221 Combined antibiotic-phage therapies to control *Pseudomonas aeruginosa* biofilms

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Pseudomonas aeruginosa is responsible for 65% of mortality in hospitals all over the world. Its prevalence is attributed to factors such as: intrinsic resistance determined by virulence factors; acquired resistance mechanisms that lead to a low susceptibility to antimicrobial agents; and ability to attach to any natural and artificial surfaces and form biofilms. The emergence of new strategies to control P. aeruginosa biofilms is becoming more evident due to their tolerance to traditional treatments and bacteriophages have been recognized as an attractive alternative for this problem. Nevertheless, despite the potential of phages as antimicrobial agents, it is well known that bacteria can quickly adapt and create new survival strategies and the emergence of phage-resistant phenotypes is inevitable. Thus, the combination of phage and antibiotic therapies could have potentially more benefits than just using phages and antibiotics alone. This work describes the combinatory treatment using P. aeruginosa phages and antibiotics (amikacin, ciprofloxacin, piperacillin and tetracycline) against biofilms. The efficacy of phages and antibiotics were evaluated by the enumeration of viable cells and the determination of biofilm biomass. All antibiotics tested showed little efficacy against biofilms which were also very tolerant to phage infection, partially due to the fast emergence of resistant phenotypes with LPS mutations. However, the association of phage and ciprofloxacin caused a 100% biofilm removal. Synergy was observed with a combined phage-ciprofloxacin treatment, and overall an additive effect was seen with piperacillin and tetracyclin. Antagonism was observed with amikacin. Nevertheless, all antibiotic-phage combined treatments caused the disruption of the biofilm matrix. The effectiveness of combined treatments with phages and antibiotics can be due to several factors, namely high burst sizes in cells exposed to antibiotics (ex. piperacillin), lower MIC values and altered surface charges of phage resistant phenotypes and disruption of the biofilm matrix induced by some of the phages which can enhance the antibiotic penetration.
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