PHAGE THERAPY AGAINST *PSEUDOMONAS AERUGINOSA*: THE NEXT MEDICAL REVOLUTION

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What is *Pseudomonas aeruginosa?*

- *Pseudomonas aeruginosa* is a Gram-negative bacteria which is responsible for 10% of all **hospital-acquired infections** and frequently affects cystic fibrosis and HIV patients.
- The severity of its infections is due to its intrinsic ability to **develop resistance** to any antibiotic and to the **formation of biofilms**, which protect the colony from the environment.
- It has been classified as a **'Priority 1'** pathogen in the development of new antimicrobials by the World Health Organisation [1].

What is Phage Therapy?

- Bacteriophages (phages) are viruses that specifically target bacteria, and phage therapy refers to the use of **lytic phages** (**Fig. 1**) to treat **bacterial infections**.
- This field commenced on the beginning of the twentieth century, but was abandoned due to the advent of antibiotics. Now, the lack of effective treatments against *P. aeruginosa* has redirected the attention to this therapeutic approach.
- The **aim of this review** is to compare the three main phage therapy modalities and discuss the current status of this technique in the European legislation.

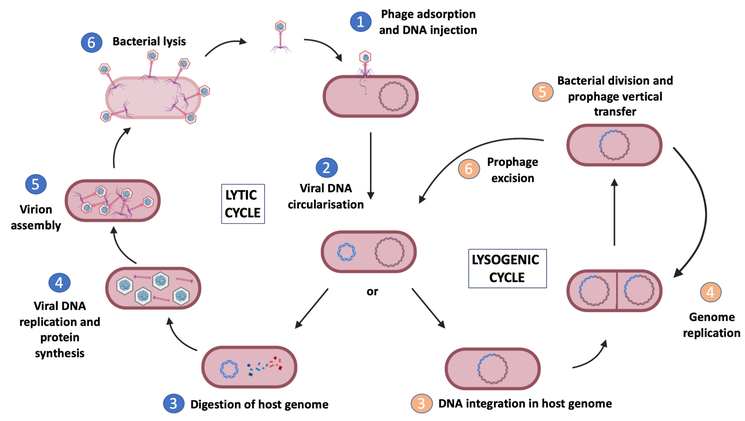


Figure 1: The lytic and lysogenic phage cycles.

Main Phage Therapy Modalities

1. Single Phage Therapy

Also called monophage therapy, it is the use of **a sole viral agent** against the strain causing the infection. It has been predominantly conducted as a **proof-of-concept** to evaluate relevant parameters of phage administration.

✓ The **administration** of phages is **safe** and can be used to clear infections in animal models. On the other hand, single phages fail to remove bacterial populations *in vitro*, suggesting a key role of the **immune system.**

✗ Monophage therapy **depends on the specificity** of the phage towards the pathogenic strain.

Directed in vitro viral evolution methodologies have been developed to obtain a precise match between both agents [2].

✗ Like antibiotics, single phages only profit from one cellular pathway, so bacterial populations can easily **become resistant**.

Polyphage therapy approaches can be used to minimise the appearance of resistant clones.

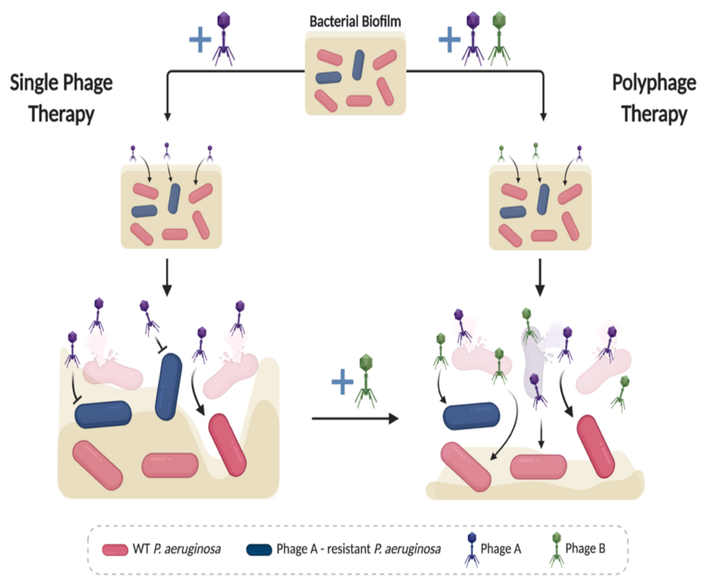


Figure 2: Monophage therapy versus polyphage approaches. The efficiency of a single phage (left) is hindered by the presence of resistant clones, represented in the same colour as the phage to which they show resistance. As a consequence, the biofilm clearance is limited. On the other hand, the administration of different viral agents provides with a broader host range, enhancing and the reduction of the biofilm and the bacterial load (bottom right). In this scenario, phages can be administered in a simultaneous or a sequential fashion.

