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Please select a day:

Home - Biofilm

[16.05.2009](#)

[17.05.2009](#)

[18.05.2009](#)

[19.05.2009](#)

[Publication only](#)

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Differences between *Pseudomonas aeruginosa* non-adapted and adapted to benzalkonium chloride: comparison of adhesion capacity, and susceptibility of biofilms to removal and surfactant attack

S.P. Lopes, I. Machado, M.J. Vieira, M.O. Pereira* (Braga, PT)

Bacteria adhesion, and consequent biofilm formation, in vitro and in vivo, are phenomena that often occur naturally but are also bacteria's strategies to protect themselves from stress factors, playing probably an important role in virulence. Furthermore, bacteria growing in biofilms are less susceptible to many antibacterial agents than their suspended counterparts. These factors emphasize the need of suitable and efficient surface disinfection procedures in order to reduce the overgrowth of resistant microorganisms in response to an ineffective course of antimicrobials. In this study, we examined the effect of adaptation of *P. aeruginosa* to a surfactant, benzalkonium chloride (BZK), on the adherence of bacterial cells to PET, conditioned and non-conditioned with BZK, and on their ability to resist to removal and BZK aggression. The assays were carried out in a PPFC. Bacterial adaptation was attained by exposing *P. aeruginosa* to gradual increasing concentrations of BZK, and selected in TSA supplemented with 4.0 mM BZK. The results show that adapted *P. aeruginosa* adhered in a more extent than the non-adapted counterpart. For both strains, the pre-conditioning of the PET surfaces significantly favored bacterial adhesion. The higher adhesion was observed with the adapted bacteria onto the conditioned PET coupons. These results highlight that the extent of adhesion is greater the higher are the stress factors. The strength of adhesion is also higher in the case of adapted bacteria since detachment only occurs with *P. aeruginosa* non-adapted. BZK application did not cause significant removal except for *P. aeruginosa* non-adapted adhered to non-conditioned PET. Nevertheless, BZK attack causes loss of viability of the cells that remained adhered to the surfaces, this loss being more notorious in the case of non adapted cells adhered in the conditioned surfaces.

Based on the results it can be said that the presence of BZK residues on the adhesion surfaces did not impair the bacterial adhesion capacity though affects the viability of the adhered cells. It can also be concluded that resistant bacteria that survived to a simple adaptation step to a common antimicrobial agent increased its adherence ability and insusceptibility to removal and antimicrobial treatment. In a disinfection point of view, these results can represent additional problems for the eradication of pathogenic bacteria with increased virulence from equipment and surfaces in the medical arenas.