Journal of
Advanced Veterinary Research
Volume 2 (2012) 140-147 **Review Article**

Phage Therapy an Effective Remedy Against Drug-Resistant Bugs and Hard to Treat Bacterial Infections-A Review

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(Recieved 2 November 2011/ Accepted 14 January 2012)

Abstract

The injudicious use of antibiotics not only in medicine but also to promote the growth of farm animals has led to the development of antibiotic resistance against many bacterial diseases. One of the remedy against such drug resistant bacterial infections is the application of phage (Bacteriophage) therapy. Phage therapy involves using phages or their products as bioagents for the treatment or prophylaxis of bacterial infections. There are two types of phages based on their type of life cycle: the lytic and the lysogenic phages. Only the lytic phages are used in phage therapy, because of the disadvantages of lysogenic pahges (Superinfection immunity, lysogenic conversion, specialized transduction). Apart from live phages the phage byproducts like phage lysins can also be used specifically against certain bacterial infections. The reports indicate that appropriate administration of living phages can be used to treat lethal infectious diseases caused by bacteria, like *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Salmonella spp*. and *Staphylococcus aureus* etc. In the coming time the phage therapy will compensate for unavoidable complications of antimicrobial therapy, particularly the appearance of multidrug resistance bacteria (super bugs).

Keywords: Phage Therapy; Lytic Phages; Lysogenic Phages; Antibiotic Resistance

Introduction

The worldwide spread of pathogenic bacteria that are resistant to a variety of antibiotics threatens to reduce modern medicine to a state reminiscent of the preantibiotic era. Although novel antibiotics are developed against such resistant bacteria, by spending extensive funds, the pathogens ultimately become resistant to such drugs. To break this vicious cycle, it will be necessary to adopt chemotherapy-independent remedial strategies to combat bacterial infections. Bacteriophages (phages) are the viruses that specifically infect and lyse the bacteria. Phage therapy, a method using phages for the treatment of bacterial infections, was introduced by Felix d'Herelle, who codiscovered phages in about 1920 (Summers, 1999). This discovery occurred about 20 years before practical application of penicillin, the first antibiotic. At the time of its discovery, phage therapy was regarded as a possible treatment method against bacterial infectious diseases (Ho, 2001; Sulakvelidze 2001). Although phage therapy was used to treat and prevent bacterial infectious diseases in the former Soviet Union and Eastern Europe (Slopek, 1987; Alisky, 1998; Weber-Dabrowska, 2000; Chanishvili, 2002), it was abandoned by the West in the 1940s with the arrival of the antibiotic era. However, the ongoing evolution of bacterial multidrug resistance has recently motivated the Western scientific community to reevaluate phage therapy for bacterial infections that are incurable by conventional chemotherapy (Barrow, 1997; Pirisi, 2000; Merrill, 2003).

History

Ernst Hankin in 1896 for the first time reported the presence of marked antibacterial activity (against *Vibrio cholera*) in the waters of Ganga and Jamuna Rivers in India. However, the bacteriophages were

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discovered by Frederick Twort and Felix d'Hérelle in 1915 and 1917, respectively (Shasha et al., 2004). The phage therapy was immediately recognized by many to be a key way forward for the eradication of bacterial infections. George Eliava, from Georgia travelled to the Pasteur Institute in Paris where he met d'Hérelle, and in 1923 he founded the Eliava Institute in Tbilisi, Georgia, devoted to the development of phage therapy. Whilst knowledge was being accumulated regarding the biology of phages and how to use phage cocktails correctly, early uses of phage therapy were often unreliable. When antibiotics were discovered in 1941 and marketed widely in the USA and Europe, Western scientists mostly lost interest in further use and study of phage therapy for some time (Hanlon, 2007). The Russian scientists continued to develop already successful phage therapy to treat the wounds of soldiers in field hospitals. During World War II, the Soviet Union used bacteriophages to treat many soldiers infected with various bacterial diseases e.g. dysentery and gangrene. Russian researchers continued to develop and to refine their treatments and to publish their research and results (Summers, 2001). There is an extensive library and research center at the Eliava Institute in Tbilisi, Georgia. Phage therapy is today a widespread form of treatment in that region. For 80 years Georgian doctors have been treating local people, including babies and newborns, with phages. As a result of the development of antibiotic resistance since the 1950s and an advancement of scientific knowledge, there has been renewed interest worldwide in the ability of phage therapy to eradicate bacterial infections and chronic polymicrobial biofilm, along with other strategies.

