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Review Article

Bacteriophage therapy: an alternative to antibiotics in the age of multi-drug resistance

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ABSTRACT

Phage therapy involves the use of specific viruses that can infect and lyse pathogenic bacteria. Since, antibiotic resistance is widely increasing among bacteria phages offer a promising field as natural, self-replicating and self-limiting antibiotics. Clinical trials are conducted to use phages against multi-drug resistant bacterial infections. The most powerful and cost-effective application of phage therapy is in the treatment of diseases such as cholera and dysentery. While phage therapy has been around for over 100 years, the treatment still is not a main part of medicine. Phage therapy has largely existed on the fringes of medicine, particularly in Western countries like the USA, where it is occasionally approved for compassionate use, on emergency basis when no other approved therapies are available. This review was undertaken to discuss, review and highlight the importance and future research of phage therapy.

Keywords: Bacterial infections, Bacteriophages, Multidrug resistance, Phage therapy.

INTRODUCTION

Bacteriophages, abbreviated as phages are viruses that infect bacteria, and occur widely in nature in soil, water, sewage, and even in

and on our body, particularly in the gut and in feces in close association with bacteria. Phages are regularly consumed in foods. The presence of high concentrations of phage particles, up to 10^8 per mL in some natural waters, suggests that they may have a role in the control of bacterial populations in such environments [1]. Prior to the discovery and wide spread use of antibiotics, it was suggested that bacterial infections could be prevented and treated by the administration of bacteriophages. These early hopes phage therapy have not been fulfilled, but these viruses have contributed much to microbiology. As phages could be grown easily on bacterial cultures, they provided the only convenient model for the study of virus-host interactions at the cellular and molecular level before the development of cell culture techniques [2].

History of phage therapy

Ernest Hankin, a British bacteriologist reported (1896) on the presence of marked antibacterial activity against *Vibrio cholerae*, which he observed in the waters of Ganges and Jamuna rivers in India, and he suggested that an unidentified substance, which passed through fine porcelain filters and was heat labile was responsible for this phenomenon and for limiting the spread of cholera epidemics [1,2]. Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis* [2]. The British microbiologist, Frederick Twort (1915) described a degenerative change in staphylococcal colonies isolated from the calf lymph, which could be transmitted serially by application of culture filtrates from the original growth [3]. The French-Canadian microbiologist, Felix d'Herelle (1917) observed that filtrates of feces cultures from dysentery patients induced transmissible lysis of a broth culture of a dysentery bacillus, and suggested that the lytic agent was a virus and gave it the name bacteriophage. The name was formed from "bacteria" and "phagein" (to eat or devour, in Greek), and was meant to imply that phages eat or devour bacteria. d'Herelle made immediate use of his discovery and used various phage preparations to treat thousands of people having dysentery, cholera and bubonic plague in India [2,3]. Richard Bruynoghe and Joseph Maisin (1921) used bacteriophages to treat staphylococcal skin disease. The phages were injected into and around surgically opened lesions, and the authors reported regression of the infection within 24 to 48 hours [4].

While phage therapy developed rapidly in Europe and USA, with phages even being produced by American pharmaceutical companies, it declined from 1940s onwards. Massive production and use of antibiotics from the 1940s onwards led to the complete disappearance of phage therapy [5]. However, it has developed significantly in Soviet countries, especially Goergia, as well as in Poland and Russia, where it is still widely practiced [6]. In France and Belgium phage therapy has continued to be used occasionally to treat patients in cases of therapeutic failure [7]. The phage therapy is gaining the importance since the beginning of 21st century due to the appearance of multi-drug resistance among bacterial pathogens [6].

A patient infected in Egypt with resistant strain of *Acinetobacter baumannii* was treated by phages that cured him in USA at University of California [8]. In England, a cystic fibrosis patient with disseminated drug resistant *Mycobacterium abbscessus* was cured by the use of phages [9]. William Smith and his

colleagues reported the successful use of phages to treat experimental *E.coli* infections in mice [10]. Soothill et al reported the utility of phages in preventing and treating experimental disease in mice and Guinea pigs infected with *Pseudomonas aeruginosa* and *Acinetobacter* [11], and they suggested that phages might be efficacious in preventing infections of the skin grafts used to treat burn patients [12]. The efficacy of a phage preparation was evaluated for the treatment of *Klebsiella pneumoniae* infection, and found to be efficacious in treating experimental infections in mice and Guinea pigs and nontoxic to animals [13]. The results of these preclinical studies were used to evaluate the safety and efficacy of the phages in treating 109 patients having *Klebsiella* infections, and found that the phage preparation was both effective with clinical improvement and bacteriological clearance and nontoxic for patients [14].

