

The use of bacteriophages against *P. aeruginosa* biofilms

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P. aeruginosa is a relevant opportunistic pathogen involved in nosocomial infections and, worryingly, it frequently shows a low antibiotic susceptibility. One of its virulence factors is associated with the ability to adhere to surfaces and form virulent biofilms. This work describes the use of newly isolated broad host lytic phages (T7-like), capable to infect antibiotic resistant *P. aeruginosa* strains, to control planktonic and biofilm cells. Furthermore, it characterizes phage-resistant mutants phenotypes which arose after biofilm treatment with phages. Two of the phages used (phages phiIBB-PAA2 and phiIBB-PAP21) caused a great biomass reduction of planktonic cells, and when tested against 24 hours old biofilms, using a MOI of 1, reduced already after 2 hours of infection the amounts of viable cells by approximately 1 - 2 log and, this reduction was further enhanced after 6 hours of biofilm infection. However, *P. aeruginosa* PAO1, after 24 hours in contact with phiIBB-PAP21, acquired resistance to the phage which led to an increase in the amount of biofilm cells. Conversely, after 24 hours, phage phiIB-PAA2 for *P. aeruginosa* ATCC10145 continued to destroy cells. Based on morphological analysis of the colonies, five different *P. aeruginosa* phenotypes were isolated, after 72 hours of *P. aeruginosa* ATCC 10145 biofilm infection with phage phiIBB-PAA2, and only one remained susceptible to the phage. Furthermore, all phage-resistant phenotypes revealed a reduced ability to form biofilms resulting in lower amounts of biomass and viable cells in 24 hours old biofilms and, after 48 hours of biofilm formation, 3 out of these 5 strains continued to form less biofilm compared to the parental strain suggesting that these strains have an attenuated virulence. Overall, the phages used in this work showed a great capacity in infecting a wide number of antibiotic resistant strains and also control biofilms. Nevertheless, we found that phage-resistant phenotypes can arise quickly after exposure of biofilms to phages.