

### 19. The role of *Candida tropicalis* biofilms on human urinary bladder cells

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*Candida tropicalis* has been reported to be one of the *Candida* species which is most likely to cause urinary tract infections in hospitalized patients mainly associated with biofilm formation on catheters.

**Objectives:** The aim of this work was to evaluate the effect of *C. tropicalis*' biofilms, formed on silicone coupons using artificial urine, on human urinary bladder cells. Enzymatic activity (haemolysin and proteinase) of biofilm cells was also assessed.

**Methods:** Two *C. tropicalis* clinical isolates (from candiduria) and a reference strain were used. Biofilms (24-120 h) were formed in artificial urine on silicone coupons, and quantified by crystal violet (CV) staining and colony formation units (CFU). Haemolysin and proteinase activity of biofilm cells were evaluated in agar plates containing blood sheep and bovine serum albumin, respectively. The biofilm effect on urinary epithelial cells was performed incubating the silicone coupons with pre-formed biofilms with a confluent layer of human urinary bladder cells. The extent of adhesion was evaluated after 2h of incubation using the CV staining method; cell viability was evaluated by MTS and cytotoxicity by LDH production.

**Results:** 24h biofilms showed differences among strains, with *C. tropicalis* ATCC 750 presenting a higher number of biofilm cells than the clinical isolates although having lower biofilm biomass. After 48h all strains reached a plateau of viable cells ( $\approx 1 \times 10^6$  CFU/cm<sup>2</sup>). *C. tropicalis* biofilm cells were able to express total haemolytic activity and high proteinase activity. Additionally, *C. tropicalis* biofilms adhered in higher extent to epithelial cells than their planktonic counterparts. Moreover, epithelial cells showed more damage when in contact with biofilms.

**Conclusions:** Thus, it is possible to conclude that *C. tropicalis* were able to cause more epithelial cell damage when in biofilm form, than planktonic cells. This highlights the importance of biofilm formation when associated to the use of urinary catheters, on *C. tropicalis* virulence.