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Fast emergence of phage-resistant *Pseudomonas aeruginosa* biofilm cells in response to the pressure exerted by bacteriophage treatment

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Antibiotic resistance constitutes currently one of the most serious threats to the global public health and it urgently requires new and effective solutions. Bacteriophages are bacterial viruses increasingly recognized as an attractive alternative to the conventional antibiotic therapies. In the present study, the efficacy of phages against *Pseudomonas aeruginosa* PAO1 biofilm and planktonic cell cultures was evaluated over the course of 48 hours. Although significant reductions in the number of viable cells were achieved for both cases, the high adaptation capability of bacteria in response to the selective pressure caused by phage treatment, resulted in the inevitable arising of phage-resistant variants. In most cases, those variants appeared later in planktonic cultures than in biofilms. Given the interest in further understanding their genetic makeup and possible mutations accumulated, some were selected for further phenotypic and genotypic characterization. The complete genomes of five *P. aeruginosa* PAO1 phage-resistant variants were sequenced and all revealed to carry mutations in the *galU* gene, which is involved in lipopolysaccharide core biosynthesis, as well as in one pil gene, which is involved in type IV pilus synthesis. Three of the *P. aeruginosa* PAO1 variants further revealed large deletions (> 200 kbp) in their genomes. Overall the results of this study reveal that the selective pressure caused by phages while targeting biofilms results in a faster emergence of resistance compared to planktonic cultures, probably due to the high genetic diversity of cells within biofilms. Furthermore phage-resistant variants seem to be quite adapted to the biofilm phenotype.