

BACTERIOPHAGE THERAPY

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ABSTRACT

Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. Lytic bacteriophage phages have been proposed as natural antimicrobial agents to fight bacterial infections in humans, in animals or in crops of agricultural importance. Phages have also been discussed as hygiene measures in food production and hospitals. Bacteriophage therapy has been used for decades, but clinical trials in this field are very few in number, leaving many questions unanswered about its effectiveness for many infectious diseases. As a consequence bacteriophage therapy is not used or accepted in most parts of the world. The increasing need for new antimicrobial therapies is driving the development of bacteriophage therapies for a number of diseases but these require the successful completion of large-scale clinical trials in accordance with US FDA or European EMA guidelines. Bacteriophage therapy is typically an active treatment requiring multiplication in the bacterial host and therefore the factors that govern its success are different from those of conventional antibiotics. From the pharmacokinetic and pharmacodynamic points of view, time of treatment, dosage depending on the site of infection and the composition of the bacteriophage formulation need careful consideration when designing clinical trials. Scientific evidence regarding inflammatory effects, potential for gene transfer and phage resistance, need to be evaluated through such trials.

INTRODUCTION

Bacteriophage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phage therapy involves the use of lytic phages to specifically kill pathogenic bacteria. It is an important alternative to antibiotics in the current era of multidrug-resistant pathogens. Phage therapy has many potential applications in human medicine, Dentistry, Veterinary science, and Agriculture.

BACTERIOPHAGE

Bacteriophages are viruses that infect bacteria. The term bacteriophage is a combination of the word "bacteria" and the Greek word "phagein" meaning to eat. They are the most abundant forms of life on the planet with an estimated population of 10^{30} – 10^{32} virions in the biome.

The major characteristic features of bacteriophage are,

- All bacteriophages contain a head/capsid and tail. Head contains

nucleic acid, covered with a protein sheath. Tail is hollow tube of protein to which tail fibres and tail plates are attached. Approximately 96% of all phages are tailed and they represent the predominant therapeutic phage type.

- The basic symmetries of bacteriophage are binary, icosahedral, helical and filamentous.
- About 96% of bacteriophages contain a double-stranded DNA (dsDNA) genome and the remaining 4% contain genome of single stranded DNA (ssDNA), or single- or double-stranded RNA (ssRNA, dsRNA).
- They are sensitive to UV rays.
- The optimum pH for phage activity is 5-6.
- Optimum temperature :37°C
- Size: 1/40th to 1/100th the size of most bacteria (20 and 200 nm)

- Phages are obligate intracellular parasites of bacteria. They cannot attack cells of more complex organisms mainly because of major differences in cell-surface components. Accordingly, natural phages are not infectious or toxic to humans or other mammals
- Phages have extreme specificity in targeting specific bacteria and bacterial strains. The host range of most phages is relatively narrow.
- Phages are self-limiting, meaning if the bacteria disappear so do the phages. They are also self-replicating at the site of infection and hence required to be administered in a limited number of small doses over a short period of time.
- Phages are safe. They are a normal part of the human environment and are commonly consumed via foods (ground beef, pork sausage, chicken, farmed freshwater fish, oil sardines, raw skim milk, cheese, yogurt, etc) and drinking water.

Classification

The International Committee on Taxonomy of Viruses (ICTV) have classified bacteriophage based on their morphology and nucleic acid genome. Thirteen families are currently recognised of which, eleven families have DNA genome and remaining two families have RNA genomes.

HISTORY

- **In 1896** Ernest Hankin, a British bacteriologist, reported on the presence of marked antibacterial activity (against *Vibrio cholerae*) in the waters of the Ganges and Jumna rivers in India, and he suggested that an unidentified substance (which passed through fine porcelain filters and was heat labile) was responsible for this phenomenon and for limiting the spread of cholera epidemics.
- **In 1915**, Frederick Twort, English bacteriologist from England, discovered a small agent that infected and killed bacteria.
- **In 1917**, Felix d'Herelle, a French-Canadian microbiologist at the Pasteur Institute in Paris recorded the discovery of a microbe that was antagonistic to bacteria and resulted in lysis and bacterial cell death. It was named as bacteriophage.
- **In 1919s**, Felix d'Herelle made first attempt to use bacteriophage

therapeutically in France to treat an epidemic of Chicken typhoid caused by the bacterium *Salmonella gallinarum* and the overall results reported that therapy helped in controlling the epidemic to great extent. He also attempted field trials against bovine hemorrhagic septicaemia in Indochina.

