mutations in ERG11 (G510E, V497I, D153E, E266N, and R265E) may be linked with the fluconazole resistance in *C. albicans*. In conclusion, to determine the molecular mechanism of this resistance, RNA expression levels of *CDR1*, *MDR1* and *ERG11* and mutations in the *ERG3* gene, should also be investigated.