

## 236 Biofilm formation on a microtiter-plate using artificial urine to mimic catheter-associated urinary tract infections

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Catheter-associated urinary tract infections (CAUTIs) are the most frequent nosocomial infections. It is now known that most of the biofilms involved in CAUTIs are polymicrobial, with disease causing (e.g. *E. coli*) and non-disease causing microorganisms (*Delftia tsuruhtensis*, *Achromobacter xylosoxidans*, *Burkholderia fungorum*) frequently co-inhabiting the same catheter. However, little is known about the effect of non-disease causing species on biofilms formation and infection outcome. In here, single-species (*E. coli*, *Delftia tsuruhtensis*, *Achromobacter xylosoxidans*, *Burkholderia fungorum*) biofilms were formed in 96-well microtiter plates in artificial urine medium. Quantification of pure biofilms formation in 96-well microtiter plates was evaluated by crystal violet (CV) staining (for quantification of biomass formed) and by CFU counts (cultivable cells) at selected time points (24h, 48h, 96h and 192h). Experiments were performed in triplicate for each one of the species used. The average absorbances obtained on biofilms using CV method revealed an increase in biomass with time. Moreover, total biomass obtained for *Achromobacter xylosoxidans* was considerably higher compared to *E. coli*, *Delftia tsuruhtensis* and *Burkholderia fungorum* pure culture biofilms. Regarding the quantification of cultivable cells after a sonication step, the variations of the number of cells present in the bacterial biofilms were very small over the time. *E. coli*, *Delftia tsuruhtensis* and *Achromobacter xylosoxidans* pure culture biofilms presented similar values for cultivability ( $\Delta\text{Log } 7.81 - 8.01$  CFUs/ml for 192 h). The *Burkholderia fungorum* pure culture biofilm presented low CFUs values compared to other three species ( $\approx \text{Log } 7.12$  CFUs/ml for 192 h). These results demonstrate that non-disease causing species can form biofilms in conditions mimicking the urinary tract and that they may hence influence the rate at which disease-causing organisms adhere and grow. Future work will attempt to explain which factors are involved in the process that selects a single pathogenic species (e.g. *E. coli*) as prevalent agent and causer of infection from a starting polymicrobial community.

Keywords: CAUTIs, single-species biofilms, 96-well microtiter plates, artificial medium urine, crystal violet, CFU counts.