- **In 1920**, Felix d'Herelle made first human trial by treating dysentery in a 12 year old boy in Paris.
- **In 1921**, Richard Bruynoghe and Joseph Maisin used bacteriophages to treat staphylococcal skin disease.
- **In 1925**, Felix d'Herelle treated bubonic plague with bacteriophage and all the patients recovered rapidly.
- **From 1925 to 1930s** Felix d'Herelle used various phage preparations to treat thousand of people in India with Cholera and bubonic plague.
- **In 1934**, a bacteriophage institute was established in Tbilisi, Soviet Georgia. The institute exist today as George Eliava Institute of Bacteriophage, Microbiology & Virology.
- **By 1930- 1940s**, discovery of penicillin hailed the beginning of the **antibiotic era** and phage therapy was largely supplanted across the developed world, with the exception of a number of Eastern bloc countries.
- **In 1952**, The Hirschfeld Institute was founded. The bacteriophage laboratory of the Institute was instrumental in developing and producing phages for the treatment of septicemia, furunculosis, and pulmonary and urinary tract infections and for the prophylaxis or treatment of postoperative and post traumatic infections.
- In 1969, Max Delbrück, Alfred Hershey and Salvador Luria were awarded the Nobel Prize in Physiology and Medicine for their discoveries of the replication of viruses and their genetic structure.
- **Since 1993**, at least 13 companies located in US, Canada, & UK were founded to develop bacteriophage therapeutics
- **In June 2009**, the first regulated randomized, double blind clinical trial was reported in the Journal of Wound Care, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The study was

approved by the FDA as a **Phase I clinical trial**.

- In August 2009, another regulated clinical trial in Western Europe (treatment of ear infections caused by *Pseudomonas aeruginosa*) was reported shortly after in the journal Clinical Otolaryngology. The study concludes that bacteriophage preparations were safe and effective for treatment of chronic ear infections in humans.
- Now, the increasing **incidence of antibiotic-resistant** bacterial strains has stimulated a resurgence in interest into these bacteria-specific viruses.

MECHANISM OF ACTION

Two different of phages are present.

1. Lytic phages
2. Lysogenic phages

Lytic phages follow the lytic infection cycle whereby they multiply in the bacterial cell and lyse the bacterial cell at the end of the cycle to release newly formed phage particles.

Lysogenic phages follow the lysogenic pathway where the phage genome will integrate as part of the host genome, replicate as part of the host genome and stay in dormant state as a prophage for extended periods of time. If the host bacterium encounters adverse environmental conditions the prophage may become activated and turn on the lytic cycle, at the end of which the newly formed phage particles will lyse the host cell.

The following phases can be distinguished in the lytic bacteriophage developmental cycle (Skurniket *al.*, 2006)

1. Adsorption of the phage on the bacterial cell by binding to a specific surface receptors.
 - Receptors: bacterial capsules, flagella, fimbriae, pili, proteins, oligosaccharides or lipopolysaccharides (LPS).
 - Adsorption is mediated by tail fibres.
 - Bacteriophages without tail fibres penetrate the host bacterium by producing polysaccharide degrading enzyme that digest components of bacterial envelope or cell wall.
 - Adsorption causes a conformational change resulting in a contraction of tail sheath and penetration of bacterial cell membrane.
2. Injection of the nucleic acid into the bacterium through the tail tube.

3. Expression of the phage early genes, synthesis of early proteins, most involved in the shutting down of the host bacterium systems and phage genome replication.
4. Replication of the phage genome.
5. Expression of the phage late proteins involved in the formation of new phage particles and lysis of the host bacterium.
6. Assembly of the phage heads and tails and packaging of the genome.
7. Lysis of the host bacterium and release of the new phage progeny.

The ability of the phages to kill the bacterial cells at the end of the infectious cycle is the cornerstone of the idea of using phages as therapeutic agents.

SOURCE OF PHAGES

Phage for a given bacterium can be isolated wherever that bacterium grows, such as in faeces, sewage, soil, hot springs oceans. Water from the Ganges (India) has been found to be a rich source of vibrio phages.

PREREQUISITES FOR PHAGE THERAPY

Levin and Bull (2004) suggested that phage therapy only needs to decrease

the numbers of infecting bacteria to a level where the host defenses can take care of the remaining bacteria. They also wrote that it is difficult to understand why a phage that replicates extremely well in the target bacteria fails when it is used for treatment. Understanding this requires a quantitative appreciation of the dynamics of the phage infection process, especially in vivo. Before attempting phage therapy several, sometimes rather demanding, prerequisites should be met:

1. Phage therapy should not be attempted before the biology of the therapeutic phage is well understood.
2. Phage preparations should meet all the safety requirements; the preparations should be free of bacteria and their components
3. Phage preparations should contain infective phage particles, thus storage of the preparations should be validated.
4. The phage receptor should be known. In a bacterial population of 10^6 - 10^8 bacteria there is a high possibility of spontaneous phage-resistant mutants deficient in the receptor or with an altered receptor.
5. The efficacy of phage therapy should be tested in an animal model. Each phage may behave differently in vivo.

