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TITLE: Candida tropicalis biofilms on catheters: formation and effect on urinary epithelial cells

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ABSTRACT

A substantial proportion of *Candida tropicalis* infections is associated with biofilm formation, especially on urinary catheters. Thus, the aim of this study was to investigate *C. tropicalis* biofilm formation on silicone catheters using artificial urine and its influence on human urinary bladder cells. This study was performed 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. First yeast biofilm was formed dynamically on silicone urinary catheters using artificial urine. Adhesion (2h) and biofilm formation (24h) were quantified by crystal violet (CV) staining, by enumeration of colony forming units (CFU), and observed under scanning electron microscopy (SEM). From the results obtained it was possible to verify that, in general, *C. tropicalis* cells formed biofilm in the entire length of the catheter. It was also observed that the clinical isolate adhered ($3.78x10^3$ CFU/ml) in higher extent than ATCC 750, ($1.49x10^3$ CFU/ml). Additionally, after 24 h *C. tropicalis* ATCC 750 biofilms presented a higher number of cells ($1.77x10^4$ CFU/ml) than the clinical isolate ($7.08x10^3$ CFU/ml) but less biofilm biomass (Abs/cm^2 = 0.33 and 0.48 for ATCC 750 and clinical isolate, respectively).Second the biofilm effect on urinary epithelial cells was performed incubating silicone coupons, with pre-formed yeast biofilms (24-120h) with a confluent layer of epithelial cells. As control, cells were also grown in suspension and incubated with epithelium. After 2 and 24 h of incubation the amount of remaining *Candida* cells was evaluated using an adaptation of the CV staining method. Furthermore, epithelial cell activity was also assessed, using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H - tetrazolium) viability assay. The clinical isolate biofilm adhered in higher extent to epithelial cells than their planktonic counterparts. Nevertheless, the reference strain grown planktonically adhered in significantly higher extent ($p<0.05$) to epithelial cells than the clinical isolate, which was confirmed through ultra structure analysis by SEM. Moreover, epithelial cells showed less metabolic activity when in contact with biofilms. Thus, it is possible to conclude that *C. tropicalis* 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 *C. tropicalis* virulence.