A genotypic analysis of five *P. aeruginosa* strains after biofilm infection by phages targeting different cell surface receptors

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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 being good alternatives to the traditional antibiotic therapies [1]. 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 level of adaptability of the bacteria in response to the selective pressure caused by phage treatment resulted in the emergence of phage-resistant variants. However, very few studies have explored this phenomenon. Here, the emergence of phage-resistant variants was tracked during the phage infection experiments. Resistant bacterial variants appeared as early as 6 hours post-phage biofilm treatment, depending on the phage used and the respective bacterial receptors. It was also found that phage-resistant variants appeared later in planktonic cultures than in biofilms, in most cases. Given the interest in further understanding the genetic makeup of these variants and possible mutations accumulated, some were selected for further phenotypic and genotypic characterization Whole genome sequencing was performed on five *P. aeruginosa* PAO1 phage-resistant variants and all revealed to carry mutations in the *galU* gene, which is involved in lipopolysaccharide core biosynthesis, as well as in one *pil* gene involved in the synthesis of type IV pilus. The sequencing analysis further revealed that three of the *P. aeruginosa* PAO1 variants carry large deletions (> 200 kbp) in their genomes. Overall, this work demonstrates that *P. aeruginosa* biofilms can survive phage attack and develop phage-resistant variants that are well adapted to the biofilm mode of growth.

References

[1] Pires DP, Vilas Boas D, Sillankorva S, Azeredo J, Phage Therapy: a step forward in the treatment of *Pseudomonas aeruginosa* infections, *Journal of Virology* 89(15), 7449-7456, 2015.