Life cycle and mechanism of bacteriolysis of phages

Besides, morphological classification, phages can be divided into roughly two groups according to their life cycle. The "lytic phage," which repeats a cycle in which self-proliferation is synchronous with destruction of bacteria (lytic cycle). The lytic cycle is completed in five steps: The adsorption of the phage to the complementary receptor site on the bacterial cell is first step, it then penetrates into the bacterial host, followed by intracellular development, maturation and finally release of virions from the host cell e.g., KVP20, KVP40, KVP241 and T-even phages. The second type of life cycle the "lysogenic cycle," has a lysogenic cycle in addition to a lytic cycle. In the lysogenic cycle, the phage genome is integrated into the bacterial genome and the phage genome multiplies cooperatively with the host bacteria without destroying it. However, by UV light or certain chemicals or a rare spontaneous event can lead to popping out of phage DNA and initiation of the lytic cycle e.g. fMR11. Bacterial strains that integrate the phage genome into their genome are known as lysogens and they are resistant to infection by phages that are genetically related to previously lysogenized phages. Some lysogenic phages have toxic genes in their genome (Bradbury, 2004; Kaneko, 1997; Yamaguchi, 2000). For these reasons, the lytic phages are thought to be more suitable therapeutic candidates than lysogenic phages.

Disadvantages of Lysogeny

I. Superinfection immunity:

Superinfection immunity means lysogenic cells are immune to reinfection by the same phage. Phage genes c1+,c11+,c111+ are responsible for it, especially c1 which codes for immunity repressor which binds with operator genes OL and OR which govern expression of Cro+ gene that is required for activation of lytic cycle. UV light causes increased levels of RecA protein that cleaves c1+ hence causes reversion to lytic cycle.

II. Lysogenic conversion:

Lysogenic conversion means the host cell may exhibit new properties. For example, Prophage of Corynebacterium diptheriae, Clostridium botulinum, Vibrio cholera, Streptococcus spp. carries genes for their toxin production and hence makes them pathogenic. Similarly colonies of Bacillus megatherium change from smooth to rough by prophage.

III. Specialised transduction:

Specialized transduction means upon excision, viral genome carries with it the adjacent genes from bacterial genome and can transduce them into the bacterial cell that is lacking those genes. It occurs due to unusual excision events. For example, phage

Lambda is a specialized transducing phage for gal and bio genes; phage80 for tryptophan genes and phage P22 is a transducing phage for proline genes in *E. coli.*

Therapy using living phages

Two types of phage therapy have been distinguished: passive (where the initial phage dose removes the pathogen) and active (where the effect is due to the in vivo replication of the phage on the pathogen). Georgian Scientists have been using it for more than 80 years. In the 1980s, Smith and coworkers undertook rigorous investigations into phage therapy for pathogenic *E. coli* infections in a veterinary context (Smith and Huggins, 1982, 1983,1987), thereby reopening this field of research in Western countries. Smith and coworkers showed that a single intramuscular dose of one anti-K1 phage is more effective for treating mice challenged with *E. coli* intramuscularly or intracerebrally than multiple intramuscular doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim plus sulfafurazole. *Staphylococcus aureus* is a pathogen of pyogenic inflammatory diseases, food poisoning, toxic shock syndrome and is also a major causative agent for opportunistic and/or nosocomial infections and often results in high mortality rates (Noble, 1998). More than 50% of clinical *S. aureus* isolates in Japan today carry multidrug resistance and are generally referred to as methicillin-resistant *S. aureus* (MRSA) (Hiramatsu, 2001; Shimada, 2002). Moreover, certain MRSA strains have already acquired low sensitivity or resistance to vancomycin, a unique antibiotic previously considered effective against MRSA, e.g., vancomycin intermediate *S. aureus* (VISA) (Hiramatsu, 1997) or vancomycin-resistant S. aureus (VRSA) (Chang *et al*., 2003; Kacica, 2004). Furthermore, *S. aureus* strains resistant to linezolid, a recently developed novel synthetic antibiotic, are already reported to be present in the United States and Europe (Pillai, 2002). Therefore, possibility of phage therapy was developed for *S. aureus* infectious disease. Recently, a staphylococcal phage (2×10^9) was shown to prevent abscess formation in a rabbit model of wound infection in which it was injected simultaneously with 8×10^7 *S. aureus* cells into a subcutaneous site. This result indicates that phages may be a valuable prophylaxis against staphylococcal infection (Wills, 2005). Furthermore, in hand-wash studies in situ, a phage-enriched wash solution resulted in a 100-fold reduction in staphylococcal numbers on human skin compared with a phage-free wash solution (O'Flaherty, 2005). These results provide strong evidence for the usefulness of living staphylococcal phages as agents for therapy, prophylaxis, and disinfection of *S. aureus* infection. There have been many published reports examining phage efficacy against experimental infections by *E. coli* (Merrill, 1996; Chibani-Chennoufi, 2004), *Pseudomonas aeruginosa* (Soothill, 1992; Ahmad, 2002), *Acinetobacter baumanii* (Soothill, 1992), *Klebsiella pneumonia* (Bogovazova *et al.*, 1991, 1992), *Enterococcus faecium* (vancomycin- resistant strain) (Biswas, 2002), *Vibrio vulnificus* (Cerveny, 2002) and *Salmonella spp*. (Toro, 2005) in animal models.

