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An Overview on the Application of Bacteriophage Therapy in Combating Antibiotics Resistance: A Review

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Abstract

The practice of phage therapy, which uses bacterial viruses (phages) to treat bacterial infections, has been around for almost a century. The universal decline in the effectiveness of antibiotics has generated renewed interest in revisiting this practice. Conventionally, phage therapy relies on the use of naturally-occurring phages to infect and lyse bacteria at the site of infection. Biotechnological advances have further expanded the repertoire of potential phage therapeutics to include novel strategies using bioengineered phages and purified phage lytic proteins. Current research on the use of phages and their lytic proteins, specifically against multidrug resistant bacterial infections, suggests phage therapy has the potential to be used as either an alternative or a supplement to antibiotic treatments. Antibacterial therapies, whether phage- or antibiotic-based, have relative advantages and disadvantages accordingly. Many considerations must be taken into account when designing novel therapeutic approaches for preventing and treating bacterial infections. Although much is still unknown about the interactions between phage, bacteria, and human host, the time to take phage therapy seriously seems to be rapidly approaching

Keywords: Antibiotic resistance; Antimicrobial; Bacteriophage; Biofilms; Multidrug resistance; Phage; Phage safety; Therapy.

INTRODUCTION

Almost a decade before the discovery of penicillin, the controversial practice of phage therapy was being developed as a treatment for bacterial infections. Phages, short for bacteriophages, are bacteria-specific viruses that have been used as a treatment against pathogens such as *Shigella dysenteriae* as early as 1919 (Chanishvili, 2012). With an estimated 10^{31}-10^{32} phages in the world at any given time (Suttle, 2007), they make up the most abundant biological entity on Earth and play a crucial role in regulating bacterial populations. Phages are responsible for the death of approximately 20%-40% of all marine surface bacteria every 24 hours (Wittebole *et al.*, 2014).

Bacteriophages (phages) are natural parasites of bacteria that have long been considered as agents for treating bacterial infections (D'herelle, 2007). Phage therapy, the use of phages as antibacterial agents, is based on the fact that phages recognize, bind to and multiply within bacterial host cells, rapidly causing cell lysis. While early studies of phage biology and therapy were unclear as to the nature of bacteriophage – many thought them to be a component of the human immune response or bacterial enzymes (D'herelle, 2007) – phage therapy has nonetheless been practiced for approximately 90 years. Early trials met with mixed results and the use of phage therapy was never universally adopted (Kutter *et al.*, 2010).

Antibiotics interact with specific bacterial cellular targets to produce selective toxicity. However, the genes for these targets can be changed through mutation and gene transfer, and these mutations can become amplified in a population through natural selection. While antibiotics are still successful in treating the majority of bacterial infections, there are notable exceptions where frontline therapies are no longer reliable (French, 2010; Gootz, 2010). Methicillin resistant *Staphylococcus aureus* (MRSA) (Ippolito *et al.*, 2010),

vancomycin-resistant *Enterococcus* (VRE) (Wang and Hsueh, 2009), and carbapenemase-producing strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter* spp. Recent reductions in the incidence of nosocomial pathogens such as MRSA (Lee *et al.,* 2011) and *C. difficile* (Hsu *et al.,* 2010) in the UK and several other countries have been achieved by improved infection control measures including better use of isolation and barrier nursing, however rates of some Gram negative multi-antibiotic-resistant infections are still on the increase and MRSA and *C. difficile* remain major problems in some settings (Goff, 2011). The need for novel antimicrobial therapies therefore remains high and indeed with the emergence of pan-resistant strains of some Gram-negative pathogens (Saleem *et al.,* 2009; Arias and Murray, 2009) it could be argued that the need has never been greater since the pre-antibiotic era.

Historical background of Bacteriophage

Bacteriophages were discovered independently by a British microbiologist Frederick Twort in 1915 and Felix de Herelle (Felix d'Herelles, 1917; Felix d'Herelle, 1949) however, the concept of bacteriophage therapy was introduced by Felix de Herelle in 1920. Many countries like France, Georgia, United States and in Europe there are several phage therapy centers are working, and dealing with various human diseases. An extensive work on phage therapy was carried out between 1920 and 1930 in USA to treat infection caused by *Streptococcus* and *Bacilli*. "Staphylogel" and bacteriophage "gel labaled" preparation were manufactured by Eli Lilly and Company. At the same time, antibiotics were discovered and widely used which caused the rejection of bacteriophages as therapeutic agent in many countries including Europe and USA. Many papers were published between 1950 and 1980 that showed benefits of bacteriophage therapy in animal models (Qadir, 2015; Samson *et al.,* 2013; Kutateladze and Adamia, 2010). Due to increase antibiotics resistance in bacteria, bacteriophage therapy was "rediscovered" with the work done by Smith and Huggins in 1980s. Presently, treatment of infection by phage is widely used in several countries namely Poland, United States, Europe (Georgia) and Russia (Kutateladze and Adamia, 2010; Chhibber and Kumari, 2012).

