Phage therapy: Review Article

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Abstract

The global emergence of multiple drug resistant strains of bacteria and rapidly developing resistance to antibiotics in both animals and humans has generated urgent requirement for alternatives. There has been a long term history of Bacteriophages being used in bacterial infection treatment, their successful and somehow clearly reported trials throughout the world (especially Georgia, Poland and to a lesser extent India), make them promising tool to combat serious Multi-Drug Resistant bacterial infections caused due to many bacterias such as Methicillin resistant *Staphylococcus aureus*(MRSA), Vancomycin resistant *Enterococci* (VRE), respiratory pathogens including *Streptococcus pneumonia* and *Mycobacterium tuberculosis*. Phages are valuable in therapeutic medicines, aquaculture, agrophages and as food additives approved by FDA. Specificity, self replication, auto dosing and other properties of phages have been exploited by researchers and scientists to replace antibiotics in use. However true efficacy of phage therapy and stable eradication of infection still requires novel developments, approaches and formal confirmation in clinical trials along with adequate regulatory framework, implementation of safety protocol and general public acceptance.

KEYWORDS: Antibiotic resistance, Bacteriophages, Phage therapy, Clinical applications

Over the last century, most important medical tool i.e. antibiotics, which have been used to treat bacterial infection from past 70 years, have now become ineffective due to spread of antimicrobial resistance. Inappropriate use of antimicrobials drives the development of antibiotic resistance (European Centre for Disease Prevention and Control (ECDC 2014). Further both overuse and misuse of medicines in humans and animals is accelerating this process. Antibiotic resistance is natural evolutionary phenomenon (WHO) performed by bacteria by exchanging their genetic material with other bacteria. Another method of acquiring resistance among bacteria against multiple antibiotics is through natural selection phenomena and developed by environmental factors (Sahoo *et al*. 2010). The report "*Antimicrobial resistance: global report on surveillance 2014*" showed that antimicrobial resistance is everywhere and has the potential to affect anyone, of any age, in any country. The Infectious Disease Society of America updated that nearly 2 million Americans per year develop hospital-acquired infections (HAIs), resulting in 99,000 deaths – the vast majority of which are due to antibacterial-resistant pathogens. With the growth of global trade and travel, resistant microorganisms can spread promptly to any part of the world. Hospitalized patients are one of the main reservoirs of resistant microorganisms. As a result of resistance, previously curable infectious diseases may one day become untreatable and spread throughout the world.

In April 2015, WHO report publication revealed that while much activity is underway and many governments are committed to addressing the problem, there are major gaps in actions needed across all 6 WHO regions. Multidrug resistant bacteria (MDR) are ‘‘an increasingly serious threat to global public health that requires action across all government sectors and society’’ (WHO Fact sheet 194 (2014)).The most important examples of multi-drug resistant strains are Methicillin resistant *Staphylococcus aureus* (MRSA) (Michel *et al*. 1997), Vancomycin resistant Staphylococcus aureus, NDM-1-producing Enterobacteriaceae [Pirnay *et al*. 2009], Salmonella and other Gram-negative bacteria.

There is an urgent need to replace synthetic antibiotics with an alternative, having low toxicity, fast production, less expensive and having few or no side effects. One may find all these qualities of synthetic antibiotics in a natural killer or superbug mainly known as bacteriophage. They are much more specific than antibiotics and offer a possible *alternative to conventional antibiotic* treatments for bacterial infection.

Bacteriophage, also called phage or bacterial virus, are a group of viruses that infect bacteria. Bacteriophages are among the most common and diverse entities in the biosphere(*Mc Grath et al., 2007).* Phages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats. When phages infect their host cell, the order of business is to replicate their nucleic acid and to produce the protective protein coat often killing bacteria in the process. As phages have an obligate requirement for a host, their abundance and distribution is dependent out on their host organisms. So maximum phages are found at the site where the majority of their hosts exist i.e sea water, where up to 9×10^8 virions per milliliter have been found in microbial mats at the surface(Wommack *et al.* 2000*)* and up to 70% of marine bacteria may be infected by phages (Prescott, 1993). Those active towards pathogenic micro flora are usually obtained from patient samples (urine, faeces, pus, etc).

Bacteriophages are hugely important to the ecology and evolution of bacteria, have enormous impacts on the global carbon cycle (which among other things controls whether climates globally warm), have been of interest to scientists as tools to understand fundamental molecular biology, as vectors of horizontal gene transfer and drivers of bacterial evolution, as sources of diagnostic and genetic tools and as novel therapeutic agents. In short, phages are perhaps the biological world's least appreciated superstars.

