

REVIEW

Phage therapy: A modern tool to control bacterial infections

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Abstract: The evolution of antibiotic-resistant in bacteria has aggravated curiosity in development of alternative therapy to conventional drugs. One of the emerging drugs that can be used alternative to antibiotics is bacteriophage therapy. The use of living phages in the cure of lethal infectious life threatening diseases caused by Gram positive and Gram negative bacteria has been reported. Another development in the field of bacteriophage therapy is the use of genetically modified and non replicating phages in the treatment of bacterial infection. Genetically engineered bacteriophages can be used as adjuvant along with antibiotic therapy. Phages encoded with lysosomal enzymes are also effectual in the treatment of infectious diseases.

Keywords: Bacteriophage therapy, antibiotic resistance, infectious diseases.

INTRODUCTION

All types of microbial agents including bacteria have the innate ability to protect themselves against naturally occurring antibiotics by exchanging their genetic material with other bacteria. Another approach of acquiring resistance among bacteria against multiple antibiotics is through natural selection phenomena. Bacterial resistance can also be developed by environmental factors (Sahoo *et al.*, 2010). The emergence of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid *et al.*, 2009; Hussain *et al.*, 2011) or through nanotechnology (Naz *et al.*, 2012; Ehsan *et al.*, 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood *et al.*, 2011), or marine microorganisms (Javed *et al.*, 2011). However, now a days, the trend is being changed from synthetic drugs to the natural drugs to control the diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al.*, 2012; Ali *et al.*, 2013; Mallhi *et al.*, 2014; Qadir *et al.*, 2014a; Qadir *et al.*, 2014b; Saleem *et al.*, 2014a), hypoglycaemic (Nisa *et al.*, 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic, spasmolytic, bronchodilator (Janbaz *et al.*, 2013a), antioxidant (Janbaz *et al.*, 2012), anti-diarrheal (Janbaz *et al.*, 2013b) and antimicrobial (Amin *et al.*, 2012; Saleem *et al.*, 2014b) properties. As a natural product, probiotics have been tried as new management tools for the control of different infectious diseases.

Although the workers are trying to get benefits from bacterial derivatives, the humankind is again inflowing in the pre-antibiotic era. To solve the problem of bacterial resistance, there is a requirement of new techniques for therapeutic treatment of resistant bacteria. Among the most credible modern approaches, the most promising technique is bacteriophage therapy or phage therapy. Bacteriophages is a class of viruses that infect bacteria. They are intracellular parasites that multiply inside bacteria and make use of their biosynthetic machinery.

Virology of bacteriophages

There are twelve families of phages which are given in table 1. They may be divided into enveloped and non-enveloped phages. Replication of phage viruses is similar to other viruses. After attachment to a specific receptor in the bacterial cell wall, the phage genome enters the cell. The capsid proteins are usually stripped off and remain outside the cell. After entry into the cell, many phage genomes are degraded and destroyed. Since phage are so prevalent in the environment, bacteria have specific mechanisms to protect themselves against infection with phage - "restriction/ modification" systems which depend on the recognition and destruction of foreign DNA. "Virulent" phages do not integrate their genetic material into the host cell chromosome and kill the host cells (lytic infection). So these viruses may be used for destruction of bacteria for treatment purpose. Whereas "temperate" phages integrate into the host DNA, causing Lysogeny.

History of bacteriophage therapy

Bacteriophages were discovered by British worker Twort in 1915 and independently, by French-Canadian worker d'Hérelle in 1917. Twort did not follow his innovation, while d'Hérelle explored their ability to utilize them for treatment purpose (d'Hérelle, 1917). In the reward of this contribution, he was honored as Professor at Yale

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University, and member of the Pasteur Institute. He became able to develop phage therapy centers in several countries like United States of America, France and Soviet Georgia.



Fig. 1: d'Hérelle

Research on bacteriophage therapy was at its peak between 1920 and 1930 in the United States of America. Eli Lilly & Company manufactured "Staphylo jel", as well as further bacteriophage "jel labeled" preparations, for management of infections by *Streptococcus* and *Bacilli*. Squibb & Sons and Abbott Laboratories also manufactured phage filtrate products. In spite of this early passion for bacteriophage preparations, the discovery of antibiotics led in rejection of bacteriophages as therapeutic agent in USA and Europe. On the other hand, the workers in Soviet Georgia followed a line of investigation and continued progress and even they are working in these days. The appearance of bacterial resistance against presently existing antibacterial drugs has made a dilemma in contemporary medical sciences, predominantly due to the associated increase in immunosuppressed patients (Carlton, 1999).

