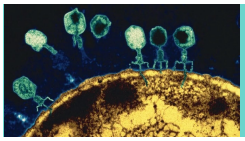

PHAGE THERAPY: THE CURE FOR SUPERBUGS

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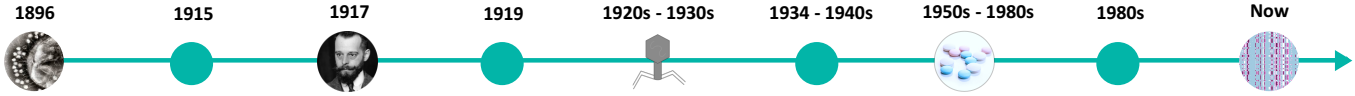
INTRODUCTION

Nowadays, antibiotic-resistant bacteria or superbugs, are one of the biggest problems in the world. The humankind is heading to a "post-antibiotic" era in which common infections and minor injuries can kill again.[1,2] So, as fewer antibiotics are being discovered, suitable alternatives need to be found. The use of lytic bacteriophages, in a process known as phage therapy, is re-emerging as a as potential therapeutic solution for tackling superbugs.

Objective:

The aim of this review is to determine whether phage therapy can be used against superbugs in order to fight a wide-range of human diseases.

TIMELINE OF PHAGE THERAPY HISTORY [3,4]



1896	1915	1917	1919	1920s - 1930s	1934 - 1940s	1950s - 1980s	1980s	Now
Ernest Hankin found lytic activity against pathogens	Frederick Twort discovered the virus of bacteria	Félix d'Hèrelle: starting of the phage era	D'Hèrelle treats dysentery using phages	Phage therapy was a promising treatment	Eaton-Bayne-Jones report about phage therapy	Antibiotic era and declining of phage therapy in western Europe	Rediscovery of phage therapy in western Europe	Progression due to the explosion of genomics and new available data

BACTERIOPHAGES AS ANTIBACTERIAL AGENTS

Phage Biology basics

- Bacteriophages or phages are viruses that infect bacteria and are harmless to eukaryotic cells.
- Phages constitute the largest biomass on Earth.
- They bind to specific receptors and incorporate their genetic material into the bacterial host cell by either a lytic or lysogenic cycle.

Phage therapy: essential steps

Phage therapy involves the use of lytic phages which specifically kill pathogenic bacteria at the site of the infection. There are many essential steps[5,6] involved in the therapy **[Figure 1]** and two typical models of phage choice that can be administered in an **oral, intravenous** or **topic** way:

- **Custom-designed preparations:** the infecting pathogenic bacteria are isolated from the patient and tested against a well characterized phage collection. It is complex and time-consuming.
- **Mainstream-production preparations:** phage cocktails (multiple phages with an *in vitro* efficacy against the target pathogen) are used. The cocktails display a broader spectrum of activity. The first therapeutic phage preparation available for a commercial use will probably be based on this approach.

The therapy success depends on the phage densities reached on the target so as to achieve the desired levels of bacterial eradication.

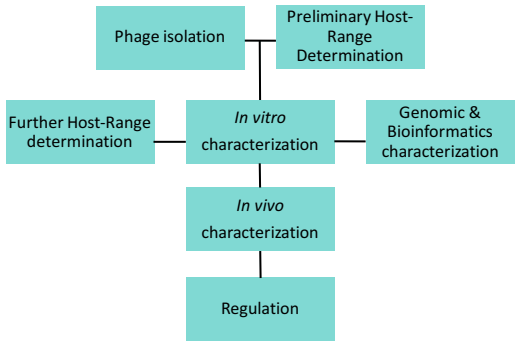


FIGURE 1.- Essential steps involved in phage therapy. In summary, phage isolation is done together with a host-range determination. Purification steps are also essential to avoid carrying toxins. This is followed by an *in vitro* characterization in association with a further host-range determination and an *in silico* characterization. For promising phages, *in vivo* characterization comes next. Finally, regulatory requirements are needed to implement the therapy on a large-scale. [Source: modified from (6) and created with Microsoft® PowerPoint]

ANTIBIOTIC THERAPY *VS* PHAGE THERAPY

Antibiotic and phage therapy are antibacterial treatments that either inhibit bacteria or lysate them. Depending on the situation, phage therapy can be an alternative to antibiotics or either applied together as synergistic therapies. Despite the great number of advantages that phage therapy has **[Table 1]**, there are also certain limitations.[7]

TABLE 1.- Comparison between antibiotic therapy and phage therapy.

ANTIBIOTICS	PHAGE THERAPY
Side effects	Fewer side effects
Unspecific towards the host	Specificity towards the host
No replication at the site of infection	Replication at the site of infection
Drug resistance	Phage resistance
Not an environmentally friendly solution	Environmentally friendly solution

[Created with Microsoft® PowerPoint]

Limitations of phage therapy

- Lack of studies and clinical data.
- Phages are self-limiting and they will only persist as long as their targeted pathogen is present.
- Highly immunogenic and rapid clearance from the bloodstream.
- Narrow host range:
  - Loss of effectiveness ⟶ Improvements with phage cocktails are being tested.
  - Preliminar selection of the phages ⟶ PCR or 16s rRNA sequencing facilitate the rapid and accurate selection of phages.

REGULATORY CHALLENGES

- Phage therapeutic preparations are only available in some countries: Georgia, Poland or Russia.
- The European Medicine agency considers bacteriophages as phage therapy medicinal products (PTMPs).[8]
- Phage therapy has been used publicly for many years and consequently the technique itself could not be patented.
- Difficulty in obtaining funding for research or clinical trials.
- Phagoburn is the first European big clinical trial in Phase I-II.

NOVEL AND FUTURE APPROACHES

ENZYBIOTICS [9]
- Combination between antibiotics and purified enzymes
- Lysins, other natural or synthetic enzymes are used

GENETICALLY MODIFIED PHAGES [10]
- Phages are genetically modified and adapted to a purpose
- Increasing of bacteriophages natural features
- Ultimate goal: to create completely synthetic phages

CONCLUSIONS

The use of phages as antibacterial agents is re-emerging as a solution for overcoming the antibiotic resistance worldwide threat. Further studies are needed for a better understanding on the therapy and its relation with the human host. Moreover, progress in the regulation framework is required before implementing the therapy on a large scale. However, there are still many difficulties and a negative public perception against viruses and its therapeutic use. The available data suggest that novel approaches, such as enzybiotics or genetically modified phages, are promising solutions for improving the future of phage therapy.

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