

**TITLE:** Prolonged exposure to fluconazole promotes morphological alterations in *Candida tropicalis*.

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**ABSTRACT:**

*Candida tropicalis* (*Ct*) is the second most frequent species of the *Candida* genus, being one of the most recurrent in terms of resistance to fluconazole (FLC). The development of FLC resistance is associated to the use of this fungistatic agent. The complex epidemiological context of *Ct* is due to the range of yeast virulence factors, including phenotypic switching. This event promotes reversible changes in colony morphology, generating variability in isogenic populations, which can originate strains with altered sensitivity to antifungals. The present study aimed to evaluate the response of a clinical isolate of *Ct* exposed to FLC for morphological variation and associated FLC sensitivity. Cells of *Ct* were inoculated in RPMI-1640 medium supplemented with a sub-inhibitory concentration of FLC (0.0625 µg/mL) and incubated for 10 days. Morphological analysis was carried out after cells plating from the 10th day of exposure, where 4500 CFU were counted to analyze frequency of change and reversibility. Distinct morphological patterns were isolated and categorized for sensitivity to fluconazole via broth microdilution and filamentation ability via cell count. Prolonged exposure to FLC allowed fungal growth over the 10 days of treatment. After plating the 10th day aliquot, there was a predominance of three morphological patterns, described in 3 groups: Smooth, Star and Micro, suggesting that exposure to the antifungal promoted a macrostructural change, that was not observed for the control (RPMI without the addition of FLC). The frequencies of altered morphological patterns were 1.8% for Star, 34% for Smooth (morphological pattern described for the clinical isolate pre-exposure), and 64.2% for Micro. Further analyses revealed that Star and Micro morphologies had reversibility rates of  $7.14 \times 10^{-2}$  for Star and  $1.14 \times 10^{-1}$  for Micro. The restoring to the original phenotype (smooth) by the revertants suggests the occurrence of phenotypic switching. Regarding the presence of filamentous forms, Smooth colony-type presented 0.29%, Star colony-type showed 3.78% and Micro colony-type presented 2.96% ( $p < 0.05$ ). Concerning sensitivity to FLC, Smooth presented MIC<sub>80</sub> = 0.125 µg/mL, Star 0.0625 µg/mL and Micro 0.125 µg/mL. Our data suggest that prolonged exposure to fluconazole may promote phenotypic switching and the raise of morphological variants with altered sensitivity to fluconazole and morphogenesis abilities in *Ct*.

**Keywords:** *Candida tropicalis*, fluconazole, morphology, stress.

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