History

The biblical Book of Kings relates how the prophet Elisha cured general Naaman’s disease by commanding him to bathe seven times in river Jordan. Since ancient times various reports show the power of rivers to cure infectious disease, but first observation was made in 1896, when the British bacteriologist Ernest Hankin reported antibacterial activity against *Vibrio cholera* in the Ganga and Yamuna rivers in india. He suggested that an unidentified substance was responsible for this

phenomenon. His work was published in French in the '*Annals of the Pasteur institute*' (Hankin *et al*., 1896). Two years later, Gamaleya, the Russian bacteriologist, observed a similar phenomenon while

working with *Bacillus subtilis* (Adhya and Merril, 2006). In 1915 Fredrerick Twort, a British microbiologist, demonstrated the presence of filterable agent in pure culture of micrococci isolated from vaccinia. This agent was unable to grow in absence of bacteria and had capacity to infect and kill the same. Similar experimental findings were reported by Felix D' Herelle in 1917 while working at the Pasteur Institute in Paris while studying patients suffering from bacillary dysentery. He gave the term "Bacteriophage". Twort did not follow his innovation; it was D' Herelle who introduced phage therapy concept by conducting deep research (Keen 2012). D'Herelle began testing his phages in human patients. Under the clinical supervision of Professor Victor-Henri Hutinel at the Hospital des Enfants-Malades in Paris, he demonstrated the safety of his phages by ingesting them. The next day, he demonstrated their efficacy by administering them to a 12-year-old boy with severe dysentery.

In 1923, two physicians from Baylor University's College of Medicine reported successful results from one of their phage therapy trials conducted in United States, and concluded that "the bacteriophage holds enormous possibilities as a new weapon for fighting infectious disease" (Ho, 2001). Research on bacteriophage therapy was at its peak between 1920 and 1930 in USA. Herelle developed phage therapy centers in many countries like France, USA and Soviet Georgia. He developed the Laboratoire du Bacteriophage in paris, which produced five phage preparations for commercial use. They were marketed by French company Robert et Carriere, which later was acquired by L'Oreal (Sulakhvelidze *et al*., 2001).

Despite the numerous successful therapeutic outcomes reported in Eastern European countries, phage therapy remained disregarded in the west. This was mainly due to the lack of a specific regutory framework (Verbeken *et al*. 2014), and due to difficulty to obtain intellectual property rights for phage products (Reardon 2014) that made the large pharmaceutical companies reluctant to invest in phage therapy. Besides these reasons the discovery of antibiotics in 1940 became the main reason behind the rejection of bacteriophages as therapeutic agent.

Bacteriophage uses

Bacteriophages are used in various fields such as food additives, biofilm disruption, agriculture, aquaculture and most important one is therapeutics application in humans and animals. A number of commercial applications of Bacteriophages have been reported.

The US Food and Drug administration(FDA) in 2006 approved the use of phages targeting the food borne pathogen *Listeria* in packaged meats and cheese, and have been given the designation Generally Regarded As Safe (GRAS)(Hagens and Loessner 2010).Phages are able to disrupt biofilm colonies of target organisms such as *Staphylococcus epidermidis* growing on silicon catheters (Chan and Abedon, 2015).

Bacteriophages are helpful in preventing food born and Zoonotic bacterial pathogens (Miller et al., 2010). Several publications reported success of experimental phage therapy with different bacteria, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Soothill, 1992), *Vibrio vulnificus* (Cerveny *et al.* 2002), *Enterococcus faecium* (Biswas *et al.,* 2002), and even *Escherichia coli* in animals such as calves (Barrow *et al*., 1998) and chickens (Huff *et al*. 2002a,b). In 2008, the US Food

Safety and Inspection Service approved a *Salmonella*-specific phage preparation to reduce the contamination level of live poultry before processing (OmniLytics, 2009). Bactericidal bacteriophages prevent the introduction of *Salmonella enterica* into the food chain and consequently reduce food poisoning among consumers (Wegener *et al.,* 2003).

Phages can be used as biocontrol agents in agriculture. Mallmann and Hemstreet (1924) observed that filtrate of the liquid collected from the decomposing cabbage inhibited the growth of the bacterium that caused the rot, *Xanthomonas campestris* pv *campestris*. Kotila and Coons (1925) demonstrated that bacteriophages isolated from the soil suppressed growth of *Pectobacterium carotovorum* subsp *atrosepticum, the* causal agent of blackleg disease of potato. Phage therapy has also been used to increase the shelf life of fruits, vegetables, meat and plants (balogh *et al*.,2010) as well as prevent the contamination of fresh cut fruit (Leverentz *et al*., 2001) and sprout seeds (Pao et al., 2004)

A study revealed that the T4-like phage ESP 732-1 was able to suppress the growth of *Enterobacter sakazakii* in infant formula milk both at 24◦C and 37◦C (Kim et al., 2007). In 2011 the FDA cleared the first bacteriophage-based product for in vitro diagnostic use. The KeyPath MRSA/MSSA Blood Culture Test uses a cocktail of bacteriophage to detect *Staphylococcus aureus* in positive blood cultures and determines methicillin resistance or susceptibility (Sullivan *et al*., 2013). Phages specific to sulphate reducing bacteria associated with fouling and MIC in Gulf of Mexico pipelines, have been sourced, characterized and found to be effective in petroleum industry (Summer and Summer, 2010). Recently advancement has been reported in the phage display technology used for the preparations of antivenoms for animal toxin neutralization (Roncolato *et al*., 2014)