(Zavascki *et al.,* 2010; Livermore *et al.,* 2011) are just a few of the bacteria which have driven the search for alternatives to antibiotic use.

Bacteriophages

Bacteriophages are the most abundant organisms on the Earth. They are ubiquitous, obligate intracellular parasites and attack the host cell, hijack the machinery and finally destroy it. Achaea and cyanobacteria are also attack by a group of viruses often called cyanophages (Clokie *et al.,* 2011; Paul and Sullivan, 2005). Bacteriophages widely occur in sewage, soil, water and marine water etc. Structurally, most of bacteriophages consist of three parts i.e. head, tail and tail fiber. The head encloses nucleic acid which can be either DNA or RNA but not both. The tail is like a hollow tube through which nucleic acid passes in to the host cytosol during infection and tail fibers help bacteriophages to attach to the bacterial surface. The size of most bacteriophages in general ranges from 22-200nm in length, with the largest bacteriophage known as T4 being about 200nm long and 80-100nm wide (Clokie *et al.,* 2011).

The most important feature of phages is their narrow host range i.e. they kill only specific bacterial strain and that makes them potential antimicrobial agents. This feature of bacteriophages is very advantageous because unlike broad-spectrum antibiotics, phage can kill specific pathogenic bacteria without affecting the balance of beneficial bacterial microflora (Koskella and Meaden, 2013).

However one drawback of this narrow bacteriophage host range is that, bacteria may develop resistance against bacteriophage. To solve this problem, phage "cocktail" i.e. a mixture of different bacteriophages is used that provides a wider antimicrobial range (Chan *et al.,* 2013). Normally lytic bacteriophages infect and kill specific bacteria and are widely used in therapy because they act on short period of time.

Furthermore, the mode of antimicrobial action of Bacteriophages is more complex than mechanism of action of antibiotics. Therefore in this chapter we are highlighting the use of bacteriophages as an alternative antimicrobial therapy and also other potential applications of bacteriophages (Qadir, 2015; Samson *et al.,* 2013; Kutateladze and Adamia, 2010).

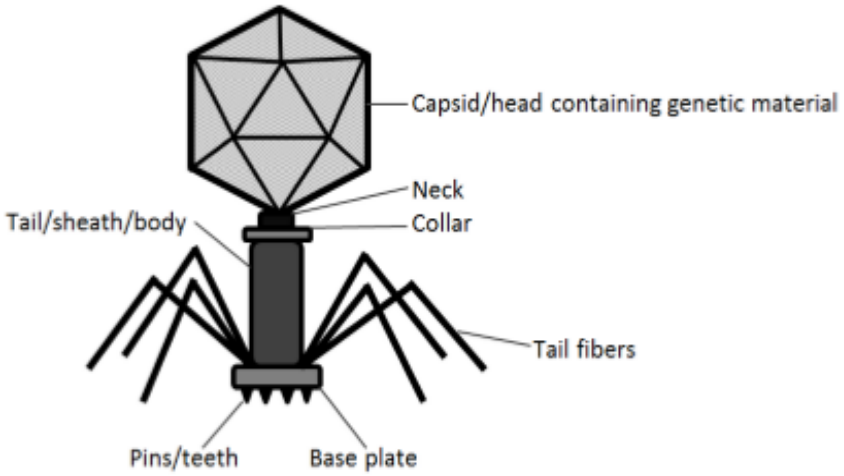

Figure 1: The anatomy of a bacteriophage.
Source: (Forterre, 2016).