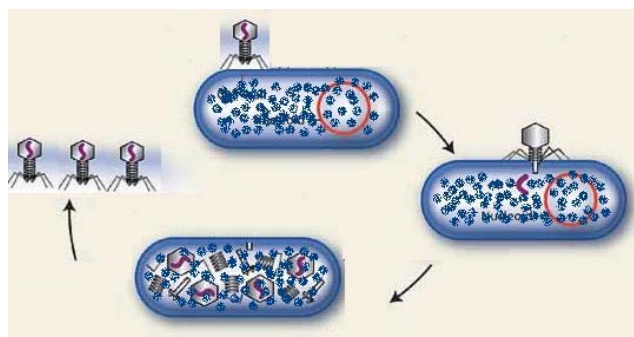


Fig. 2: Mechanism of action of phage-therapy

Case studies

Between 1950 and 1980, nearby a diminutive published work is available on the area under discussion. During this time, papers began to appear representing the usefulness of bacteriophage therapy in animal models. For example, phages were shown to be effective in rescuing rats from fatal systemic infections (induced with *E. coli*) in rescuing calves and lambs from fatal diarrhea (induced

with *E. coli*), in rescuing chicks from fatal diarrhea (induced with *S. typhimurium*), and in preventing destruction of skin grafts in burned rabbits by *Pseudomonas aeruginosa*. Smith and Huggins demonstrated that, in rats inoculated with a lethal intramuscular dose of *E. coli*, a single injection of a phage preparation was more effective than multiple injections of antibiotics (chloramphenicol, tetracycline, etc). This work was replicated in 1996 by Levin and Bull, who used mathematical modeling in a population dynamics approach to study the titers of phages and bacteria in the animals (Levin and Bull, 1996).



Fig. 3: Medi Phage-Phage therapy product by Special Phage Services (Pvt.) Ltd.

Commercial phage preparations

Initially five phage preparations were created against various bacterial infections in commercial laboratory of d'Hérelle in Paris. The preparations were named as Bacté-rhino-phage, Bacté-intesti-phage, Bacté-pyo-phage, Bacté-coli-phage and Bacté-staphy-phage. The Eli Lilly Company produced seven phage preparations against *Escherichia coli*, *Streptococci*, *Staphylococci*, and for other various infectious pathogens for human use. These commercially available phages preparations are sterile bacteriologically manufactured in broth cultures and are produced against specific type of bacteria e.g. Ento-lysate, Neiso-lysate, Colo-lysate and Staphylo-lysate. These preparations are used to treat various lethal infections such as diabetic wounds, infection of upper respiratory track, abscesses, different chronic infections, and many other infections including mastoid infections (Summers, 1999).

Comparison of phages and antibiotics

From a noteworthy point of view, it is resolute that antimicrobial activities of lytic phages have similar effects as of antibiotics. However phages have more therapeutic efficacy than antibiotics in the treatment of infectious diseases in human (Meladze *et al.*, 1982; Sulakvelidze *et al.*, 2001) and in animals. For example, in one study, the

patients suffering from infectious lethal diseases of pleura and lungs (due to *Staphylococcus aureus*) were used as a sample for treatment. The patients were divided into two groups having 223 individuals in Group A and 117 individuals in Group B. Patients of Group A were given bacteriophages and patients of Group B were given antibiotics. The purpose was to determine the effectiveness of bacteriophages over antibiotics. The results were obtained and observed under general condition of the patients. No side effects were observed in individuals that were given bacteriophages and 82% individuals have completely recovered in phage treated group and 64% recovery in antibiotics treated group. Also there is a minimum chance of development of resistance related to bacteriophage therapy as in case of antibiotics (Meladze *et al.*, 1982).

