

TITLE: Structural cell changes of a clinical isolate and morphological variants of *Candida tropicalis* to fluconazole exposure.

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ABSTRACT:

Candida tropicalis (*Ct*) is an opportunistic human pathogen. The ability to switch morphology through both polymorphism and phenotypic switching (PS) allows rapid adaptation of yeasts to host niches. *Ct* is the second most isolated of the *Candida* genus in Brazilian hospitals exhibiting increased resistance to azoles. In this sense, changes in the structural composition and dynamics of the cell wall play an important role in the development of antifungal resistance. Therefore, the aim of this study was to evaluate structural variations in a clinical *Ct* isolate and its variants derived from PS following exposure to fluconazole (FLC). For this purpose, the clinical isolate 49.07 (parental) and two morphotypes: Crepe (C) (structured morphology) and Revertant of Crepe (RC) (parental-like morphotype) were subjected to analysis of cell wall porosity using Tris-HCl, DEAE-Dextran and Poly-L-lysine buffer; plasma membrane integrity using 0.4% trypan blue solution in a microtiter plate with decreasing concentrations of fluconazole (64 - 0.125 µg/mL) and quantification of wall components - a fluorometer analysis using the dyes calcofluor white, aniline blue and concanavalin A (to quantify chitin, β-glucan and mannan, respectively) pre and post exposure to FLC in solidified medium (YPD + 1 µg/mL FLC). Following exposure to FLC, RC showed the lowest porosity (51.15%), compared to C (62.14%) and Parental (64.85%) (p<0.05). Analyses of cell wall components, revealed that all three morphotypes exhibited decreased chitin content following exposure to FLC, however, the decrease was significantly lower for the RC (63.83±11.55), which maintained a chitin content twice as high as for Parental (33.77±6.05) and C (31.87±9.105). No changes were observed for the amount of β-glucan and mannan. Interestingly, in the membrane integrity data, an increase in the number of damaged cells was observed in Parental, but also in the population of cells with intact plasma membrane tested at the concentration of 0.125 µg/ml of FLC. A similar behavior was observed in RC and C, which showed an increase in damage, but also in the number of cells with preserved membrane in the presence of 2 µg/mL of FLC, showing an adaptive character of the yeast to the antifungal compared to control without FLC. Thus, it is concluded that the PS affects the structure and composition of the cell wall, in addition to promoting changes at the membrane level, which may be a determining factor for resistance in *Ct*.

Keywords: *Candida tropicalis*, fluconazole, cell wall, plasmatic membrane.

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