Phage Therapy

Phage therapy combats bacterial infections of humans (or animals) with the goal of reducing bacterial load. Findings show that appropriate administration can be used to treat lethal infectious diseases caused by *Escherichia coli, Vibro cholerae, Staphylococcus aureus* and *Salmonella spp*. (Matsuzaki *et al.,* 2003). Phages can be delivered topically, orally, directly into body tissues, or systemically. A second means in which therapies differ is in terms of delivery. Phage - infected bacteria may be employed as a means of delivering phages to intracellular pathogens (Broxmeyer *et al.,* 2002). Finally, the normal ability of lytic phages to destroy infected bacteria may be exploited, as is generally the case with phage therapy, or alternatively phages may also be engineered to deliver non-phage genes coding for antibacterial agents (Westwater *et al.,* 2003). The success of phage therapy is a function of elementary principles of phage-bacterial infection. One must first recognize a phage that can infect a bacterium, and the effectiveness relies on the extent of phage bacterial infection. Secondly, if treatment is initiated during early stages of infection, it may be essential to compensate, by using a larger phage dosage, due to the inability of too-few bacteria to successfully expand phage population (Kasman *et al.,* 2002).

The advent of antibiotic resistant bacteria has grown into a major problem for clinician over the last 2-3 decades. In United State, antibiotic resistant bacteria infect approximately 2 million people each year of which at least 23,000 people have been documented to die annually (CDC, 2013). In recent times, bacteria such as *Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococus faecium, Klebsiella pneumonia* have developed multi-drug resistance and cause major healthcare disaster in ICUs (Wittebole *et al.,* 2014; Blair *et al.,* 2015). The development of severe infectious diseases resistant to antibiotics has been an issue of serious concern and this in turn has led to the discovery of alternative approaches that could replace these conventional antibiotics. Steady decline in the discovery of new class of antibiotics, has enhanced the scope of bacteriophage as an alternative to conventional antimicrobial drugs. Therefore, bacteriophage can be employed as the therapeutic agent to combat infections caused by multi-drug resistant bacteria (Gill and Hyman, 2010; Lu and Koeris, 2011).

Bacteriophages as Antimicrobial

Bacteriophage therapy is widely used to treat severe infections caused by multi-drug resistant pathogenic bacteria in human, animals and plants and it is now also employ to enhance the shelf-life of meats, vegetables, fruits and stored plant parts (Reardon, 2015; Ahmed *et al.,* 2012). Mzia Kutateladze, who heads the scientific council at the Eliava Institute in Tblisi, Georgia, says that antibiotic resistance is driving more Western patients towards phage-therapy clinics in Europe and America. The US National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, now list phages as a research priority to address the increasing drug resistance bacteria. Thus there is increasing demand for isolation and characterization of more bacteriophage against major clinical threats (Kutateladze and Adamia, 2010).

Mode of Delivery of Bacteriophages

The success of bacteriophage-derived therapeutics and biosensors will ultimately rely on suitably robust, reproducible delivery technologies. Delivery of suitably-engineered phage has permitted isolation of allergens inducing IgE production using high throughput screening technologies (Ryan *et al.*, 2011). Phages can also be engineered to bear target-specific peptides or proteins for biorecognition, and thus may have application in development of novel chemical and biological sensors that may provide quantitative or semi-quantitative data through exploitation of a chemical or biological recognition element (Mao *et al.*, 2009).

The potential routes of administration of phages include topical, oral, rectal, and parenteral; topical administration to chronic wound infections is the most frequently reported route. One product used at the Eliava Institute is Phage BioDerm, a biodegradable polymer wound dressing impregnated with ciprofloxacin, benzocaine, chymotrypsin, bicarbonate, and 6 lytic phages (Pyophage) with activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus species,* and *Proteus species* (Markoishvili *et al.*, 2002). Other potential means of topical administration include sprays, aerosols, lozenges, mouthwash, suppositories, bandages, eye drops, and tampons. Intrapleural administration and bladder irrigation are also feasible.

- **Oral Administration of Phage**

Gastrointestinal infection and systemic infections are successfully treated with oral delivery of phage (Stanford *et al.*, 2010). The main difficulty with phage delivery through this route is that phage can be inactivated in the highly acidic condition of the stomach. To avoid such problem, polymer microencapsulated phages are used that protect phage from inactivation by acid and also enhance efficacy of phages. Other way of neutralizing acidity of stomach is use of sodium bicarbonate or sodium bicarbonate mineral water before administration of phages (Ryan *et al.*, 2011).

- **Local Administration of Phage**

This is the most successful route of phage administration where phage suspensions are directly applied on the infected area (Qadir, 2015). In addition to above routes, phage can also be administered to humans intravenously (IV), intaperitonial (IP), intramuscular (IM), and subcutaneous (SC) methods (Ryan *et al.*, 2011).

