

Persisters

In the news Yesterday, on our labs Today – the world together for a safer Tomorrow

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Biofilms can constitute environmental refuges for bacterial pathogens. Embedded in those microbial structures, pathogens can gain adaptive advantages over planktonic bacteria resulting in their environmental persistence. In a clinical arena, the disinfection and rising practices using antimicrobials at sub-MIC can change the environment conditions and represent a selective pressure for bacterial resistance.

In this work, the behavior to disinfection of planktonic and sessile *P.aeruginosa*, non- and adapted to benzalkonium chloride (BZK), was investigated. Adaptation was attained exposing *P. aeruginosa* (ATCC 10145) to TSB containing gradual increasing concentrations of BZK. Adapted bacteria were selected in TSA also supplemented with increasing doses of BZK. Thus, type strain, *P. aeruginosa* adapted in TSB with 0.9 mM BZK (A0.9) and *P. aeruginosa* selected in TSA with 4.0 mM BZK (A4) were used as model bacteria. MIC's and Antibiotic inhibition halos (Ciprofloxacin – 5 µg and Gentamicin – 10 µg) were determined. Biofilms were developed in cell-culture plates, normal and preconditioned with BZK, being after exposed to BZK (1.0 mM) and Ciprofloxacin (1.0 µg/ml) for 30 min. Mass and Biofilm activity were determined in the initial adhesion period (2 h and 6 h) and in a mature biofilm (24 h of growth).

The adaptation of *P. aeruginosa* to BZK was easily attained, after 6 cycles of re-inoculation every 48 h (to reach the stationary phase of growth).

The planktonic data showed that adapted cultures (mainly A4) become less susceptible to BZK and Ciprofloxacin (any MIC was observed). Reduced antibiotic inhibition halos were also noticed suggesting a cross-resistant phenomenon.

Biofilms formed by persisters revealed to be less thick, even losing mass and activity along time, but much more stable when exposed to ciprofloxacin, especially when developed in the presence of BZK residues. Biofilms attack with BZK promoted biomass fixation. The biofilms formed by A4 on preconditioning plates were less sensitive to Ciprofloxacin attack, revealing even an increase in biofilm mass and biofilm activity when compared to A4 biofilms without antibiotic attack.

This study shows that the presence of BZK residues in the adhesion surfaces after unsuitable disinfection and rinsing procedures may easily lead to bacteria adaptation and biofilm development. Biofilms, despite being thinner and less active, are much more stable and difficult to eradicate.