2. Polyphage Therapy

Polyphage therapy is the **combination of two or more phages** with different properties and host ranges in a single suspension (**cocktail**).

✓ **Cocktails** designed on the basis of host range, genomic information or individual *in vitro* efficiencies result in a **more acute reduction of the bacterial load** in comparison with **each phage alone** [3].

✓ The **lysing efficiency is higher** when phages are administered **sequentially**, rather than **simultaneously**, due to potential antagonistic interactions between them.

✗ **Sequential** polyphage therapy is not yet viable because it requires a high level of **personalised medicine** and **regular visits to ambulatory centres** to receive the different doses.

Simultaneous therapy is currently the best option to rapidly clear acute infections, while sequential strategies could be used in prophylactic treatments.

3. Phage-Antibiotic Complementation

The combination of phages and antibiotics is based on the idea that the **interaction between both agents** can result in a combined **therapeutic outcome greater than the addition of their individual effects**.

The **order of the administration** of the phages and antibiotics is crucial to achieve a positive complementation.

- In simultaneous approaches, antibiotics reduce the population density to an extent in which phages cannot replicate as efficiently.
- Sequential modalities where phages are introduced first and antibiotics in second place achieve better lysing efficiencies.

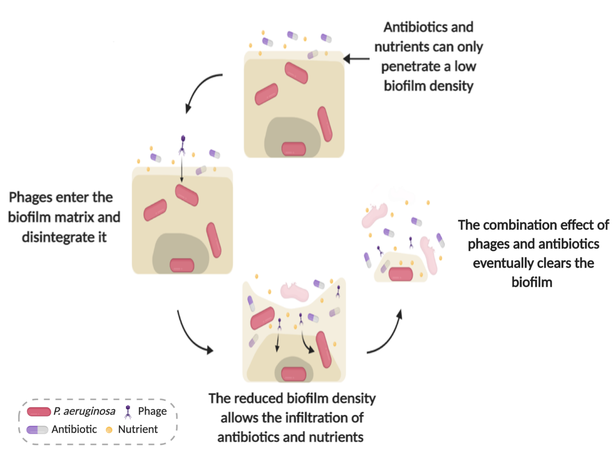
Regardless of the modality, the joint application of phage and antibiotics performs **better than phage-only approaches** [4].

The **selection of both agents** is also important and must be done carefully to achieve a positive complementation. Some antibiotics strongly bind to the extracellular biofilm matrix and are therefore unsuitable for this strategy.

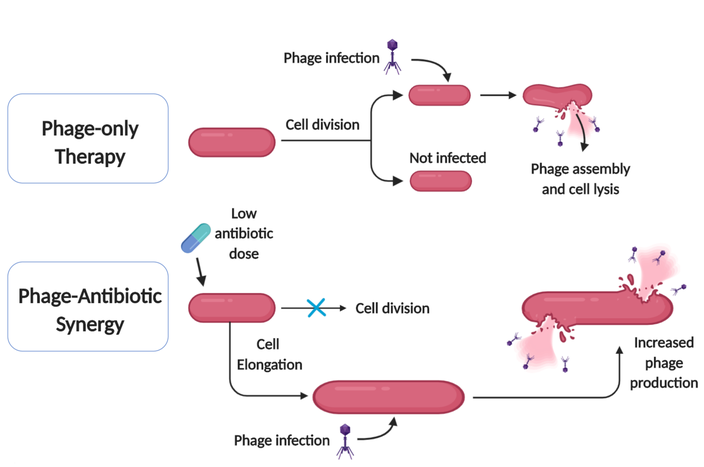
Phage-Antibiotic Complementation has barely been tested in animal models, so there are **not enough *in vivo* evidence** to confirm these observations.

There are **three main ways** in which phages and antibiotics can result in an additive complementation.

