TITLE: ANTIFUNGAL ACTIVITY OF AMPHOTERICIN B, ITRACONAZOLE, MICAFUNGIN, AND FLUCYTOSINE AGAINST BIOFILMS OF *Candida glabrata* ISOLATED FROM PATIENTS WITH OPORTUNISTIC CANDIDIASIS

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ABSTRACT:
Candida glabrata is an emerging opportunistic human fungal pathogen in public and private Brazilian hospitals. Several virulence-associated attributes are involved in its pathogenesis, including the adhesion of the organism to host cells and/or tissues as well as medical device surfaces, which leads to biofilm formation. Biofilm is an important virulence factor, as it increases the resistance to antifungal therapy, as well as protects fungal cells from the host immune response and other stress conditions. This study evaluated the in vitro antifungal susceptibility profile to four antifungal agents on planktonic cells and biofilm cells in 24 clinical strains of *Candida glabrata* sensu stricto identified by sequencing of ITS1-5.8S-ITS2 region of the rDNA. The minimal inhibitory concentrations (MIC) of the drugs on planktonic cells were determined by broth microdilution, according to the CLSI recommendations. The antifungal susceptibility on pre-formed biofilm growing cells was evaluated through the quantification of metabolic active fungal cells able to reduce 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT). The MIC values for amphotericin B ranged from 0.06 to 1 μg/ml in planktonic cells and from 1 to > 16 μg/ml in pre-formed biofilms. For itraconazole, planktonic MIC values ranged from 0.06 to 0.25 μg/ml and biofilm MIC values from 0.5 to > 16 μg/ml. For micafungin, MIC values ranged from 0.015 to 0.12 μg/ml in planktonic cells and from 0.06 to 0.5 μg/ml in the cells present in the biofilm. Regarding flucytosine, all strains had the same MIC value (0.12 μg/mL) in planktonic cells whereas the biofilm MIC values ranged from 8 to 128 μg/mL. Amphotericin B and micafungin were able to penetrate the barrier imposed by the biofilm and inhibit the cellular growth of some clinical strains of *C. glabrata*, while itraconazole and flucytosine were not efficient to prevent biofilm growth. These results demonstrated that biofilm-forming clinical strains of *C. glabrata* present a considerable resistance to different antifungal classes, especially regarding azole (e.g., itraconazole), nucleoside analog (e.g. flucytosine), polyene (e.g., amphotericin B), and echinocandin (e.g., micafungin), in a descending order. New antifungal strategies involving inhibition of biofilm formation are plausible approaches to combat biofilm–associated *C. glabrata* infections.

Keywords: antifungal, biofilm, *Candida glabrata*

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