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CONTROL OF MIXED BIOFILMS WITH BENZALKONIUM CHLORIDE: EFFECT OF DIFFERENT APPLICATION STRATEGIES

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In real world, microorganisms are often associated in complex communities (usually comprising more than one microbial species) and exposed to stress factors that can lead to the development of bacterial resistance and thus to the failure of the traditional sanitation procedures. In mixed biofilms, bacteria may present symbiotic relationships and/or distributions that confer them best conditions to survive. Moreover, in multi-species biofilms, cell-cell events and the production of different polymers may play a significant role in the formation of more cohesive biofilms. The complexity of mixed biofilms may increase the antimicrobial resistance phenomena, raising thus the difficulty in eradicate biofilms. So, the investigation and characterization of mixed cultures in planktonic and sessile state, as well as their responses to antimicrobial products, is of increasing importance for basic research as well as for ecological, medical and biotechnological applications.

The main goal of this study was to evaluate the behaviour of mixed biofilms when facing antimicrobial stress and compare it with the responses obtained with the respective single biofilms. For that purpose different antimicrobial application strategies were implemented.

In this scenario, single and mixed biofilms were developed using Gram+ (*Staphylococcus aureus*) and Gram- (*Pseudomonas aeruginosa*) bacteria from collection. Both types of biofilms were formed in microtiter plates for 24 h in the presence of several concentrations of benzalkonium chloride - BZK (a cationic surfactant with marked biocidal character) being then submitted to BZK attack. Biofilms were characterized in terms of accumulated mass and activity. Single and mixed suspended growth curves with several doses of BZK were also made, in order to establish the minimum inhibitory concentration (MIC) defined as the minimum concentration where no suspended microbial growth was detected.

In planktonic growth, *S. aureus* was the strain that presented higher susceptibility to BZK, emphasised by the low MIC value, possibly due to its gram+ character. However when this bacterium grew together with *P.aeruginosa*, the MIC of BZK increased 5-fold. This evidence suggests that, when developed together, *S. aureus* planktonic growth is favoured by the presence of *P.aeruginosa*. The data obtained with the sessile studies showed that biofilms (single and mixed) were more affected by the presence of BZK during biofilm formation than when it was used to treat the established biofilms. In both situations, BZK efficacy is more notorious in the reduction of biofilm activity that in the reduction of the biomass accumulated. Comparing single and mixed biofilms, binary biofilms seemed to be less susceptible to the action of BZK since biomass and activity reduction were only verified when high concentration of the antimicrobial were used. These results seem to point out that when developed together within a biofilm, bacteria establish favourable microbial relations that gives them additional resistance to the aggressive action of antimicrobials. This can represent a drawback since the favoured physiology of binary biofilms could augment its resistance to sanitation. This study highlights for the need of developing suitable biofilm control strategies based on multispecies biofilms approaches and gives emphasis to the question of the complexity in this type of micro niche.