PHARMACOKINETICS/ PHAGEKINETICS (Payne et al.,2003)

- Little detailed research has been performed on the fate of phages in animals and humans
- The pharmacokinetics are complicated due to the self replicating nature of phage.
- Critical parameters that affect phage therapy are the phage adsorption rate, burst size, latent period and initial phage dose, also density-dependent thresholds and associated critical times should be considered. Another critical parameter is the clearance rate of the phage particles from the body fluids by the reticuloendothelial system.
- Addition of antibiotics in parallel with phage may hamper the phage efficacy.

DELIVERY MECHANISMS OF BACTERIOPHAGES (Ryan et al., 2011)

1. Parenteral delivery of bacteriophages

Parenteral delivery of bacteriophages in experimental animal studies has proven to be one of the most popular and successful of all delivery methods for bacteriophages because of the immediate distribution of phages into the systemic circulation. However, recent studies have highlighted that the specific site of administration – intramuscular (IM), subcutaneous (SC) or intraperitoneal (IP) – has a significant influence on the success of phage therapy.

2. Oral delivery of phages

Oral delivery of bacteriophages has proven successful in the treatment of gastrointestinal infections and systemic infections. The main issue with delivery of bacteriophages *via* the oral route is phage stability in the highly acidic and proteolytically active environment of the stomach. Protection of phages from gastric acidity by methods such as polymer microencapsulation may enhance the efficacy of orally administered phages.

3. Local delivery of phages

Local delivery of phages has proven very successful in wound healing. The development of hydrogel and impregnated wound-healing formulations has increased the success rates of phage therapy in topical applications. An example of a successful commercially available product is the Phagebiodermsystem developed by the Eliava Institute in Georgia, which targets *P. aeruginosa*, *S. aureus* and *Streptococcus* spp. This formulation consists of a cocktail of

bacteriophages and antibiotics impregnated in a stabilised hydrogel system for topical application. Recently, local phage therapy for areas other than the skin has also received attention (i.e. otic and oral applications).

4. Topical administration of phages

5. Otic phage administration

6. Dental phage administration

7. Inhalation of bacteriophages

The application of inhalation technologies to phage therapy has been one of the most recent advances within the field. The development of modern inhalation and process technologies has allowed great advances in this field. Recently, nebulisers have been used to deliver bacteriophage solutions.

CLINICAL TRIALS

Phage therapy has been tested in humans, animals and plants, with varying degrees of success, and has also been proposed as a means to decontaminate carcasses and the environment. The first regulated randomized, double blind clinical trial was reported in the Journal of Wound Care in June 2009, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The study was approved by the FDA as a Phase I clinical trial. Study results satisfactorily demonstrated safety of therapeutic application of bacteriophages, however it did not show efficacy. Another regulated clinical trial in Western Europe (treatment of ear infections caused by *Pseudomonas aeruginosa*) was reported in August 2009. The study concluded that bacteriophage preparations were safe and effective for treatment of chronic ear infections in humans. Additionally, there have been numerous animal and other experimental clinical trials evaluating the efficacy of bacteriophages for various diseases, such as infected burns and wounds, and cystic fibrosis associated lung infections etc.

ADVANTAGES OF PHAGE THERAPY

1. Bactericidal agents

Bacteria that have been successfully infected by obligately lytic phages are unable to regain their viability. By contrast, certain antibiotics are bacteriostatic, such as tetracycline, and as a consequence may more readily permit bacterial evolution towards resistance.

Auto “dosing”: Phages during the bacterial-killing process are capable of increasing in number specifically where hosts are located, though with some limitations such as dependence on relatively high bacterial densities.

2. Low inherent toxicity

Phages consist mostly of nucleic acids and proteins, they are inherently nontoxic. However, they can interact with immune systems, at least potentially resulting in harmful immune responses, though there is little evidence that this actually is a concern during phage treatment. Nonetheless, it can be imperative for certain phage therapy protocols to use highly purified phage preparations to prevent anaphylactic responses to bacterial components, such as the endotoxins that can be found in crude phage lysates.

3. Minimal disruption of normal flora

Owing to their host specificity—which can range from an ability to infect only a few strains of a bacterial species to, more rarely, a capacity to infect more than one relatively closely related bacterial genus. Phages only minimally impact health-protecting normal flora bacteria.

4. Narrower potential for inducing resistance

The relatively narrow host range exhibited by most phages limits the number of bacterial types with which selection for specific phage-resistance mechanisms can occur. This contrasts with the substantial fraction of bacteria that can be affected by most chemical antibiotics. In addition, some mutations to resistance negatively impact bacterial fitness or virulence due to loss of pathogenicity-related phage receptors.

5. Lack of cross-resistance with antibiotics

Because phages infect and kill using mechanisms that differ from those of antibiotics, specific antibiotic resistance mechanisms do not translate into mechanisms of phage resistance. Phages consequently can be readily employed to treat antibiotic-resistant infections such as against multi-drug-resistance *Staphylococcus aureus*.

