# Phage therapy: To combat Antibiotic resistant bacteria

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## Abstract

Centers for Disease Control (CDC) and World Health Organization (WHO) have alert about increasing antimicrobial resistance of pathogen against medications that were once successful for the treatment of infectious diseases. Universal decline in effectiveness of antibiotics is the global health concern. For effective prophylaxis measures to combat bacterial infections, revisiting phage therapy appears to be a constructive replacement. The bacteriophages are successfully used as the therapeutic agent for the treatment of diarrhea and infections caused by *Shigella*, *E.coli*, *Vibrio*, and *Staphylococci* and *Streptococci* respectively. Various clinical trials have established the reliability and dependability of phage cocktails towards bacterial pathogens. Oral application of phages use has found no adverse effects on humans. However, there are various regulatory constraints for the commercialization of phages. High specificity of phages towards their bacterial host and continuous evolution of different receptors by the bacteria during treatment, seeks the preparation of phage cocktails a mandate for medical sciences. However, these phage cocktails are generally not accepted by Food and Drug Administration. Regulatory constrains complicate the preparation and distribution of phage cocktails difficult for pharmaceutical companies who wish to have sole rights over the 'preparation'. Regulatory and patenting frameworks need to be made in accordance with changing needs of the therapeutic world so that many lives can be saved from bacterial infections.

## Introduction

Bacteriophage (or simply phage) is defined as virus that specifically binds to bacterial cell, invading the host machinery and cause subsequent death of the cell. The discovery of bacteriophages can be traced back to 1896, when a British bacteriologist, Ernest Hankin, hypothesized that an unidentified substance that limited the spread of cholera epidemics in the river Ganges and Yamuna in India is a heat labile substance that can pass through fine porcelain filters (Wittebole *et al.* 2014). He claimed that this heat labile substance posses antibacterial activity. In 1898, Gamaleya, a bacteriologist in Russia detected an identical process against *Bacillus subtilis*. However, both of these investigators were not able to further explore their findings until Frederick Twort, observed the similar phenomenon almost after 20 years. Trained English bacteriologist Frederick Twort in 1915 observed that sometimes bacterial colonies were lysed, dissolved and disappeared and this phenomenon could be transmitted from colony to colony. This lysis causing agent was able to pass through porcelain bacterial filters, but was heat labile. From these observations, Twort stated that this lytic agent could be a virus. Another microbiologist Felix d'Herelle from Canada rediscovered the phenomenon in 1917 and coined the termed 'Bacteriophage' which literally means bacteria eater or devour. Every day, about 20-40% of bacteria on sea surface is being killed by the bacteriophages.

These natural predators of bacteria are among the most abundantly found organisms in the biosphere (Ceyssens *et al.* 2010). Structurally, a phage has nucleic acid bound to the viral protein coat. The genomic material of phages can either be double stranded or single stranded RNA or DNA. Morphologically, phages can be filamentous, pleomorphic, and may be tailed. As per the International Committee on Taxonomy of Viruses (ICTV), there are about 10 families of bacteriophages and phages have been classified under caudovirales. Tailed and sessile phages are grouped in caudovirale.

The intracellular, obligate parasites of bacteria having diverse life cycles are called as bacteriophages. Phages can multiply in bacteria following lysogenic life cycle or lytic life cycle (Guttman *et al.* 2004). In lytic life cycle (virulent), a phage enters the bacterial cell taking over the cell's replication mechanism, it multiplies vegetatively making viral proteins and viral DNA, and then lyses (disintegrated host cell) the cell, allowing the new progeny to leave and infect

other cells. Some phages (temperate phages/ avirulent) can grow vegetatively by integrating their genome into host chromosome and replicating with the host for many generations.