The effectiveness of phage administration for the control of fish diseases and for food disinfection has also been documented. Nakai et al. (1999) saved the lives of cultured fish challenged by *Lactococcus garvieae* and *Pseudomonas plecoglossicida*, which are fish pathogens (Park *et al*., 2000; Nakai and Park, 2002). Phages were also shown to be effective for the elimination of food poisoning pathogens such as *Listeria monocytogenes* (Leverentz *et al*., 2003, 2004), *Campylobacter jejuni* (Atterbury *et al*., 2003, Goode *et al*., 2003) and *Salmonella spp.* (Leverentz et al., 2001) from the surface of foods.

Phages are currently being used to treat post-burn bacterial infections, which are a major problem for those recovering from the trauma of third-degree burns. Within 24 hours, burn patients can start suffering from opportunistic bacterial attacks. As an alternative to treating post-burn bacterial infections by antibiotics, bacteriophages have been in use in certain parts of the world, such as at Tbilisi in Georgia and in Poland, and this approach has now been more widely recognized. Soothill (1994) demonstrated that use of phages could improve success of skin grafts by reducing the underlying *P. aerugenosa* infections. It has been shown that bacteriophage therapy has an 80% success rate against *Enterococcus* infections and up to 90% against *S. aureus*, *P. aeruginosa*, *E. coli* and *Klebsiella pneumoniae* (Soothill, 1994). *P. aeruginosa* is the most common post-burn infection, and it is known to be notoriously resistant to a variety of antibiotics. For the most effective treatment of post-burn infections, a cocktail of bacteriophages is

sprayed at the site of burns; this will reduce the chance of the bacteria developing resistance against the different bacteriophages. Bacteriophage solutions or aerosols can also be used to treat the surfaces and instruments in operating rooms as well as the skin of the surgical patient (prior to surgery). The bacteriophages can also be used to treat the chronic infections, refractory to the treatment of antibiotic therapy. Antibiotics fail to cure many chronic infections caused due to biofilm formation. Biofilms are complex mixtures of microbes that typically resist the effects of antibiotics formed in chronic infections like: chronic and acute urinary tract infections and cystitis, ear infections (otitis media), chronic sinusitis (rhino sinusitis), skin infections, intestinal infections, prostatitis and associated sexual problems. It is also effective in infections where blood circulation is poor e.g. osteomyelitis, diabetic foot, tropic ulcers, bed sores.

Animal studies

Mice:

In 1980s a careful series of phage therapy experiments in various animals was conducted, which resumed the tradition of the mouse experiments from the early 1940s. The trial was started with a K1 E. coli meningitis mouse model. Low doses of phage, given intramuscularly, protected mice against a massive dose of pathogen applied in the opposite muscle at the same time (Smith and Huggins, 1982). The anti-K1 phage was in vivo more efficient than a large number of antibiotics. Multiplication of the phage occurred in the animal, and phage was disseminated from the site of inoculation into the blood and the spleen, where it was sequestered. However, phage treatment could not be delayed for more than 5 h after the pathogen challenge without loss of activity. Intramuscular phage also protected against intracerebral pathogen challenge. Only phages recognizing the K1 antigen were protective. Phages with high in vitro lytic activity were also the most effective in conferring protection in vivo. The results of Smith and Huggins were reproduced recently (Bull *et al.*, 2002). Human volunteers showed a very similar faecal phage excretion pattern to mice (Bruttin and Brüssow, 2005). More than 10% of the orally applied phage was recovered from the faeces. When the volunteers were put back on phage-free drinking water, faecal phage titres quickly dropped below the threshold of detection when no infective *E. coli* strain was present in the gut.