6. Rapid discovery

Phages against many pathogenic bacteria are easily discovered, often from sewage and other waste materials that contain high bacterial concentrations.

7. Formulation and application versatility

Phages, like antibiotics, can be versatile in terms of formulation development, such as being combined with certain antibiotics. They are also versatile in application form, as liquids, creams, impregnated into solids, etc., in addition to being suitable for most routes of administration.

8. Biofilm clearance

Phages have a demonstrated ability to clear at least some biofilms, perhaps owing to a potential to actively penetrate their way into biofilms by lysing one bacterial layer at a time, or due to the display of biofilm exopolymer-degrading depolymerases.

Major advantages of phage therapy over antibiotics

Phage Therapy	Antibiotics
Specificity: Very specific for targeted bacteria; therefore no probability of developing secondary infections.	Target all microorganisms. This affects the microbial balance leading to serious secondary infections.
Phages replicate exclusively at the site of infection and are thus available where they are most needed.	Quickly eliminated from the body, antibiotics do not concentrate at the site of infection.
No side effects have been described for over 90 years of use.	Multiple side effects: intestinal disorders, allergies, and secondary infections (yeast infections).
Resistance: Rare <i>in vivo</i> to targeted bacteria. Phage-resistant bacteria remain susceptible to other phages having same host.	Resistance to antibiotics is not limited to targeted bacteria (Multi-resistant strains)
Relatively rapid process (can be done in weeks) available for selecting new Phages.	Developing new antibiotics is time-consuming, very expensive process and takes several years.

DISADVANTAGES OF BACTERIOPHAGE THERAPY

In phage therapy, the following problems remain to be solved

- Inactivation of administered phages or lysis by a neutralizing antibody and allergic reactions to them.
- Appearance of mutants resistant to phages
- Capture and transfer of bacterial toxin genes by phages.
- Insufficient purity of phage preparations
- Narrow host range of phages

APPLICATIONS OF PHAGE THERAPY

1. Food safety

- Natural, non-toxic, safe and effective means for significantly reducing or eliminating food contamination with pathogens like *Listeria monocytogenes*, *Campylobacter jejuni* (Goodridge, L. and Bisha, B. 2011).

2. Veterinary Applications

- Safe and effective preventive/treatment modality against numerous bacterial infections of animals.
- Some examples include:
 1. *Salmonella* infections in poultry
 2. *Escherichia coli* infections in mice, poultry, calves, piglets, and lambs
 3. *Clostridium difficile* infections in hamsters
 4. *Acinetobacter baumannii* infections in mice
 5. *Pseudomonas aeruginosa* infections in mice
 6. *Staphylococcus aureus* infections in mice and cows.

3. Environmental sanitation

Hospitals and food processing units to kill pathogenic bacteria

4. Human therapeutics

Viable, alternative for the treatment of multi drug resistant pathogenic bacteria.

COMMERCIAL PHAGE -BASED PRODUCTS

Product	Description	Company
AgriPhage™	Targets bacterial spot or bacterial speck on crops, with specific formulations for strains of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> or <i>Pseudomonas syringae</i> pv. <i>Tomato</i>	Omnilytics
BioTector	Animal feed for control of <i>Salmonella</i> in poultry	Cheiljedang Corporation
EcoShield™	Targets <i>Escherichia coli</i> O157:H7 contamination in foods and food processing facilities	Intralytix
FASTPlaque-Response™	Rapid detection of rifampicin resistance in smear-positive sputum specimens containing <i>M. tuberculosis</i>	Biotech Laboratories/Lab21
FASTPlaqueTB™	Rapid detection of <i>Mycobacterium tuberculosis</i> in human sputum samples	Biotech Laboratories/Lab21
ListShield™	Targets <i>Listeria monocytogenes</i> contamination in foods and food processing facilities	Intralytix
LISTEX™ P100	A food processing aid that targets <i>L. monocytogenes</i> strains on food products	EBI Food Safety
MRSA/MSSA Blood culture test	Determining of <i>Staphylococcus aureus</i> methicillin resistance or susceptibility directly from blood cultures	Microphage
MRSA Screening test	Identifies methicillin-resistant <i>Staph. aureus</i> (MRSA) for use in Infection Control programs	Microphage
MicroPhage MRSA/MSSA test	Differentiation of methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) <i>Staph. Aureus</i>	Microphage

SUMMARY

Bacteriophages have been used to control pathogenic bacteria in man and animals with varying degrees of success for over 80 years. They have also been a cornerstone of modern molecular biology and genetics. The recent surge in interest in phage therapy, the successful results in animal models, the shift towards clinical trials and the reducing investment in antibiotics are all indications that this therapy is a viable alternative to antibiotic treatment.

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