

TITLE: MICROPLATE ASSAY FOR THE FORMATION OF A MIXED BIOFILM OF *PSEUDOMONAS AERUGINOSA* AND *CANDIDA ALBICANS*

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ABSTRACT: *Candida albicans* and *Pseudomonas aeruginosa* are biofilm-forming pathogens commonly found colonizing central venous catheters and mechanical ventilation devices, being associated mainly with cases of pneumonia and bloodstream infection. The co-infection by these microorganisms presents higher mortality rates when compared to those caused by a single microbial species. Therefore, it is crucial to analyze their interactions. In this work a protocol was developed to study the *in vitro* formation of mixed biofilms of *P. aeruginosa* PAO1 and *C. albicans* SC5314, compared to the monomicrobial biofilm. For the monomicrobial biofilm formation assay, a fungal suspension (1×10^7 CFU / mL) or bacterial (1×10^8 CFU / mL) in RPMI 1640 medium buffered with 0,16M MOPS was dispensed separately into the wells of a microplate of 96-flat bottom wells. For the mixed biofilm formation assay, suspensions of *C. albicans* and *P. aeruginosa* were dispensed into the same well. The microplates were incubated at 37 °C under shaking (150 R.P.M.) for 96h. The biofilm biomass was quantified every 24 h by crystal violet staining (CV) at 590 nm. The polymicrobial biofilm of *C. albicans* and *P. aeruginosa* was robust in 24 h of incubation and remained with the same biomass until 96 h. Similar behavior was observed for the monomicrobial biofilm of *C. albicans*; in contrast, the monomicrobial biofilm of *P. aeruginosa*, under the same culture conditions, showed the lowest biomass in 96 h of incubation. It is known that species of microorganisms can interfere with one another's growth in a co-culture. In our model of polymicrobial biofilm this interaction is likely to occur; therefore, more studies are being conducted in order to understand how these interactions occur and to develop strategies to combat nosocomial infections caused by polymicrobial biofilms of *C. albicans* and *P. aeruginosa*.

KEYWORDS: *Candida albicans*, *Pseudomonas aeruginosa*, Mixed biofilm; polymicrobial biofilm, Nosocomial infections; Microplate Assay

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