Chickens:

E. coli causes severe respiratory infections in broiler chickens. In one study, phages were applied by aerosol spraying, followed by injection of 10^4 cfu *E. coli* directly into the thoracic air sac (Huff *et al*., 2002). Aerosol containing 10^7 phage forming units (pfu) of two phages halved the mortality when the challenge was done on the day of phage spraying. When the dose of the phage was increased to 10^8 pfu significant protection against infection was still observed up to 3 days after phage spraying. Another study documented efficacy of phage applied intramuscularly against lethal *E. coli* infections for chickens. When phage and bacteria were given in equal numbers, no morbidity was observed at all in chicken, but 100-fold lower phage titers also conferred significant protection, demonstrating the in-vivo multiplication of the phage. Intramuscularly administered phage also protected against intracranial *E. coli* infection. Phage therapy was even effective when given at the onset of clinical symptoms (Barrow *et al*., 1998).

Calves:

Subsequently, Smith and colleagues infected calves with a natural bovine enteropathogenic *E. coli* strain causing high lethality. Convincing evidence for the efficacy of phage therapy was obtained in an extremely carefully documented series of experiments (Smith and Huggins, 1982, 1983; Smith *et al*., 1987). Diarrhoea could be prevented by phage given 1–8 h after infection. When phage application was delayed until the onset of symptoms, phage had no effect on diarrhoea, but still largely prevented death (Smith and Huggins, 1983). Phage titers increased in the faeces over time, with a concomitant decrease in the enteropathogen counts. In sacrificed animals this observation was confirmed at all anatomical levels of the gastrointestinal tract. Phage counts were 10-fold lower in mucosal scrapings than in the luminal content. Phage was not recovered from blood or spleen. Phage-resistant cells were observed in most of the calves, but their titres generally remained low. Upon reinoculation into new calves, the mutant cells were less competitive

than the parental strain.

Calves held in a room previously occupied by phage-exposed calves could no longer be infected with the enteropathogen, coming close to d'Herelle's initial idea of 'infectious protection' by phages. Also, spraying the litter of the calves in the room with a high or low dose of phage (10^6 pfu.) prevented an infection of the calves with the pathogenic *E. coli* strain, applied either before or after transfer to the phage-inoculated room. When substantial pathogen counts were measured in the faeces, phage appeared with titres 10- to 100-fold higher than the bacterial counts. Phage survived in the room for up to a year and at least 100 days longer than the pathogenic bacteria, and was also more resistant to phenolic disinfectants than the enteropathogen (Smith *et al*., 1987b).

Utilization of phage byproducts to treat bacterial infections

I. Phage Lysin:

Most tailed phages produce peptidoglycan hydrolase (endolysin or lysin) to release their progeny at the final stage of multiplication. Amidase (N-acetyl-muramyl-l-alanine bond), endopeptidase (crosslinking peptide bond), or muramidase or lucosaminidase (sugar chain) may be released, depending on the cutting site (Jado *et al*., 2003). Lysin is able to degrade peptidoglycan even if it is made to react from outside the cell wall (Nelson *et al*., 2001; Loeffler *et al*., 2001; Cheng *et al*., 2005). Although penicillin and cephalosporin antibiotics inhibit peptidoglycan synthesis, lysing the bacterial cell upon cell division, phage lysin destroys the Peptidoglycan directly, exerting a bacteriolytic effect within several seconds of administration. It can also destroy the cell walls of nongrowing bacterium which are insensitive to many antibiotics. The simultaneous administration of two lysins that have different peptidoglycan cutting sites has a synergistic effect (Loeffler *et al.*, 2003; Schuch *et al*., 2002). Interestingly, except for the lysin of an enterococcal phage (Yoong *et al*., 2004), lysin is fairly specific for bacterial species as well as phages themselves, indicating that phage lysin can very likely eliminate the targeted bacteria without disturbing the normal flora.

In vivo efficacy of lysin treatment has been examined using mice challenged by *Streptococcus pyogenes* (Loeffler *et al.*, 2003), *S. pneumoniae*, *Bacillus anthracis*, (Yoong *et al*., 2004) and group B streptococcus (Yoong *et al*., 2004). Lysin treatment was shown to be effective not only against localized infections in the nasal cavity or vagina, but also against systemic infections. Similar results were obtained using a staphylococcal phage lysin.