1 Enhanced Biofilm Penetration



2 Phage-Antibiotic Synergy



3 Genetic Trade-Off

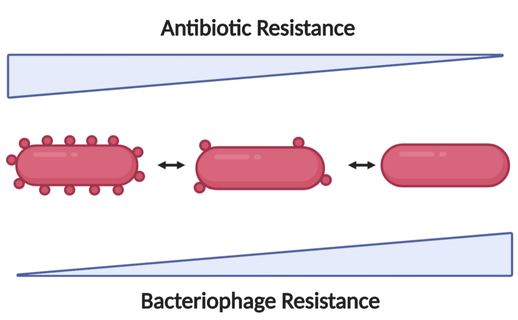


Figure 3: Three main mechanisms of Phage-Antibiotic Complementation.

1) Phages can produce enzymes to disintegrate the biofilm extracellular matrix, exposing to antibiotics and nutrients previously embedded cells. The resumption of their metabolic activity turns them into a target for phages and antibiotics.

2) The bacterial production of phages can be significantly enhanced by sublethal concentrations of certain antimicrobials, in what it is known as Phage-Antibiotic Synergy (PAS). Low doses of antibiotics can arrest bacterial division, leading to cell elongation or filamentation. This altered morphology concentrates the supplies required for bacteriophage assembly and thus improve its efficiency.

3) The selective pressure imposed by the phages can lead to a genetic trade-off. In this scenario, the presence of efflux pumps in the bacterial membrane confers antibiotic resistance, but serves as an entry point for the phages. Conversely, the loss of these proteins makes the cell susceptible resistant to phages but susceptible to antibiotics. *Source: Adapted from [5]*

Regulatory Hurdles in the Consolidation of Phage Therapy

In Europe, bacteriophages are classified as **Investigational Medicinal Products (IPM)**, which equals them to classical pharmaceuticals [7]. As a consequence, they need:

1) To successfully pass through the Phase I, II and III of **human clinical trials**.
2) To be produced under **Good Manufacturing Practice (GMP)** guidelines. This entails that the final phage stock would need to have a label indicating detailed characteristics of the virus, such as its whole genomic sequence.

Phages, unlike antibiotics, **cannot be individually characterised** in detail because they can differ greatly between members of the same species regarding host range, mode of action and stability.

As they are public domain, phages are protected from a very limited form of **Intellectual Property Rights**, which hinders investment from the private sector.

The **main consequence of the legal limbo** of phage therapy is a negative feedback loop.

- The private sector does not invest because the current legislation is too rigid.
- The sanitary authorities are not incentivised to legislate on phage therapy due to the lack of research and phage products.

Concluding Remarks

- **Monophage therapy** has been useful in early experimentation, but is not reliable as a remedy against infections caused by *P. aeruginosa*.
- **Polyphage therapy** ameliorates some of the disadvantages of monophage therapy, but its most potent modality is still unavailable.
- **Phage-antibiotic complementation** represents the most promising strategy, as the additive outcome of both agents can vastly surpass their individual effect.
- To create an incentive to the research on phage therapy, **new dynamic regulatory structures** should be created.

Relevant References

[1] World Health Organization, "Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics," 2017 ; **[2]** B. H. Burrowes, I. J. Molineux, and J. A. Fralick, "Directed In Vitro Evolution of Therapeutic Bacteriophages: The Appelmans Protocol," *Viruses*, vol. 11, no. 3, 2019 ; **[3]** Y. Yang *et al.*, "Development of a Bacteriophage Cocktail to Constrain the Emergence of Phage-Resistant *Pseudomonas aeruginosa*," *Front. Microbiol.*, vol. 11, no. March, pp. 1–12, 2020 ; **[4]** W. N. Chaudhry, J. Concepcion-Acevedo, T. Park, S. Andleeb, J. J. Bull, and B. R. Levin, "Synergy and Order Effects of Antibiotics and Phages in Killing *Pseudomonas aeruginosa* Biofilms," *PLoS One*, vol. 12, no. 1, pp. 1–16, 2017 ; **[5]** B. K. Chan, P. E. Turner, S. Kim, H. R. Mojibian, J. A. Elefteriades, and D. Narayan, "Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*," *Evol. Med. Public Heal.*, vol. 2018, no. 1, pp. 60–66, 2018 ; **[6]** European Commission, "Directive 2001/83/EC," *Off. J. Eur. Communities*, vol. L 269, no. September 2000, pp. 1–15, 2000.