Phage therapy has agricultural applications to eradicate *Salmonella* from chickens to prevent human infections and to treat diarrhoeal diseases of live-stock such as cattle and fish. Phages can also be used as biocontrol agents in agriculture and petroleum industry [15].

Mode of action of therapeutic phages

Phages exhibit two types of life cycle: 1. Virulent or lytic cycle- lysis of the bacterial cell by phage 2.Temperate or lysogenic cycle- integration of phage DNA into the bacterial chromosome. Phages are excellent vehicles for horizontal gene transfer by transduction. So, they find wide use in recombinant-DNA technology to construct mutants and to transfer genes of interest from one bacterium to another [5]. In context of therapeutic use, only virulent phages with lytic cycle are relevant. In the virulent cycle, the phage attaches specifically to the surface of a susceptible host bacterium, and injects its DNA into host cell. A given phage will be able to attach itself to a given bacterial species. Phage DNA replicates in host cell cytoplasm via bacterial enzymes, and synthesizes proteins and lipids needed to form capsids. After assembly of these different components into daughter phages (progeny), the bacterium will be lysed releasing between 50 and 200 new phages, which can attach themselves to new bacteria to start the virulent/lytic cycle again (Fig 1). In therapeutic use, it is therefore necessary to isolate active phages against the bacteria responsible for patients' infection, amplify them, and administer them in such a way that they come into contact with the pathogen. Phages, multiplying in contact with the host bacteria, will spread as long as there are cells left to infect [16]. Once, the pathogenic bacteria are eliminated, the phages, which cannot survive without a host, will then be degraded. They could be used as an alternative to antibiotic use, or used together. Some studies show their synergistic potential and a reduced acquisition of resistance in bacteria [17]. They could be used to decolonize patients before surgery, or to decolonize carriers of *Staphylococcus aureus* in immunosuppressed patients [18]. Phage therapy is more effective than antibiotics in the treatment of biofilm infections, especially *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [19].

