TITLE: THE GENE EXPRESSION PROFILES OF SUSCEPTIBLE AND RESISTANT STRAINS OF *Aspergillus fumigatus* AFTER EXPOSURE TO AZOLES APPRAISED BY RNA-seq

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ABSTRACT:

In immunocompromised patients, aspergillosis is a prevalent invasive fungal disease; *Aspergillus fumigatus* is the most common etiological agent. The first-line treatment of invasive aspergillosis relies on the administration of azoles. However, azole-resistant *Aspergillus* strains are emerging from many parts of the world. Some studies have reported a role in azole resistance of other genes from the ergosterol biosynthesis pathway, some transcriptional factors, or overexpression of drug efflux pumps. Up-regulation of drug efflux could represent the second most abundant azole resistance mechanism in *A. fumigatus*. In such a context, this study aimed to investigate differentially expressed genes between resistant and susceptible strains after exposition to azoles by RNA-seq. Two pairs of clinical *A. fumigatus* isolates (resistant and susceptible) from the same patient were cultured in AMM (*Aspergillus* Minimal Medium) under agitation and incubated for 30 and 120 min with itraconazole or voriconazole. These cultures were filtered and submitted to RNA extraction, and a cDNA library was built for RNA-seq. After bioinformatics analysis, it was observed that the number of differentially expressed genes (DEGs) increased in 120 min of treatment with azoles, including 1100 and 751 up-regulated genes, and 772 and 637 down-regulated genes after treatment with itraconazole and voriconazole, respectively. A functional and gene ontology enrichment analysis of these DEGs revealed that processes related to nucleic acid metabolism, transcription, RNA processing, ribosome synthesis, biosynthesis of amino acid, and organization of cellular components showed more relevance in resistant strains. In contrast, primary metabolism was observed in susceptible ones, as hydrolase activity, glycolipid metabolism, and amino acid degradation. Gene expression of putative drug efflux transporters was investigated, and 109 DEGs related to transporters were found, most of them expressed after 120 min of treatment. These transporters have been categorized into smaller groups, like genes expressed exclusively in resistant or susceptible strains, when treated with itraconazole or voriconazole. Further investigation has been developed about these genes and the ones involved in the ergosterol biosynthesis pathway to elucidate possible differences between resistant and susceptible strains that promote the survival of the fungus in the patient.

Keywords: RNA-seq, transcriptome, *Aspergillus fumigatus*, resistance to azoles, drug transport

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