Pharmacokinetics of bacteriophages

It was evident by an experiment on mice that was compromised by a burn wound injury and was given a dose of *Pseudomonas aeruginosa* phage (containing three different *Pseudomonas aeruginosa* phages) by three different routes; intraperitoneal (i.p.), intramuscular (i.m.) and subcutaneous (s.c.) route; suffering with infection of *Pseudomonas aeruginosa* indicating that route of administration is of great importance and imparts in efficacy of treatment. Intraperitoneal route provides 87% protection. The pharmacokinetics of bacteriophages suggests that dose delivered to blood, spleen, and liver by intraperitoneal route were delivered earlier, and were delivered for more sustained period of time than doses given by intramuscular or subcutaneous route (Catherine *et al.*, 2007).

There are large numbers of reports about phage therapy but very few of them could have elaborated and defined the pharmacokinetics of therapeutic phages. Many experiments were performed to evaluate the therapeutic efficacy of bacteriophages but the kinetics of blood clearance have had not been well described (Jumpei *et al.*, 2009). So, for further development in therapeutic efficacy, information on bacteriophages blood kinetics is one of the most important criteria to determine optimal therapeutic approach (Caldwell, 1996; Merrill *et al.*, 2006). After a single oral dose, phages get resorbed and go into blood stream within 2 to 4 hours and to internal organs in time duration of 10 hours (Sulakvelidze *et al.*, 2001). With reference to the data available about phages reveals that phages can reside in human body circulation for very long period of time; as long as for many days (Kochetkova *et al.*, 1989).

After administration, the level of bacteriophages decreased rapidly in first 8 to 12 hours and then gradually decreased and eventually disappeared in three days, depicts a pattern just like of two-compartmental model. The initial phase of rapid decrease from 8 to 12 hours (considered as alpha-phase) is due to distribution of

phages to organs and second phase (considered as beta-phase) is due to elimination of phages (Jumpei *et al.*, 2009).

Table 1: Families of Bacteriophages

Family	Genera	Example
Enveloped phages		
Plasmaviridae	Plasmavirus	Acholeplasma phage
Cystoviridae	Cystovirus	Ø6
Lipothrixviridae	Lipothrixvirus	Thermoproteus phage 1
Non-Enveloped phages		
Inoviridae	Inovirus	Coliphage fd
	Plectrovirus	Acholeplasma phage
Leviviridae	Levivirus	Coliphage MS2
	Allolevirus	Coliphage Qbeta
Microviridae	Microvirus	Coliphage ØX174
	Spirovirus	Spiroplasma phages
Myoviridae		Coliphage T4
Corticoviridae	Corticovirus	PM2
Podoviridae		Coliphage T7
Siphoviridae	Lambda phage group	Coliphage lambda
Sulpholobus shibatae virus	-	SSV-1
Tectiviridae	Tectivirus	Phage PRD1

It was determined that reticuloendothelial system is involved in elimination of bacteriophages from the human body (Jumpei *et al.*, 2009); but the potential net control of innate immunity on elimination of bacteriophages is not evident. The initially discovered bacteriophages that were studied by d'Helle were rapidly cleared and have shorter duration of action. Later studies made the researchers successful in making phages of longer duration of action that are long circulating mutant phages (Vitiello *et al.*, 2005; Capparelli *et al.*, 2006).

Therapeutic phages were assumed to kill their target bacteria by their bactericidal activity by replicating inside the host cell via lytic cycle. On this basis, we can argue that clinical application of phage therapy is preferred due to the extent that they differ in their pharmacokinetics of self-replicating property that is not a characteristic of chemical drugs, small molecules or antibiotics.

Mode of administration

Phages can be given through following routes of administrations (Ryan *et al.*, 2011):

i) Oral route

Phages may be given three times a day before eating. And

a few minutes earlier before the administration of phages; The neutralization of gastric acid is done by oral administration of some “stomach acid neutralizing agent” like sodium bicarbonate or bicarbonated mineral water.

ii) Local route

Phages can also be applied locally by applying moist and phage containing dressings on infected areas.

iii) In form of suspension (Drops)

In this form phages are applied to nasal mucosa, eye and middle ear.

iv) IV Route

Phages have been administered to humans intravenously as well. Phages are also available in form of creams, tampons, aerosols and rinses.