**History of Bacteriophages**

Prior to the use of antibiotics, bacteriophages were looked upon as the better option to prevent/or treated bacterial infections. Clinical studies were not carried out so extensively in the US and Western parts of Europe. However, phages carried on with to be the part of therapeutics in Russia and Eastern Europe. In 1919, French troops suffered an epidemic of severe hemorrhagic dysentery. d'Herelle's was able to cure this dysentery through bacterium-free filtrates of the patients' fecal samples mixed and incubated with *Shigella* species isolated from the patients. Through his studies d'Herelle's persuaded the idea that bacteriphage was a living agent not an enzyme (Twort, 1920). This was probably the first instance where bacteriophage was practically used for therapeutic use. Professor Victor-Henri Hutinel, a Pediatrican cured the severe bacterial dysentery of a 12 year old boy by administering him antidysentery phage isolated by d'Herelle's. After few days, the boy recovered. Three more patients of bacterial dysentery were nursed with preparation. They too recovered within 24 h of treatment. Apart from the biotherapeutic potential of bacteriophages, bacteriophage can be successfully used to curb microbial food spoilage. In 2006, a commercially available phage based product called as ListShield$^{TM}$ was used to inhibit the of *Listeria monocytogenes* in poultry and meat product. Phages can be used as a successful way to inhibit food borne bacterial pathogens such as *Campylobacter*, *Listeria*, *Salmonella*, *Lactococcus* , canned food, meat and fish respectively. Recently the technique of phage therapy has moved one step further, which includes treating intracellular infections using phage enzymes like lysins.

**Need of Bacteriophages**

The application of antibiotics to cure bacterial diseases is a common practice. However, long term use of these antibiotics can cause the target bacteria to become less responsive to the antibiotics. Antimicrobial resistance is phenomenon of resistance of pathogen against a medication that was once successfully used to treat that disease. World Health Organization (WHO) has warned about the comprehensive reduction in success rate of antibiotics as the grave universal health concern. Causative agents of a number of bacterial diseases including

methicillin-resistant *S. aureus*, tuberculosis, pneumonia, blood poisoning, gonorrhoea, and foodborne diseases have become difficult to treat with antibiotics. For the immunosupressed patients with low immunity or compromised immunity such as patients suffering from acquired immunodeficiency syndrome (AIDS), patients in intense care units, and transplant recipients, antibiotic resistance is a great concern both in terms of increased mortality rates and high medical costs. Studies reveal that in US, about 2 million illnesses and 23000 deaths occur per annum due to complications by antibiotic resistant infections. Globally, seven lakh people die from antibiotic resistant infections per annum and this death number is likely to reach 10 million by 2050 (Laxminarayan *et al.* 2013). Because of the above reasons, there has been less research for the commercialization of antibiotics. The approval rate of antibiotics for pharmaceutical use by Food and Drug Administration (FDA) in USA has shown a steady decline. From 16 in early 1980s, only 6 new novel pharmaceutical antibiotics were approved in last decade (Luepke *et al*. 2017). When mankind needs new prophylaxis measures to combat bacterial infections, revisiting phage therapy appears to be an effective alternative. Studies report that phage therapy provides many advantages like

(i) Ease of discovery and characterization
(ii) Relative safety
(iii) Low "killing titers"
(iv) Inactivity towards body's normal flora
(v) Low toxicity and minimal side effects
(vi) In situ amplification within the target host cell

**Steps involved in Phage therapy**

**(1) Choose the Correct Phage –** Bacteriophages offer dual therapeutic potential because of its ability to target bacteria and no effects on the normal flora of human body. While choosing the bacteriophage against a certain bacteria, it should be taken into consideration that a bacteriophage can kill only certain specific strains or species of bacteria. Only a few limited numbers of phages from environment can be used as therapeutic phages. Many bacteriophages exhibit the ability to act as a prophage inside bacterium and assist in the horizontal gene transfer (Oyaski & Hatfull 1992). This precludes their use in therapy.

Factors that should be taken care of while choosing a phage for therapeutic efficacy comprise:

- Killing potential
- wide host range
- adsorption kinetics
- efficiency in propagation
- stability during storage
- Penetration ability into encapsulated cells and biofilms
- Ease of purification.

The criteria of phage selection involve isolation of bacteriophage from water sample collected locally which is likely to be contaminated with large quantities of bacteria and bacteriophages. The water samples from sewage and effluent outlets can be used for bacteriophage isolation. The samples taken are applied to the target bacteria. If the bacteriophage sample is able to kill the bacteria, the mixture is centrifuged to collect the phages sample. The collected phage sample is tested for its lysogenic or lytic activity, and vegetatively replicated using cultures of the target bacteria.