II. Protein antibiotics:

Some small phages such as fX174 or Qb, which have single stranded DNA or RNA, respectively, do not have the genes for holin or lysin proteins, which are expressed by tailed phages to degrade peptidoglycan as described earlier in this article. Instead, they produce a protein that inhibits a step in murein monomer synthesis. The fX174 gene product, gpE, inhibits MraY, which catalyzes the formation of the first lipid-linked murein precursor, and Qb gpA2 inhibits MurA, which catalyzes the first step in the murein biosynthesis pathway. Inhibition of synthesis of the cell wall is thought to be a general strategy in small phages that do not produce holin or lysin; their inhibitory gene products are known as "protein antibiotics." If a method can be developed to transport them efficiently into the host cytoplasm through the cell membrane, they would be useful as antibacterial agents.

Advantages of phage therapy over antibiotics

Phage therapy can be very effective in certain conditions and has some unique advantages over antibiotics. Bacteria also develop resistance to phages, but it is incomparably easier to develop new phage than new antibiotic. A few weeks versus years are needed to obtain new phage for new strain of resistant bacteria. As bacteria evolve resistance, the relevant phages naturally evolve alongside. When super bacterium appears, the super phage already attacks it. We just need to derive it from the same environment. Phages have special advantage for localized use, because they penetrate deeper as long as the infection is present, rather than decrease rapidly in concentration below the surface like antibiotics. The phages stop reproducing once a specific bacteria they target are destroyed. Phages do not develop secondary resistance, which is quite often in antibiotics. With the increasing incidence of antibiotic resistant bacteria and a deficit in the development of new classes of antibiotics to coun-

teract them, there is a need to apply phages in a range of infections.

Application of Bacteriophages

Collection: In its simplest form, phage treatment works by collecting local samples of water likely to contain high quantities of bacteria and bacteriophages, for example effluent outlets, sewage and other sources. The samples are taken and applied to the bacteria that are to be destroyed which have been cultured on growth medium. The bacteria usually die, and the mixture is centrifuged. The phages collect on the top of the mixture and can be drawn off. The phage solutions are then tested to see which ones show growth suppression effects (lysogeny) and/or destruction (lysis) of the target bacteria. The phage showing lysis are then amplified on cultures of the target bacteria, passed through a filter to remove all but the phages, then distributed.

Treatment:

Phages are "bacterium specific" and it is therefore necessary in many cases to take a swab from the patient and culture it prior to treatment. Occasionally, isolation of therapeutic phages can typically require a few months to complete, but clinics generally keep supplies of phage cocktails for the most common bacterial strains in a geographical area. Phages in practice are applied orally, topically on infected wounds or spread onto surfaces, or used during surgical procedures. Injection is rarely used, avoiding any risks of trace chemical contaminants that may be present from the bacteria amplification stage and recognizing that the immune system naturally fights against viruses introduced into the bloodstream or lymphatic system.

Distribution:

Phages can usually be freeze dried and turned into pills without materially impacting efficacy. In pill form temperature stability up to 55^{o}C, and shelf lives of 14 months have been shown. Other forms of administration can include application in liquid form. These vials are usually best kept refrigerated. Oral administration works better when an antacid is included, as this increases the number of phages surviving passage through the stomach. Topical administration often involves application to gauzes.

Problems to overcome

In phage therapy, the following problems remain to be solved: (i) inactivation of administered phages or lysin by a neutralizing antibody and allergic reactions to them, (ii) liberation of endotoxins as a consequence of widespread lysis of bacteria within the body. (iii) The negative public perception of viruses.

Regarding the first problem, decreases in the therapeutic effect with multiple administrations have not been shown, nor have side effects such as allergies been observed for phages or lysin, although antibodies against them have been detected in mouse blood (Stone, 2002). To circumvent this problem, nevertheless, phages or lysins with different antigenicities or with low immunogenicities could be prepared. Liberation of endotoxins is a minor effect and also such effects may be observed when antibiotics are used. The third problem can be overcome by increasing the awareness among people about the benefits of phage therapy.

Conclusion

Phages will not be the panacea of medicine, but phage therapy research will gain momentum because traditional antibiotic research has come to a stop. Appropriately selected phages can easily be used to help prevent bacterial diseases in humans or animals, with potential for alternative applications and special interest for developing countries. Much of the evidence strongly shows that appropriately administered phage therapy is very effective for treatment and prevention of many kinds of bacterial infectious diseases, especially those caused by multidrug-resistant bacteria. Currently, many pathogenic bacteria have acquired multiple drug resistance, which is a serious clinical problem. Although some problems remain to be solved, many experts are of the opinion that phage therapy will find a niche in modern Western medicine in the future.

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