Phage safety

Phages are harmless from clinical point of view. Phages have been administered to humans through different routes as discussed above. Non associated serious side effects are reported with the use of bacteriophages. Phages are present in very large amount in our common non-polluted drinking water and food (as high as 2×10^8 bacteriophage per ml) and we regularly consume phages in our daily diet (Leverentz *et al.*, 2001). But before using phages therapeutically it is compulsory to ensure that phages are safe in every aspect. There are number of modern techniques used recently to improve the bacteriophage therapy.

i) Use of phage lysin

As the resistance against the antimicrobial drug is increasing, it has become necessary to explore & evaluate new techniques and to conduct more research in the field of phage therapy. Researches are moving on to understand the biochemistry and potency of phage lytic enzymes because they are very important in lysis of host cell and believe in the advantages of phage therapy when given in combination with enzymes. However, there is a problem in this combination therapy: the enzymes are very specific in their nature and in lysis of particular species. But the workers are trying both to increase the activity and antibacterial spectrum by production of chimeric enzymes.

ii) Phage therapy in combination with antibiotics

Another valuable approach in the therapeutic use of bacteriophages is to give phage therapy in combination with the antibiotics. A detailed study is required, related to the specific phage and bacterium system behaviour and their specific properties in order to understand the safe use of phage therapy.

iii) Genetically altered phages

Another development in the field of bacteriophage

therapy is the use of genetically modified and non-replicating phages in the treatment of *Helicobacter pylori* and *Pseudomonas aeruginosa* infections.

When phages or antibiotics act on target cell, a side effect occurs in addition to their therapeutic effect, which is the release of endotoxin by the cell wall of gram negative bacteria. These endotoxin induce general pathological procedures e.g. signs of septicemia. To counter this problem, a new and alternative approach has been explored by using genetically engineered non-lytic and non-replicating phage that is encoded with specific protein which is harmful for bacteria to fight with a bacterial infection that result in the release of less endotoxin (Chen *et al.*, 2010).

iv) Engineered bacteriophage as adjuvants

In order to make phage therapy more effective, engineered bacteriophages are designed in such way to potentiate expression of proteins and attack the gene networks of bacteria on which the antibiotics can not target directly. It was studied that the suppression of SOS gene network of *Escherichia coli* by the genetically modified engineered bacteriophage increases the ability of antibiotics e.g. quinolones to act on them more effectively. So in this way phages also act as adjuvants. In addition to quinolones, these genetically altered bacteriophages act as a strong adjuvant for many other antibiotics e.g., β -lactams and aminoglycosides. If engineered bacteriophages act on non-SOS gene networks, it also has proved to be effective antibiotic adjuvant (Lu and collins, 2009).

Problems faced by phage therapy

There are some problems in the use of bacteriophages. These are being discussed here briefly.

i) The clearance of bacteriophages by Reticulo-endothelial system

The clearance of bacteriophages by Reticuloendothelial system that lowers the level of phages to a level which is not enough to provide suitable combating behaviour with the infection is a problematic issue in the use of bacteriophages therapeutically. To solve this problem, the scientists used a special method serial passage based on natural selection phenomena, attenuate the phages and then select the phages having ability to stay in blood circulation for a longer period of time (Sulakvelidze *et al.*, 2001).

ii) The production of neutralizing antibodies

The production of neutralizing antibodies against phages is also a retarding factor in the therapeutic use of phages *in vivo*. After intravenous administration of phages, the production of antibodies is well documented (Stroj *et al.*, 2000). However, after oral and local administration, this effect is not much pronounced. This problem occurs in

chronic infections but in case of acute infections this is not a considerable problem because the rate of action of phages is much faster than the production of neutralizing antibodies.

iii) The development of resistance

The development of resistance in infectious bacteria against certain phage type may also lowers the effectiveness of phages therapeutically, although the rate of developing resistance is 10 folds less than that of antibiotics (Eaton and Jones, 1934). This problem can be countered by using multiple phages in a single preparation.

iv) Intracellular pathogens

In case of infections that are caused by intracellular pathogens e.g. *Salmonella* species in which multiplication of pathogen takes place inside the cell and make pathogens unapproachable by phages. In these types of cases, phages are not found to be effective. So the use of bacteriophages in intracellular infections is limited.

CONCLUSION

In short, bacteriophages have much individuality due to which they are very striking therapeutically active agents. Bacteriophages are very effective in lysis of specific infectious pathogen due to high specificity in their action. They have proved very safe in therapeutic use and are rapidly adaptable to combat with the newly mutant bacterial species. But there is still a need of further extensive research in the field of bacteriophages that will entail to discovery of new techniques against rapidly increasing, resistant and mutant strains of bacteria.

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