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EFFECT OF BENZALKONIUM CHLORIDE RESIDUES ON THE INITIAL ADHESION OF ADAPTED *P. AERUGINOSA*

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Bacteria have developed different strategies to sense, respond and adapt to chemical antimicrobial compounds. One of them involves bacterial adhesion to solid surfaces and subsequent biofilm formation. The biofilm lifestyle, that is usually resistant to antimicrobial treatment, is a phenomenon well noticed in almost all industrial and medical arenas. In the latter, the existence of pathogenic bacteria, that easily adhere to a surface and form biofilms, can give rise to equipment contamination and persistent infections. For instance, *Pseudomonas aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. This bacterium, often found in a biofilm, attached to some surface, can frequently cause life-threatening infections under conditions where the host is injured and/or has the immune system compromised.

Benzalkonium chloride (BZK) is a quaternary ammonium compound (QAC) used as a general clinical disinfectant and antiseptic in health care facilities and domestic households. QACs are bacteriostatic at low concentrations and bactericidal at high concentrations, thus their indiscriminate and improper use may favor the development of adaptive resistance. Therefore, they have been pointed out as the possible cause for the selection and persistence of bacterial strains with antibiotic and biocidal resistance.

With this study it was aimed to characterize the adhesion ability and biofilm formation of *P. aeruginosa* non-adapted and adapted to BZK on polyethylene (PET) surfaces non-conditioned and pre-conditioned with BZK solution. For that purpose, bacterial hydrophobicity and changes in surface properties after conditioning were evaluated by means of contact angles determination. The initial bacterial adhesion was followed up along time (2, 4 and 6 h) by the quantification of the adhered biomass, through crystal violet (CV) and respiratory activity, using XTT. The adaptation of *P. aeruginosa* to BZK was attained after 6 cycles of re-inoculation every 48 h selected in TSA also supplemented with increasing doses of BZK.

The free energy of adhesion of the bacteria with the PET surfaces, calculated trough the hydrophobicity components, revealed that the precontioning of the substrata increases the potential initial adhesion of *P.aeruginosa*, being this more significant for the adapted bacteria. Thus, it can be stated that, in the thermodynamic point of view, the initial adhesion of the *P. aeruginosa* adapted and non-adapted to the PET is favoured by surface preconditioning. Moreover, BZK preconditioning did not prevent or impair *P.aeruginosa* adhesion. In fact, the initial adhesion data showed that the accumulation of biomass increased with time, being the non-adapted bacterial cells more able to attach and establish a biofilm. The presence of BZK residues in the adhesion surfaces (preconditioning) promotes the attachment of the bacterial cells, since more biomass was determined. Concerning the activity of the adhered cells, preconditioning seem not cause any disturbance of cells, since equal or higher cellular activity was determined.

The overall results suggest that preconditioning of PET surfaces with BZK promotes the attachment and activity of the bacterial cells, especially of the adapted bacteria. These evidences suggest that when the bacteria were previously exposed to a sub-lethal concentration of a chemical antimicrobial compound, they acquired some insusceptibility to that compound allowing their growth when facing adverse conditions. This study allowed gaining insights about the impact of unsuitable disinfection and rinsing procedures that might instigate the residues deposition, leading to bacteria adaptation, bacterial adhesion and biofilm development.