Role of oxygen-restricted environments in biofilm growth and susceptibility profiles of traditional and atypical bacterial species in cystic fibrosis

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It has been widely demonstrated that different predisposing "local environmental" conditions exist within the cystic fibrosis (CF) airways. Steep oxygen gradients are frequently detected in CF airways and may give rise to hypoxic or even anaerobic zones, providing nutritionally rich growth environments where bacteria may penetrate and accommodate, and being also responsible for the increase of antimicrobial resistance of most microorganisms. The aim of this study was to investigate the influence of aerobic, microaerophilic and anaerobic environments in biofilm growth and susceptibility patterns of the conventional CF-pathogen *P. aeruginosa* and other two atypical species related with CF.

Single biofilms formed by *Pseudomonas aeruginosa*, and two uncommon CF-related bacteria, *Inquilinus limosus* and *Dolosigranulum pigrum*, were formed *in vitro* under environments with distinct oxygen availability, and their biomass and respiratory activity were further evaluated. The planktonic and biofilm susceptibility patterns to eight clinically relevant antibiotics were also determined under the same oxygen conditions, by measuring the minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC), respectively.

The results obtained showed that both bacterial species, the traditional and unusual, were able to grow under the distinct environments, with *D. pigrum* demonstrating a great capability to develop biofilms with higher amount of biomass and higher respiratory activity, particularly when formed under microaerophilic atmospheres. Concerning the susceptibility profiles, all the planktonic cultures showed antibiotic tolerance under aerobic environments, decreasing their resistance under oxygen-restricted environments. As expected, biofilms were notoriously more difficult to eradicate than the same bacteria in the planktonic state (MBECs higher than MICs) which was independent of the oxygen availability within the surrounding environment. Furthermore, the biofilm consortia involving the atypical species were particularly more resistant to most antibiotics than the biofilms formed by the traditional pathogen *P. aeruginosa,* revealing the ineffectiveness of most antibiotics when applied to those atypical biofilms.

This study suggests that the environment where bacteria grow is of great importance, demonstrating that the biofilm formation of traditional and other non-conventional bacteria is favored by restricted-oxygen atmospheres, as occurs in CF airways, which also makes biofilms more resistance to antibiotics. The recognition of CF lung as an environmental habitat, comprising niches ranging from oxic to anoxic, that can be occupied and colonized by a set of different microbial species, could lead to a better understanding of the clinical repercussions that these CF-associated infections can origin and may assist improvements in the management of CF and eventually in the treatment of other pathologies.

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