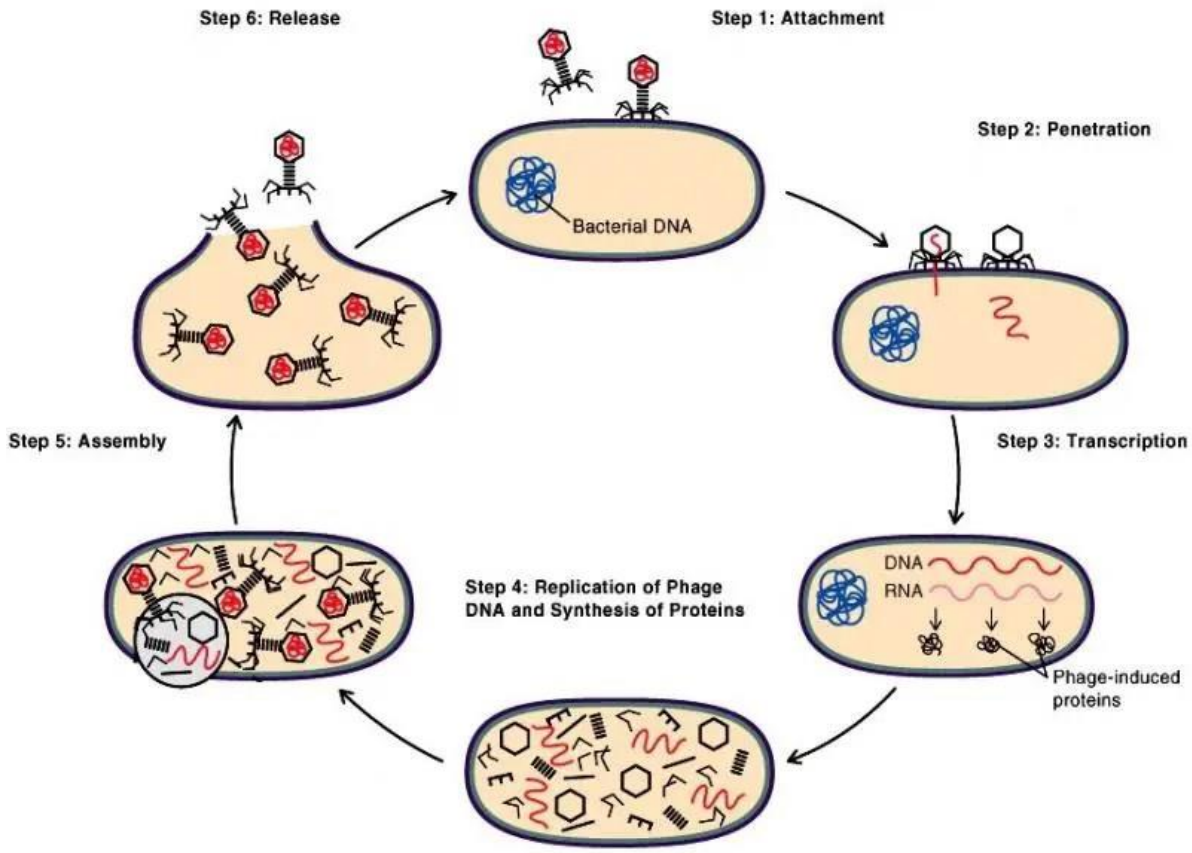

Fig 1. Virulent (lytic) replication cycle of phage [20]

It is suggested that phages enter into blood stream within 2 to 4 hours after a single oral dose, and found in the internal organs such as liver, spleen, and kidney etc., in approximately 10 hours. Administered phages can remain in the human body for several days [16]. Some therapeutic phages have some unique unidentified genes or mechanisms for lysing target bacteria effectively. A gene from anti-*Salmonella* phage has been identified, which is responsible for the phage's potent lethal activity against *Salmonella* enteric serovar Typhimurium strains [21]. In one study, a unique mechanism has been described for protecting phage DNA from the restriction-modification defenses of *S. aureus* host strain [22].

Comparison of prophylactic and therapeutic use of phages and antibiotics

Though lytic phages are similar to antibiotics, phages have some advantages over antibiotics and phages have been reported to be more effective in treating certain infections in humans and animals. The phage action is very specific affecting only the targeted bacterial species without disturbing the normal bacterial flora, and chances of developing secondary infections are avoided. Antibiotics target both pathogenic and normal flora, which disturbs microbial balance in the patient leading to secondary infections. Since phage action is highly specific, the etiologic agent must be identified before initiating phage therapy. Thus,

antibiotics are more effective than phages when the identity of the etiologic agent is not known [23]. Phages can be given in a single dose, since the phages can replicate themselves and are not diluted out by body fluids. Phages replicate at the site of infection and are thus are available where they are most needed. Whereas antibiotics are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection. The phages grow exponentially at the site of infection and require less frequent phage administration to achieve an optimal therapeutic effect. In cases of intestinal infection, phages along with the feces will prevent the spread of infection, if feces contaminate food and water [24].

Antibiotic administration may have multiple side effects, including intestinal disorders, allergies, and secondary infections, while no serious side effects have been noticed with phage therapy. A few minor side effects noticed after phage therapy may be due to endotoxins liberated from lysed bacteria. However, such side effects are also seen following antibiotic administration [25]. Phage-resistant bacterial mutants that develop are much less virulent than the parent wild types. Phage resistant bacteria remain susceptible to other phages having a similar target range, while resistance to antibiotics is not limited to targeted bacteria, because of their broad-spectrum activity [26].

In the rare case that a phage therapy doesn't work, there are plenty of other phages to choose from because they are so common in nature. Antibiotics are limited while there are many phages. Selecting new phages against phage resistant bacteria is relatively a rapid process; a specific phage therapy can be made and matched to bacterial infection within a few days or weeks. Developing a new antibiotic against antibiotic resistant bacteria may take several years [27].

Safety profile of therapeutic phages

It is important to ensure further the safety of therapeutic phages that they do not carry out generalized transduction, do not possess genes for drug resistance, toxins, and other bacterial virulence factors. Phages have been administered to humans orally, in tablet or liquid formulations (10^5 to 10^{11} PFU/dose), locally in creams, rinses (skin, eye, ear, nasal mucosa etc.), as aerosols or intrapleural injections, rectally, and intravenously to a lesser extent, and there have been no reports of serious complications associated with their use [23]. Phages can be freeze-dried and turned into pills. Patients generally receive mixtures (cocktails) of phages that target bacteria in different ways [25]. Because of their absolute safety phage phiX174 has been used to monitor humoral immune function in adenosine deaminase-deficient hosts [28] and to determine the importance of cell surface-associated molecules in modulating the humoral immune response [29].

