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 emergent 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 mail 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) under oxygen-atmospheres resembling CF airways. For this, those bacteria were grown in dual- and three-species populations with *P. aeruginosa* 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 changed 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 *D. pigrum* and/or *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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