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Paper Poster Session
Update in fungal resistance and susceptibility

Molecular genetic analysis of the 14-alpha lanosterol demethylase gene in azole-resistant pathogenic fungi

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Background: Fungal pathogens cause life-threatening infections in immunocompetent and immunocompromised individuals. The most prevalent fungal pathogens are *Candida* spp. and *Aspergillus* spp. Azole drugs are the first line of antimycotic therapy. Antifungal drug resistance is a confounding factor that negatively impacts clinical outcome for patients with serious mycoses. Recent years have seen the development of molecular technology that is ideally suited for the assessment of drug resistance mechanisms. The target of the azoles is the 14-alpha lanosterol demethylase enzyme generated by the gene *erg11* in yeasts and the *cyp51* in filamentous fungi. Reduction of the susceptibility to azole is mainly triggered by point mutation in this gene. Nowadays, multiple point mutations in *erg11*/*cyp51* are identified. However, not all of them are accounted for the drug-resistant phenotype and there are geographical differences.
The aim of the study is a molecular genetic analysis of the *erg11*/*cyp51* nucleotide sequence for azole-resistant strains of *Candida* spp. and *Aspergillus* spp. isolated from patients in the Russian Federation, which can serve as a basis of molecular diagnostic platforms suitable for rapid detection of drug resistance.

Material/methods: All *Candida* and *Aspergillus* isolates were identified by sequencing of internal transcribed spacer region (ITS) and a fraction of the β-tubulin gene. Antifungal susceptibility testing was carried out according to the EUCAST microdilution method.
The nucleotide sequences of pathogenic *Candida* spp. and *Aspergillus* spp. *erg11*/*cyp51* were aligned with reference sequence from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). Optimal oligonucleotides sequences were selected for amplification and sequencing of *Candida* spp. and *Aspergillus* spp. *erg11*/*cyp51*. Specificity of the oligonucleotides was verified by BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/).

Results: We currently included in the study 12 and 31 resistant clinical *Aspergillus* spp. and *Candida* spp isolates, respectively, which belong to the following species: *A.terreus, A.flavus*, *A.niger* and *A.calidoustus*, *C.albicans* and *C.glabrata*.
A resistant clinical isolate *A.terreus* had the most nucleotide polymorphism when analyzing nucleotide sequences *cyp51* gene of the *Aspergillus* fungi, it also revealed the following amino acid substitutions: S8T, L26F, K64R, Q270R, V336A, H362D, I365L, H366P, V390M, Q419H, A478F, P498A compared with susceptible strains and the standard NCBI gene sequence database.
The *erg11* gene of the resistant strains *Candida* spp. bears the following mutations: T315C, T348A, A357G, A383C, C658T, A1020G, C1110T, A1440G, T1470C, compared with the standard sequence

of the wild type gene X13296. Mutations T315C, A357G, C658T, A1020G, C1110T, A1440G, T1470C do not change the amino acid sequence of the protein product. Missense mutations T348A and A383C lead to substitutions D116E, K128T.

Conclusions: The study of the mutations in *erg11*/*cyp51* is promising to determine molecular genetic characteristics of azole resistant *Aspergillus* and *Candida* isolates that may lead to development of the original system for testing antimycotics resistant in human fungal pathogen.