Phage therapy Versus Antibiotic therapy

Both antibiotics and phages function as antibacterials that disrupt bacterial colonies through lysis or inhibition, yet several key differences make each antibacterial more or less appropriate depending on the situation.

- **Safety**

Adverse reactions to antibiotics are well documented and include instances of anaphylaxis, nephrotoxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity, as well as a number of gastrointestinal and hematological complications (Granowitz *et al.*, 2008). The majority of adverse reactions are allergic reactions; in these rare instances the anaphylaxis is associated with specific classes of antibiotics or is the product of high tissue concentrations (Shehab *et al.*, 2008). In contrast to the comprehensive literature on antibiotic safety, phage therapy has only recently gained attention by western medicine and, as a result, much of the available information on phage safety is new. Although oral phage administration is generally considered to be safe (Merabishvili *et al.*, 2009), a major consideration for phage therapy is the translocation of phage across the intestinal epithelium where they subsequently circulate within the blood.

- **Specificity**

In stark contrast to antibiotics, phages tend to be specific towards both species and strain. In certain situations this can be a major advantage, considering the well-documented, collateral effects of broad-spectrum antibiotics on commensal gut microbes, which are notorious for secondary outcomes such as antibiotic-associated diarrhea and *C. difficile* infection (Rea *et al.*, 2016). Other consequences of antibiotic perturbations in the gut microbial community include risk of asthma, obesity, and diabetes (Metsala *et al.*, 2015; Cox and Blaser, 2016). The current understanding of collateral damage due to phage therapy is limited, but, compared to antibiotics, phage therapy has been reported to result in less perturbation of the gut microbiome while still effectively reducing gut carriage of pathogens such as *Shigella sonnei* and uropathogenic *E. coli* (Galtier *et al.*, 2016). The strain and species specificity of antibacterial compounds offers many advantages, it comes with a number of inherent constraints. By targeting a single pathogen, phage therapy could be less effective against infections such as infected burn wounds, which are often colonized by more than one strain of bacteria (Servick, 2016).

This can be accounted for by creating phage cocktails infective against a range of known pathogens, but the success of this approach depends on knowledge of which pathogens are being treated. Logistically, host specificity significantly impacts treatment development and testing, and also limits the possibility of large-scale production and distribution, a distinct advantage of broad-spectrum antibiotics.

- **Biofilm Penetration**

Antibiotic therapy is highly effective with planktonic bacteria, such as *V. cholerae* and *Yersinia pestis*, yet is limited in treating biofilm-based bacterial infection. Phages, however, are equipped with enzymes (e.g., EPS depolymerase) on the exterior of the capsid that degrade the extracellular polymeric substances (EPS) and disperse bacterial biofilms, allowing the phage to access bacteria embedded within the EPS matrix (Abedon, 2015). The phage progeny released upon completion of the lytic cycle propagate the dispersal of the biofilm through the removal of biofilm-embedded bacteria in subsequent layers (Abedon, 2015). In order to penetrate dense biofilms, high doses of antibiotics are typically required to observe any inhibition of bacterial growth, yet complete eradication is rare and regrowth of colonies begins after the end of antibiotic treatments (Amorena *et al.*, 1999). Although low concentrations of many antibiotics are generally considered non-toxic, high concentrations can result in tissue toxicity (Abedon, 2015).

Gabisoniya and colleagues (Gabisoniya *et al.*, 2016) at the Eliava Institute of Bacteriophages in Tbilisi, Georgia found that the application of phages on in vitro colonies of the pathogen *P. aeruginosa* not only prevented additional biofilm formation by the pathogen but also degraded existing biofilm. Phage treatments have eliminated biofilms formed by *L. monocytogenes*, *P. aeruginosa*, and *Staphylococcus epidermidis* on the surface of medical devices (Motlagh *et al.*, 2016). These findings are highly relevant to the problem of persistent infections caused by implanted medical devices such as catheters, lenses, and prostheses where biofilm formation is common.

CONCLUSION

The available literature on the use of phages and phage-derived proteins for combating bacterial infections, specifically those of multidrug-resistant bacteria, increasingly shows promise for the prospect of phage therapy as either an alternative or a supplement to antibiotics. However, discrepancies in recent findings on the immunomodulatory effects, the host range, and the potential for horizontal gene transfer make it abundantly clear that we need a better understanding of the interaction between phage, microbiome, and human host before implementing phage therapy on a large scale.

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