Phage-derived lytic proteins

The phage encoded lytic enzymes are functionally similar to the antimicrobial eukaryotic enzyme lysozyme. Phages employ two major proteins which work together for the lysis of the bacterial host: transmembrane protein holing and peptidoglycan cell wall hydrolase called endolysin (lysin). Phage lysins alone are capable of bacterial cell lysis, whereas holins are not. Therefore lysins have been considered as antimicrobial agents. These lysin proteins are fast acting, potent, and inactive against eukaryotic cells [30]. Lysins have successfully saved mice from bacteremia caused by multi-drug resistant

Acinetobacter baumannii [31], *Streptococcus pneumoni*ae [32], and MRSA [33]. Combining phage lysins and antibiotics may be more effective at eliminating infections than by using antibiotics alone [34]. It is unlikely that bacteria will develop drug resistance to lysins because lysins target sites on peptidoglycan cell wall critical for bacterial viability [35]. Recombinant phage lysin proteins produced by genetic engineering are easier for mass production and administer than actual phage preparations [9].

The future of phage therapy

In an era of multidrug resistance against antibiotics, phage therapy has emerged as an alternative with already proven cases of clinical success. The use of phage therapy creates additional challenges such as the need of increasing phage collections of reference phage banks, development of efficient phage screening methods for the fast identification of the therapeutic phage, establishment of efficient phage therapy strategies that tackle biofilms, set-up of phage production protocols that assure quality and safety of phage preparations, and the guarantee of stability of phage preparation during storage and transport [36]. Two distinct phage therapy approaches had been developed. Broad-spectrum phage cocktails, which were supposed to target the majority of bacteria suspected to cause certain infectious diseases. The other approach is selecting one or more phages targeting the specific bacterial pathogen isolated from the patient's infection site [37].

CONCLUSION

Phage therapy has many applications in human medicine, dentistry, veterinary science, and agriculture. Phages are natural killers of bacteria and possess many characteristics that make them good therapeutic agents either alone or in combination with antibiotics. They are highly specific and safe, and very effective in lysing targeted pathogenic bacteria, and are modifiable to against the emergence of newly arising bacterial infections. Phage therapy appears to be a promising alternative in the treatment of certain multi-drug resistant bacterial infections.

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REFERENCES

1. Clokie MRJ, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage 2011;1(1):31-45.
2. Abedon ST, Thomas-Abedon C, Thomas A, Mazure H. Bacteriophage prehistory: Is or is not Hankin, 1896, a phage reference? Bacteriophage 2011;1(3):174-178.
3. Cui Z, Guo X, Feng T. *et al.* Exploring the whole standard operating procedure for phage therapy in clinical practice. J Transl Med 2019;17:373.
4. Summers W. On the origins of the science in Arrowsmith: Paul de Kruif, Félix d'Hérelle and Phage. J Hist Med Allied Sci 2001;46:315–332.
5. Summers W. The strange history of phage therapy. Bacteriophage 2012;2(2):130–133.
6. Myelnikov D. An Alternative cure: the adoption and survival of bacteriophage therapy in the USSR, 1922-1955. J Hist Med Allied Sci 2018;73(4):385-411.
7. Patay O, McCallin S, Mazure H, Liddle M, Smithyman A, Dublanchet A. Clinical indications and compassionate use of phage therapy: personal experience and literature review with a focus on osteoarticular infections. Viruses 2019; 11:l8.
8. LaVergne S, Hamilton T, Biswas B, Kumaraswamy M, Schooley RT, Wooten D. Phage Therapy for a Multidrug-Resistant *Acinetobacter*

baumannii Craniectomy Site Infection. Open Forum Infect Dis 2018;5(4):ofy064.