Clinical application in humans

The practice of phage therapy in humans begins in France since 1919, when D'Herelle first successfully treated several children at the Hospital des Enfants Malades in Paris who were suffering from severe dysentery, using the phage he had first isolated from the stools of soldiers he had observed at the Pasteur Institute (Sulakvelidze and Kutter, 2005). His study on phages role was laid out in series of books**,** five of which have been translated in English from French (Abedon *et al*. 2011). Several reviews of phage therapy in English have recently been published (Alisky *et al*. 1998 and Carlton, 1999). While the first human therapeutic phage trial was conducted by D Herelle , the first article documenting phage therapy was on research credited in Belgium by Bruynoghe and Maisin in 1921 (Bruynoghe and Maisin 1921).This article described its efficacy in *Staphylococcus* treatment. In 1931 herelle published his work related to cholera treatment.

Commercialization of phage began in france. Theodore Mazure produced the first commercial phage cocktails—BactéColi-Phage, Bacté-Intesti-Phage, Bacté-Dysentérie-Phage, BactéPyo-Phage and Bacté-

Rhino-Phage. The use of phages as well as their clinical reports continued until 1979. Henri de Montclos, Chief of Clinical Microbiology for 10 years, along with his research team produced anti-Staphylococcal vaccines and therapeutic phages until the early 1990s. A full monograph issue of the journal La Médicine in 1936 explains various phage applications in infected humans. It describes the treatment review for various diseases such as acute colitis, typhoid fever peritonitis, sepsis, prostate and urinary tract infections, furunculosis, and otolaryngology.

Many review papers explain the clinical trials in humans against typhoid and skin infections (Tsulukidze 1936 and Gougerot and Peyre 1936). Poland, particularly in association with the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, founded in 1954 treated patients successfully by phage therapy This work has been more thoroughly documented than any other in the English-language literature, mainly in the Institute's own journal in the earlier years and much of the work is available at their web site, www.aite.wroclaw.pl, and/or at www.evergreen.edu/phage. Treatment was performed by physicians from throughout the region, using phages specifically selected and prepared for each patient from the large Institute collection, and with detailed records kept. Every one of their 550 patients from 1981–1986, included in a series of overview articles and specific discussions of particular conditions, reported cure rates for specific infection types ranged from 75 to 100%(Abedon *et al*., 2011). Although the western countries disregarded phage therapy, the use continued in eastern countries and large number of reports were published over time, mainly in Poland and Georgia(former USSR). The first phase I randomized controlled trial conducted in the united states was published in 2009. It evaluated the safety of a cocktail of phages directed against *S.aureus*, *E.coli* and *Pseudomonas aeruginosa* in 42 patients with chronic venous leg ulcers (Wittebole *et al*., 2014) Another randomized trial was coducted in UK, to check the efficacy of phage against chronic Pseudomonas aeruginosa otitis (Letkiewicz *et al*., 2010). A detailed report on randomised trial also comes from Belgium, Australia and India. Earlier this year phage therapy was highlighted as one of seven approaches to "achieving a coordinated and nimble approach to addressing antibacterial resistance threats" in a 2014 status report from the National Institute of Allergy and Infectious Diseases (NIAID).

Advantages of phages over antibiotics

Bacteriophages as the name itself suggest specifically kill or lyse the bacteria, thus also became a potential tool in biotechnology, research and therapeutics. Phages specifically kills closely related bacteria and have minimal or no effect on normal microbiota of body whereas antibiotics effect the entire microbiota of body. As antibiotics spread in whole body through blood circulation, their concentration reduces at the site. Besides, phages have autodosing capacity by self replication at the confined site thus small amount of dose is enough to control or treat infection. Phages replicate with their host so rise in number of bacteria also increases phage number unlike the antibiotics. As the mechanism of antibiotic and phage to kill or infect bacteria is different, chances of resistance development against phages are very low.

Phages thus have been readily used to treat antibiotic resistant infections (Carlton, 1999: Kutter *et al*., 2010) such as against multi-drug-resistance *Staphylococcus aureus* (Gupta and Prasad, 2011).

Bacteriophages are ecofriendly natural antibacterials able to regulate bacterial populations by the induction of bacterial lysis. They are active against gram positive as well as gram negative bacteria including MDR bacterias. Unlike antibiotics phages are easily discovered from sewage and other waste materials which are heavily concentrated with bacteria. Ecofriendly nature and low cost are other important advantages of phages over the antibiotics.

Future outlook

Phages, natural bacteria eating viruses can be used in phage therapy to treat various bacterial infections. They are ready to replace antibiotics which are in use since 1940. Low cost production and specificity makes them interesting tool and also attracts the pharma companies. Further they have many advantages over antibiotics, for the success of phage therapy, certain limitations should be overcome for eg. some important issues of them are that they need adequate regulatory framework, implementation of safety protocol and most important is to raise the awareness among general public and acceptance by them.

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