Photodynamic Antimicrobial Chemotherapy (PACT) decreases the viability of biofilm produced by Candida albicans

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Candida albicans is an opportunistic fungal able to produces both superficial and systemic infections in immunocompromised patients. It has been reported that biofilms produced by C. albicans are resistant to different antifungal drugs and, that, the infections related to biofilm formation are, frequently, refractory to the conventional treatments. Photodynamic antimicrobial chemotherapy (PACT) is a potential antimicrobial therapy, which combines visible light and a nontoxic dye, known as a photosensitizer, producing ROS, which can kill the treated cells. In this work, we investigate the effects of PACT, using Toluidine blue (TB), as a photosensitizer on 24 hour old biofilms produced by C. albicans. It was observed that PACT, using TB was able to decrease the viability of the 24 hour old biofilms produced, in a TB concentration dependent manner. The inhibition promoted by PACT (0.1 mg/ml TB) was in the order of 30%, 40% and 50% in 24 hour old biofilms submitted to incubation times of 1, 2 and 3 hours, after PACT, respectively. At the same time, the increase in the ROS production was observed after PACT. Our results suggest that the inhibition observed in the viability of the 24 hour old biofilms, by PACT, can be related to the increase in ROS production, increasing the cell permeability and leading to the damage in 24 hour old biofilms produced by C. albicans.

Keywords: Candida albicans; Photodynamic antimicrobial chemotherapy; PACT; biofilm

Polymicrobial biofilms in cystic fibrosis – the role of atypical bacteria in the consortia and impact in antibiotic treatment

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Cystic Fibrosis (CF) is characterized by high rates of morbidity and mortality caused by pulmonary microbial infections. Pseudomonas aeruginosa is typically the prevailing pathogen in the airways of CF patients. However, an emerging and diverse microbial community inhabiting CF lungs has been disclosed, but how it interacts and contributes to the polymicrobial consortia with CF-common pathogens is still to be revealed.

The main goal of this study was to address the behavior of two CF-atypical bacteria, Inquilinus limosus (IL) and Dolosigranulum pigrum (DP), when associated to P. aeruginosa (PA) in variable oxygen conditions and biofilms were thoroughly characterized for biomass, activity, CFU numbers, antibiotic resistance profiles and relative distributions of bacterial populations.

Dual-species consortia were of difficult eradication, with most antibiotics being ineffective in reducing biofilm-bacteria, particularly under low-oxygen atmospheres. Regarding microbial composition, these biofilms presented similar bacterial proportions, whereas P. aeruginosa and D. pigrum dominated the three-species consortia, with I. limosus being the smallest representative population. In general, biofilm compositions changes as a result of antibiotic treatment, with alterations being dependent on the antibiotic, concentration and oxygen condition implemented. P. aeruginosa and I. limosus dual-biofilms exhibited higher antibiotic resistance, with I. limosus persisting and occupying a significant portion together with P. aeruginosa in the overall biofilm after antibiotic treatment. Interestingly, the three-species biofilms displayed higher sensitivity, with P. aeruginosa and P. aeruginosa dominating and I. limosus populations declining in most cases. This suggests that the preponderance of D. pigrum in the biofilm was decisive to decrease I. limosus and lead to an increase in overall sensitivity of the biofilm to a large number of antibiotics. PNA FISH allowed the direct observation of the location and distribution of the three-species species within the biofilms, corroborating the dominance of D. pigrum and P. aeruginosa within the mixed-species consortia and facilitating the understanding of the real complex interactions among the bacterial species.

Data highlighted that emergent species are able to establish polymicrobial consortia with common pathogens in the airways of CF patients, modulating different social activities into those communities and impacting the CF therapeutics.

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Keywords: cystic fibrosis; polymicrobial infection; biofilm; Inquilinus limosus; Dolosigranulum pigrum

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