**Challenging mono and dual-species biofilms with phages and antibiotics**

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# Abstract

Pseudomonas aeruginosa is a relevant opportunistic pathogen, and worryingly it frequently shows a low antibiotic susceptibility. This bacterium is responsible for 65% of mortality in hospitals all over the world. One of its virulence factors is associated with the ability to adhere to surfaces and form virulent biofilms. This work describes the results from several years of investigation using P. aeruginosa phages alone and combined with antibiotics or other phages against single and mixedspecies biofilms. The mixed species biofilms of P. aeruginosa reported herein were formed with fungi (Candida albicans) since these two microorganisms co-inhabit a wide variety of environments and the interactions between them can result in huge medical and economic impacts; and with Acinetobacter baumannii also an opportunistic pathogen associated with nosocomial infections. In both mixed species biofilms there was observed an inhibitory effect of P. aeruginosa since the levels of C. albicans and A. baumannii were highly reduced in the presence of P. aeruginosa. In P. aeruginosa - C. albicans biofilms, the Pseudomonas phages were able to attack their host population; however, as soon as the phages had killed P. aeruginosa, the numbers of viable C. albicans cells increased rapidly. Furthermore, C. albicans' morphology and virulence were significantly affected in the presence of P. aeruginosa. In P. aeruginosa – A. baumannii biofilms, phages applied only to one of the hosts decreased, as expected, that specific population already after 6 h. Nevertheless, while after treatment of the mixed species biofilms with P. aeruginosa phages we observed a growth of A. baumannii, the same did not occur when the biofilms were only treated with the A. baumannii phage. The use of both phages was effective and reduced significantly the numbers of viable cells of the mixed population biofilm. Despite the potential of the phages used in this work 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. Indeed, we observed the rapid appearance of Pseudomonas aeruginosa resistant phenotypes following 24h of biofilm contact with phages and those phenotypes exhibited altered LPS structures. Thus, the combination therapy of phages and 4 commonly used antibiotics in the treatment of P. aeruginosa infections was also evaluated. The results obtained proved that certain antibiotics and phages have potentially more benefits and even act synergistically compared to just phages or antibiotics alone.

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