Ciprofloxacin susceptibility patterns of planktonic and sessile *S. aureus, E. coli*, and *P. aeruginosa* – effect of the exposure time

M. Parente, I. Machado, M. O. Pereira

IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering
University of Minho, Campus de Gualtar, 4710–057 Braga, Portugal.

Several aspects of human infections within a clinical arena are related to biofilm development. Various surfaces, like indwelling devices and medical equipments, are prone to biofilm formation, causing subsequent pathogenesis. The application of antimicrobial agents is one of the main strategies to eradicate biofilms. However, the action of antibiotics could be inefficient duo to the high tolerance of biofilmes comparatively to the bacteria in planktonic state. Thus, it is of upmost importance the use of suitable antimicrobials with high efficacies to eradicate biofilms and to control nosocomial infections. Ciprofloxacin (CIP) is a broad spectrum fluoroquinolone antibiotic, often use against both Gram-positive and Gram-negative bacteria, that causes inhibition of bacterial cell division by the inhibition of DNA gyrase and topoisomerase IV.

With this study it was aimed to analyze the antimicrobial efficacy of CIP against *S. aureus*, *E. coli* K-12, and *P. aeruginosa* PAO1, in planktonic cultures and in biofilm state, and also to characterize the time–kill kinetics of ciprofloxacin in pre-established 1-day-old biofilms.

In planktonic cultures, the CIP susceptibility patterns were achieved by the broth microdilution method and by the determination of the number of viable cells. In sessile bacteria, the anti-biofilm activity of CIP was assessed using a standardized biofilm assay, quantifying the biofilm mass, through crystal violet, the respiratory activity, using the XTT reduction assay, and the number of viable cells. The time-kill kinetics of CIP were attained exposing 24-hour-old biofilms of each bacteria to ciprofloxacin (6 ug/ml) over time (until 24 h), and determining biofilm metabolic activity, biomass and cellular viability at regular time intervals.

Ciprofloxacin displayed dose-dependent activity against both bacteria in planktonic and biofilm states. The MIC values ranged between 0.185 ug/ml for *S. aureus*, 0.5 ug/ml for *E. coli* and 0.75 ug/ml for *P. aeruginosa*. These values revealed that it was needed a 4-fold increase in CIP dose to cause growth inhibition of Gram- bacteria comparatively to the Gram+ strain. The presence of CIP during biofilm formation did not kill totally the biofilm-associated cells neither eradicated the biomass adhered. CIP exhibited only a bacteriostatic effect for all strains studied emphasizing that this antibiotic is more effective on suspended than on biofilm cells.

The application of a constant dose of 6 ug/ml of CIP against pre-formed biofilms revealed an evident time-dependent effect in the antibiotic action, since a gradual reduction of biofilm activity, biomass and cell number occurred over time. However, the time of CIP exposure needed to reduce the biofilm characteristics varied from each bacterial species. In fact, the antibacterial effect was evident after 4 h of exposure of the antibiotic with the *S. aureus*, around 8 h of exposure with the *E. coli* and 6 h of exposure with the *P. aeruginosa* pre-formed biofilms. Once more, ciprofloxacin only presented bacteriostatical activity against all the bacterial biofilms, even when the exposure time reached 24 h.

In the range of experimental concentrations and exposure times tested, CIP didn´t reveal to be effective against bacteria cells neither in the pre-established biofilm nor during the process of biofilm development, in spite of the improved reduction of the metabolic activity, biofilm biomass and in the number of viable cells of *S. aureus*, *E. coli* and *P. aeruginosa* biofilms. Alternative treatments or combination of antibiotic therapies should be studied for the implementation of more effective ways of eradication biofilm-associated infections.

Keywords Biofilms; Planktonic Growth; Fluroquinolones; Ciprofloxacin; Exposure Time; Susceptibility patterns

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