Bacteriophages: a solution against the increase in antibiotic resistance in bacteria

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INTRODUCTION

The **appearance of resistances** in bacteria is an evolutionary phenomena, although the excessive exposure to antibiotics makes it a **growing problem**[1] . There is a group of bacteria called **ESKAPE**, which comprises *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae species*, that is very problematic in community and hospital settings due to their **resistance to multiple antibiotics**; they have been considered by the Infectious Diseases Society of America (IDSA) as **priority targets** for antimicrobial research[2].

The increase and continuous spread of resistances lead to the necessity of **new compounds** for the treatment of bacterial infections. However, there has been a **lack** of new antibiotics since the 1980s[3]. This fact and the appearance of new antibiotic-resistant bacteria has encourage the search of **new techniques** to solve the problem.

Bacteriophages are proposed as a potential tool for treating infectious diseases as they are bacterial viruses that replicate exponentially until the death of bacteria[4].

Objective: To perform a comparative analysis (critical points of phage therapy and advantages in front of antibiotics) and show some examples of phage therapy.

MATERIALS & METHODS

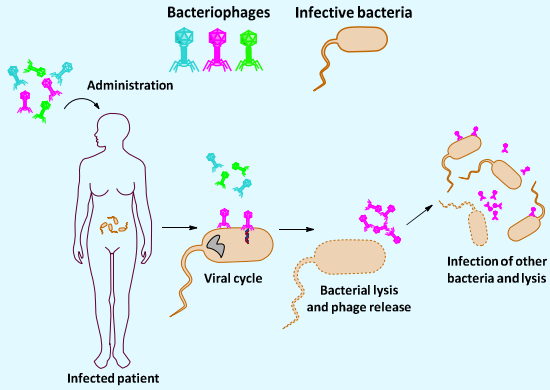

Figure 1: Phage therapy requires the presence of infective bacteria in a patient and bacteriophages that kill this bacterium species. Bacteriophages are administrated to the patient (the way of administration depends on the infection). Bacteriophages arrive to bacteria and start their cycle. They replicate and provoke bacterial lysis. Then, the released bacteriophages can infect other bacteria and repeat the process.

COMPARATIVE ANALYSIS

CRITICAL POINTS OF PHAGE THERAPY

Toxicity

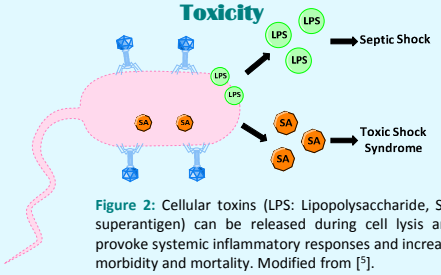

Figure 2: Cellular toxins (LPS: Lipopolysaccharide, SA: superantigen) can be released during cell lysis and provoke systemic inflammatory responses and increase morbidity and mortality. Modified from [5].

Approach to reduce toxicity[5]:

Engineered phages deficient in lysins and/or non-replicative → Reduce endotoxins + Inflammation mediators

Phage choice

1. Individual phage chosen by isolation of infectious pathogen
2. Cocktail of phages[6]

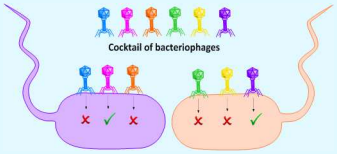

Figure 3: Cocktail of bacteriophages. The use of different phages simultaneously allows targeting a wider number of bacterial species. Modified from [5].

Pharmacokinetics

Critical parameters: absorption rate, burst size, latent period, initial phage dose, density and clearance rate of the phage particles from the body fluids by the reticuloendothelial system (RES) [4].

Solution to the clearance by RES:

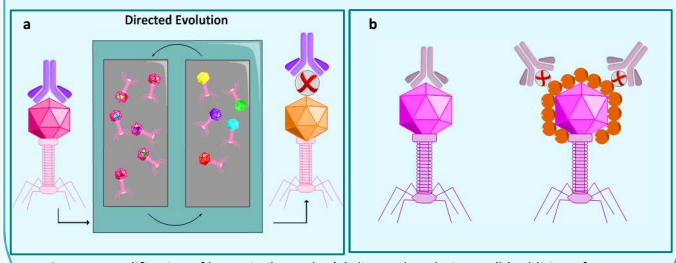

Figure 4: Modification of bacteriophages by (a) directed evolution or (b) addition of a coat, so as to avoid the inactivation and clearance. Modified from [5].

Interaction with non-target tissue

Phages can interact with non-target tissue, although these interactions do not produce side effects[7].

Efficacy

Just few bacteriophages are efficient as therapeutic agents[6].

Bacterial resistances to bacteriophages

Bacterial resistances can appear by many mechanisms
Avoidance: cocktail of phages or phage + antibiotic[6]

Phage manufacturing

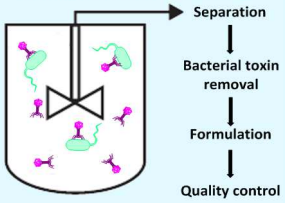

Figure 5: Bacteriophage manufacture has multiple steps: production, purification, formulation, quality control, among others. The process is complicated as the cell lysis releases endotoxins and other cellular toxins and by the need of multi-phage cocktails. Modified from [5].

ADVANTAGES

Table 1: Comparison between antibiotics and bacteriophages

Characteristic	Antibiotics	Bacteriophages
Host specificity[6]	Broad	Narrow
Solved infections[5]	Difficult infections are not solved	Difficult infections: biofilms, persisters, antibiotic resistant bacteria
Side effects[5]	Affect normal microflora	No serious but possible release of endotoxins
Concentration in time[6]	Decrease Eliminated from the body	Self-regulating tools Multiplication in presence of host bacteria Decrease when bacteria are eliminated
Synthesis[6]	Synthetics or semisynthetics	Ecologically pure
Isolation and characterization of new phages[5]	Slower and more expensive	Faster and cheaper

EXAMPLES OF PHAGE THERAPY

Example 1[4]

Bacteria: *S. aureus*
Bacteriophage: ΦMR11
Organism: mice
Result:
- 80% untreated mice died within 24 hours post-infection
- Mice treated instantly after infection were protected.
- Immune response not implied
- Phage disappear after elimination of bacteria

Example 2[5]

Bacteria: *Pseudomonas*
Bacteriophage: cocktail
Infection: chronic otitis
Organism: adult humans
Result: decrease in *Pseudomonas loads*

Example 3[4]

Bacteria: vancomycin-resistant *E. faecium*
Bacteriophage: ENB6
Organism: mouse model
Result: administration between 45 minutes and 5 hours after the infection saved all mice.

Example 4[7]

Bacteria: *P. aeruginosa* & *S. aureus*
Bacteriophage: Pyophage & Sb-1
Infection: respiratory infection
Organism: seven-year old patient
Result: both infections removed

Other example (No ESKAPE)[8]

Bacteria: *Salmonella enterica*
Bacteriophage: cocktail
Organism: chicken and mice
Result: reduction of bacteria was obtained when animals were treated frequently and especially, before the infection of *Salmonella*

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