136 Impact of environments with distinct oxygen availability on biofilm growth and susceptibility patterns of traditional and emerging species in Cystic Fibrosis

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One of the main manifestations of cystic fibrosis (CF) is a decreased clearance of mucus with concomitant bacterial lung infections. Infections are caused by a wide variety of organisms, which includes not only the typical "residents" but also other uncommon bacteria. It has been widely demonstrated that steep oxygen gradients exist within the mucus layers of cystic fibrosis, giving rise to hypoxic/anaerobic areas where bacteria may colonize and proliferate. In this study, it was aimed to investigate the responses of conventional and atypical bacterial species related with CF, when exposed to environments with different oxygen availability, in terms of biofilm growth and antibiotic susceptibility patterns. Single biofilms of the traditional pathogen *Pseudomonas aeruginosa*, and two uncommon pathogenic bacteria, *Inquilinus limosus* and *Dolosigranulum pigrum*, were formed *in vitro* under aerobic, microaerophilic and anaerobic environments in microtiter plates, and their biomass and respiratory activity were further evaluated. The planktonic and biofilm susceptibility patterns were also tested against eight clinically relevant antibiotics under the same conditions, by measuring the minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC), respectively. All organisms showed ability to grow under milieus with distinct oxygen availability, however, *D. pigrum* developed biofilms with a higher amount of biomass and respiratory activity, particularly those formed under microaerophilic conditions. The susceptibility patterns of planktonic cultures revealed antibiotic tolerance of microorganisms under aerobic environments, decreasing their resistance under environments with oxygen depletion. However, MBEC data were significantly higher than MIC values for most antibiotics, revealing that the bactericidal activity was significantly disturbed once biofilms are established. Moreover, the biofilms formed by the atypical species surprisingly exhibited significant multidrug resistance comparing with *P. aeruginosa* biofilms, which was independent of oxygen availability in the environment. This study enabled to conclude that restricted-oxygen atmospheres, as occurs in CF airways, may favor the growth and colonization of other microorganisms that are not conventional, making biofilms more resistant to antibiotics. Thus, CF must be regarded as an environmental habitat where the existence of hypoxic or anaerobic regions affects the ability of the bacteria to allocate, proliferate and resist to antibiotics. A more detailed knowledge on this area might hence be crucial for the success of infection treatment.

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