Title: Effect of *Candida tropicalis* in planktonic and biofilm form on urinary epithelial cells

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Abstract

*Candida tropicalis* has been reported to be one of the *Candida* species which is most likely to cause bloodstream and urinary tract infections in hospitals being responsible for a high rate of patients’ mortality. Adhesion to host surfaces (epithelial cells and medical devices), as well as biofilm formation, are considered the first step to initiate *Candida* infection. Hence, the colonization of indwelling devices like urinary catheters by *C. tropicalis* poses a critical problem. Therefore, more knowledge has to be acquired in order to understand and prevent the formation of these biofilm infections. AIM: The aim of this study was to investigate the influence of *C. tropicalis* growth form (planktonic or biofilm) in its adhesion to TCC-SUP cells (human urinary bladder). MATERIALS AND METHODS: This study was conducted with one isolate of *C. tropicalis* obtained from a patient with candiduria admitted to the intensive care unit at the University Hospital in Maringá, Paraná, Brazil and *C. tropicalis* ATCC 750 was also used, as a control. Adhesion assays were performed incubating one silicone cupon with pre-formed *C. tropicalis* 24h biofilm or 1 ml of *C. tropicalis* cell suspension (1.0 x 10⁷ cells/mL), at 37°C, on a confluent layer of
epithelial cells. The extent of adhesion was evaluated after 2 h of incubation using an adaptation of the crystal violet staining method. Moreover, cell viability was also assessed, after contact with yeasts, either by trypan blue staining and using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) viability assay. Samples were also observed under scanning electron microscopy (SEM). RESULTS: From the results obtained it was possible to verify that, in general, Candida cells adhered to epithelium (Fig 1). Furthermore, the clinical isolate biofilm cells adhered in higher extent than planktonic cells. Nevertheless, comparing both strains, it can be highlighted that the reference strain grown planktonically adhered significantly more (p<0.05) to epithelial cells than <i>C. tropicalis</i> from candiduria, which was confirmed through ultra structure analysis by SEM (Fig. 1). <i>C. tropicalis</i> in biofilm form caused higher epithelial cells death than their planktonic counterparts (Fig. 2). Moreover, epithelial cells showed less metabolic activity when in contact with biofilms. CONCLUSIONS: Thus, it is possible to conclude that <i>C. tropicalis</i> were able to cause more epithelial cell death when in biofilm form. This highlights the importance of biofilm formation, associated to the use of urinary catheters, on <i>C. tropicalis</i> virulence.

![Graph](image1)

**Fig. 1:** (I) <i>C. tropicalis</i> extent of adhesion to TCC-SUP cells after 2 hours of incubation measured by crystal violet staining, expressed as absorbance/cm² (Abs/cm²). BCT750 - <i>C. tropicalis</i> ATCC 750 biofilm; BCT69 - <i>C. tropicalis</i> clinical isolate biofilm; CT750 - <i>C. tropicalis</i> ATCC 750 planktonic; CT69 - <i>C. tropicalis</i>
clinical isolate plantonic. (II) Scanning electron microscopy of yeasts adhered to TCC-SUP:. A) C. tropicalis from candiduria and B) C. tropicalis ATCC 750.

Fig. 2: Death of the cells of the epithelial cell (TCC-SUP). The cell number was quantified by Trypan blue after 2h of incubation to TCC-SUP cells. CEU - epithelial cell TCC-SUP; CS - silicone coupon; BCT750 - C. tropicalis ATCC 750 biofilm; BCT69 - C. tropicalis from candiduria biofilm; CT750 - C. tropicalis ATCC 750; CT69 - C. tropicalis from candiduria.