Motility and biofilm formation ability of isolated vs collection *P. aeruginosa*: effect of single and combined antimicrobial application

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Biofilms can be defined as communities of microorganisms attached to a surface. During biofilm development, sessile cells acquire physiological characteristics differentiating them from planktonic cells which include an increased resistance to antimicrobial treatment. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium, considered as the normal bacterial flora of the pharynx, mucous membranes and skin, and is widely investigated for its high incidence in clinical environments and its ability to form strong biofilms. When this organism is isolated from clinical settings, efforts should be made to substantiate its clinical relevance often using a collection strain as control. The current treatment to eradicate *P. aeruginosa* favours the use of empirical antimicrobial combinations, balancing the potential for greater toxicity against the lower emergence of antimicrobial resistance and the greater killing that might be achieved by combination therapies acting synergistically. Studies showed that co-application of antimicrobial agents in some cases allowed improvement of biofilm destruction compared with single drug attack. With this work it is aimed to characterize and compare isolated and collection *P. aeruginosa* regarding its motility and biofilm formation ability and how this is affected by single or combined antimicrobial treatment.

P. aeruginosa from collection (ATCC 10145) (PAC) and *P. aeruginosa* isolated (PAI) from a medical device (endoscope) were used as biofilm producers. Biofilms were formed for 24 h in 96-well plates, being then non- and treated with 1 mM of Benzalkonium chloride (BZK), 1 µg/mL of Ciprofloxacin (Cip), and a combination of both, for 30 min. BZK is applied as a clinical disinfectant and antiseptic in health care facilities and domestic households and Cip is an antibiotic used to treat urinary tract infections. Crystal Violet (CV) staining together with XTT, were used to assess total attached biomass and respiratory activity, respectively. To evaluate bacterial motility, swimming, swarming, and, twitching assays were performed.

The data revealed that resulting PAI biofilms have less attached biomass and respiratory activity when compared to PAC biofilms. Also it was shown that alone none of the antimicrobial agents selected are effective on biomass clearance. BZK seems to favor biomass accumulation whereas Cip slightly affects only the PAI biofilm. Moreover, when combined, BZK and Cip seem to improve the detachment of both strains biofilms. As regards respiratory activity, when exposed to Cip attack only the PAI strain activity was affected. On the other hand, BZK have promoted the PA and PAI biofilms activity decrease. Furthermore, the combined action of antimicrobial agents affects both biofilms activity. Regarding bacterial motility, results showed that the motility effects are mainly notorious in PAI, which have revealed larger diameters of the migration zones in all the assays. The flat colony suggests that these strain spread mostly by swimming motility.

This study allowed understanding that the combination of two antimicrobial agents might not be an advantage in what concerns to *P. aeruginosa* biofilm removal, and thus to surface disinfection. Comparing both strains, it can be concluded that PAI have less ability to attach the surfaces and to develop biofilm than PAC. Taking into account that PAI is constantly under stress conditions provided by the exposition on its natural environment, this isolated strain probably developed resistance mechanisms that may led to phenotypic changes at locomotive appendix level (flagella and pili). These cell surface structures where shown to play an important role in the early events of biofilm development in a wide variety of surfaces, namely by making possible the flagellum-mediated swimming movement and further *P. aeruginosa* surface contact.

Keywords: Biofilm; *P. aeruginosa*; Antimicrobial agents; Motility, Locomotive appendixes

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