**(2) Treatment-** Clinical practices involve oral application or topical application on infected wounds or spread onto surfaces. Rarely, phage can be injected into the bloodstream or lymphatic system. The risks of trace chemical contaminants from the bacteria amplification stage should be avoided at any risks.

**(3) Administration-** Phages can be efficiently turned into consumable pills by freeze-drying without reducing their efficiency. These pills have the shelf lives of 14 months and stability at high temperature 55 °C. Storage in vials in refrigerated form is also possible. Oral administration of phage with an antacid increases the number of phages surviving the passage through the stomach. Topical administration application with gauze that are laid on the area to be treated. Drip therapy can also be used.

**(4) Pharmacology -** In 1940s, phage theraphy as a biocontrol agent against bacteria was highly discarded due to the emergence of antibiotics. But increasing resistance of bacteria towards these antibiotics has turned scientist attention towards ‘pre-antibiotic’ era. Phage therapy is a two step procedure. First step involves the phage penetration to target bacterium cell. This

is followed by killing of bacteria. Both these steps represent phage - environment interactions not only between bacteria and phages but also between phages and human body tissues including normal flora of the body. Pharmacology of phages can be studied under two broad terms: pharmacodynamics and pharmacokinetics. Pharmacokinetics explains the impact of body on a drug, its movement through various body parts and body compartments. Pharmacodynamics, on the other hand, is an explicit description of an effect of drug on the body. With the better understanding of pharmacology, i.e Pharmacokinetics and Pharmacodynamics the success rates of phage therapy can be improved rationally (Abedon and Thomas-Abedon, 2010).

**Clinical trails**

Clinical trials have well established the reliability and dependability of phage cocktails towards bacterial pathogens. In a study by Sarker *et al.* (2015), 'coliphage product' or 'placebo' was oral administrated for four days to children suffering from acute bacterial diarrhea. Clinical and functional test assessment; coliphage and Escherichia colititers and enteropathogens, confirmed the oral safety. Use of oral phages showed no adverse effects. Genome sequencing did not identify the presence of any virulence genes. When taken orally, coliphages revealed a safe gut movement in children, but fall in improving diarrhea outcome. This may be possibly due to very less titers of *E. coli* that may require large phage doses and insufficient phage coverage. More studies need to be focused on *in vivo* interactions between phage and bacterium and the role of *E. coli* phages in treating childhood diarrhea for successful implementation of Phage therapy.

McCallin *et al*. (2018) examined the make-up of a phage cocktail made by a Russian pharmaceutical company, 'Microgen' that has made manufactured to target *Escherichia coli* and *Proteus* infections. Safety testing of the phage cocktail does not reveal the presence of any undesired genes. Result of the study depicts no associated side effects when phage was orally administered. The clinical test of a therapeutic bacteriophage preparation resulted in safe treatment of chronic otitis (Wright *et al.* 2009). No treatment related adverse events were reported.

**Regulatory and safety concerns**

The use of phages for therapeutic use involves various regulatory constraints. One the major issues highlights whether medicinal products of phage therapy medicinal products requires

marketing consent or not. The high specificity of phages towards their bacterial host and continuous evolution of different receptors by the bacteria during treatment, seeks the preparation of phage cocktails a mandate for medical sciences. Also, because a single disease may be caused by multiple bacterial strains which may keep on varying, effective treatment of a disease needs preparation of phage cocktails. However, these phage cocktails are usually declined by Food and Drug Administration (FDA) U.S. FDA should provide some relaxation in regulatory laws on combination drug cocktails so as encourage commercial corporate world to invest resources in this area. Also, European Union (EU) needs to provide relaxation for the novel regulatory framework addressing the licensing of commercial PTMPs (Fauconnier, 2017). Patent issues further complicate the preparation and distribution of these phage cocktails difficult for pharmaceutical companies who wish to have sole rights over the 'preparation'. Regulatory and patenting frameworks need to be made in accordance with changing needs of the therapeutic world so that many lives can be saved from bacterial infections.

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