9. Dedrick RM, Guerrero-Bustamante CA, Garlena RA et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med 2019;25:730–733.
10. Smith HW, Huggins MB. Successful treatment of experimental Escherichia coli infections in mice using phages: its general superiority over antibiotics. J Gen Microbiol 1982;128:307–318.
11. Soothill JS. Treatment of experimental infections of mice by bacteriophage. J Med Microbiol 1992;37:258–261.
12. Soothill JS. 1994. Bacteriophage prevents destruction of skin grafts by Pseudomonas aeruginosa. Burns 1994;20:209–211.
13. Bogovazova GG, Voroshilova NN, Bondarenko VM. The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental Klebsiella infection. Zh Mikrobiol Epidemiol. Immunobiol 1991;4:5.
14. Bogovazova G G, Voroshilova NN, Bondarenko VM, Gorbatkova GA, Afanas'eva EV, Kazakova TB, et al. Immunobiological properties and therapeutic effectiveness of preparations from Klebsiella bacteriophages. Zh Mikrobiol Epidemiol Immunobiol 1992;3:30–33.
15. Ferriol-González C, Domingo-Calap P. Phage Therapy in Livestock and Companion Animals. Antibiotics (Basel) 2021;10(5):559.
16. Chaudhry WN, Concepcion-Acevedo J, Park T, Andleed S, Bull JJ, Levin BR. (2017) Synergy and order effects of antibiotics and phages in killing Pseudomonas aeruginosa biofilms. PLoS ONE 2017;12(1):e0168615.
17. Narasimhaiah NH. Therapeutic potential of staphylococcal bacteriophages for nasal decolonization of Staphylococcus aureus in Mice. Adv Microbiol 2013;3(1):52–60.
18. Bogovazova GG, Voroshilova NN, Bondarenko VM. The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental Klebsiella infection. Zh Mikrobiol Epidemiol Immunobiol 1991;4:5–8.
19. Pires DP, Meneses L, Brandão AC, Azeredo J. An overview of the current state of phage therapy for the treatment of biofilm-related infections. Curr Opin Virol 2022;53:101209.
20. Brives C, Pourraz J. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. Palgrave Communications 2020;6:100.
21. Adamia RS, Matitashvili EA, Kvachadze LI, Korinteli VI, Matoyan DA, Kutateladze MI, Chanishvili TG. The virulent bacteriophage IRA of Salmonella typhimurium: cloning of phage genes which are potentially lethal for the host cell. J Basic Microbiol 1990;30(10):707-16.
22. Andriashvili IA, Kvachadze LI, Vashakidze RP, Adamia RS, Chanishvili TG. Molecular mechanisms of DNA protection from restriction endonucleases in Staphylococcus aureus cells. Mol Gen Mikrobiol Virusolol 1986;8:43–45.
23. Chernomordik AB. Bacteriophages and their therapeutic-prophylactic use. Med Sestra 1989; 6:44–47.
24. Sulakvelidze A, Alavidze Z, Morris Jr, JG. Bacteriophage therapy. Antimicrobial Agents and Chemotherapy 2001;45(3):649-659.
25. Prins JM, Deventer SJ, Kuijper EJ, Speelman P. 1994. Clinical relevance of antibiotic-induced endotoxin release. Antimicrob Agents Chemother 1994;38:1211–1218.
26. Silver LL, Bostian KA. Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrob Agents Chemother 1993;37:377–383.
27. Brives C, Pourraz J. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. Palgrave Communications 2020;6:100.
28. Ochs HD, Buckley RH, Kobayashi RH, Kobayashi AL, Sorensen RU, Douglas SD, Hamilton BL, Hershfield MS. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency Blood. 1992;5:1163–1171.
29. Ochs HD, Nonoyama S, Zhu Q,Farrington M, Wedgewood RJ. Regulation of antibody responses: the role of complement and adhesion molecules. Clin Immunol Immunopathol 1993;67:S33–S40.
30. Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther 2017;8(3):162-173.
31. Lood R, Winer BY, Pelzek AJ, Diez-Martinez R, Thandar M, Euler CW, Schuch R, Fischetti VA. Novel phage lysin capable of killing the multidrug-resistant gram-negative bacterium Acinetobacter baumannii in a mouse

bacteremia model. Antimicrob Agents Chemother 2015;59:1983-1991.

32. Witzenrath M, Schmeck B, Doehn JM, Tschernig T, Zahlten J, Loeffler JM, Zemlin M, Müller H, Gutbier B, Schütte H, Hippenstiel S, Fischetti VA, Suttorp N, Rosseau S. Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia. Crit Care Med 2009;37:642-649.
33. Schmelcher M, Shen Y, Nelson DC, Eugster MR, Eichenseher F, Hanke DC, Loessner MJ, Dong S, Pritchard DG, Lee JC, Becker SC, Foster-Frey J, Donovan DM. Evolutionarily distinct bacteriophage endolysins featuring conserved peptidoglycan cleavage sites protect mice from MRSA infection. J Antimicrob Chemother 2015;70:1453-1465.
34. Wang Q, Euler CW, Delaune A, Fischetti VA. Using a novel lysin to help control Clostridium difficile infections. Antimicrob Agents Chemother 2015;59:7447-7457.
35. Roach DR, Donovan DM. Antimicrobial bacteriophage-derived proteins and therapeutic applications. Bacteriophage 2015;5:e1062590.
36. Diana P Pires, Ana Rita Costa, Graça Pinto, Luciana Meneses, Joana Azeredo, Current challenges and future opportunities of phage therapy. *FEMS Microbiology Review*s 2020;44(6):684–700.
37. Pirnay JP, Verbeken G, Ceyssens PJ, Huys I, De Vos D, Ameloot C., et al. The magistral phage. Viruses 2018;10:E64.