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

Nilgun Karabicak*, Dilek Guldemir†, Rıza Durmaz‡

1Türkiye Halk Sağlığı Kurumu, Mycology Reference Laboratory, Ankara, Turkey, 2Türkiye Halk Sağlığı Kurumu, Molecular Microbiology Reference Laboratory, Ankara, Turkey, 3Yıldırım Beyazıt Üniversitesi, Ankara, Turkey

Background: The cytochrome P450 lanosterol 14alpha-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 ≤2 μg/mL, n=3 R; MIC ≥8 μg/mL, n= 6 non-WT; MIC >0.5 μg/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.