P. aeruginosa antimicrobial resistance and cross resistance: the role of sessile growth and induced tolerance

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The bacterial phenotypic evolution due to external pressures has been gradually recognized and several molecular mechanisms can account for the bacterial adaptative responses to antimicrobial compounds. The slow metabolism of biofilm cells and the decreased susceptibility resulting from sub-lethal exposures to antimicrobials can contribute to the arising of resistant microorganisms. So, in this work it is aimed to determine which factor (sessile growth or induced bacterial tolerance) is the main cause of antimicrobial resistance and/or cross-resistance to antibiotics.

Benzalkonium Chloride (BZK) is a quaternary ammonium compound that is widely used as surface disinfectant. Ciprofloxacin (Cip) is an antibiotic prescribed to treat urinary tract infections. Induced resistance was attained by exposing biofilms of P. aeruginosa, developed in glass wool for 5 days, to increasing doses of BZK (0.3 to 0.9 mM) for 6 days. The bacteria used in the experiments were: *P. aeruginosa* from collection (PA) and collected from biofilms (PAB); and *P. aeruginosa* adapted to 0.9 mM BZK in planktonic (PA09) and sessile growth (PAB09). The Minimal inhibitory concentration (MIC) for all the strains was established by growing the bacteria for 24h in serial dilutions of BZK and Cip, being after plated on TSA. The minimal bactericidal concentration for biofilms (MBC) was attained by growing biofilms in TSB, exposing them to serial dilutions of BZK and Cip for 24h and then allowing biofilm regrowth in fresh media. Released bacteria from those biofilms were platted in TSA, being MBC the doses of the drug that impaired biofilm regrowth.

Cells collected from biofilm (PAB and PAB09) showed a BZK MIC value similar to the value obtained for the PA. However, the MIC value of Cip for the PAB09 was about 2-fold higher. Concerning MBC, biofilms formed by PA had a 3-fold increase in BZK MBC and a 6-fold in Cip MBC, when compared with MIC. The biofilms developed by PAB and PAB09 revealed an MBC similar to the biofilms formed by PA and PA09, both for BZK and CIP.

The MIC and MBC values obtained in this study revealed that the induced tolerance of bacterial cells in planktonic state, promoted by the growth in BZK increasing doses, does not seem to significantly affect resistance to BZK nor cross-resistance to Cip. On the other hand, the biofilm mode of life appears to confer more resistance to bacteria and even cross-resistance to other products, emphasising that the sessile mode of growth has major impact in the development of bacterial insusceptibility to antimicrobial compounds.

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