



Bacteriophages an Alternative to Antibiotics for the Treatment of Bacterial Infections: Systematic General Review

Syed Mansoor Ahmed^{1*}, Syed Junaid Ahmed²

¹Department of Biotechnology, Mirpur University of Science and Technology, Mirpur, Pakistan

²Department of Pharmacy, Birmingham City University, Birmingham, United Kingdom

ABSTRACT

Bacteriophages (BPs) also known as peptible bacteria without causing any harm to humans, animals or any other bacteria that are beneficial. Because of this, it is supposed that they can be used to treat bacterial infections either alone or can supplement the treatment of bacterial infections with antibiotics. In view of serious health problems of antibiotics resistance by the bacteria, researchers are seriously looking for alternative non-bacterial methods. Among this phage therapy seems to be an interesting option. Here we reviewed different research papers from PubMed, ResearchGate relating to bacteriophages as an alternative therapy for bacterial infections. All the research that has been done so far indicates that it is rational to use phage therapy as an alternative to antibiotics; however few problems still have to be overcome before implementing it to a large scale.

Keywords: Bacteriophages; Alternatives of antibiotics; Phage therapy; Phage cocktail; Multidrug resistant; Bacterial infection

INTRODUCTION

Wide use of antibiotics to treat bacterial infection led to an increase in resistance in bacteria against these antibiotics. Bacterial resistance to antibiotics is not new to the medical world. In fact the first case of penicillin resistance to *Staphylococcus aureus* was reported in the 1940's [1]. Extensive use of antibiotics and the natural evolution of bacteria over time has led to an increase in numbers of resistant strains. This becomes one of the most serious threats to global health these days. According to a report, at least 2.8 million people are infected with antibiotic resistant

bacteria, and more than 35,000 people die as a result each year just in the US [2]. Many research activities are carried out for alternative methods to treat bacterial infection. Among these, phage therapy seems an interesting and very good option, in which Bacteriophages (BPs) also known as phages that are viruses are used to infect and kill bacteria (**Figure 1**).

Received:	04-March-2022	Manuscript No:	IPBMBJ-22-12554
Editor assigned:	07-March-2022	PreQC No:	IPBMBJ-22-12554 (PQ)
Reviewed:	21-March-2022	QC No:	IPBMBJ-22-12554
Revised:	10-October-2022	Manuscript No:	IPBMBJ-22-12554 (R)
Published:	17-October-2022	DOI:	10.36648/2471-8084.8.12.107

Corresponding author Syed Mansoor Ahmed, Department of Biotechnology, Mirpur University of Science and Technology, Mirpur, Pakistan; E-mail: mansoor_shah125@yahoo.com

Citation Ahmed SM, Ahmed SJ (2022) Bacteriophages an Alternative to Antibiotics for the Treatment of Bacterial Infections: Systematic General Review. *Biochem Mol Biol J.* 8:107.

Copyright © 2022 Ahmed SM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



Figure 1: T-4 Bacteriophage virus as seen through electron microscopy.

It was first noticed by scientist Ernst Hankin in 1896 that water of Ganga and Yamuna rivers in India shows reasonable anti-bacterial activity against *Vibrio cholera* and suggested that cholera epidemics spread was limited due to this activity [3]. Bacteriophages were first recognized by Twort and d'Herelle in 1915 and 1917 [4]. These bacteria based viruses are very abundant in the biosphere, roughly about 10 times more than the total bacterial cells [5]. They are extremely specific in killing bacteria without any harmful effects to humans, animals and plants [6]. Rigorous activities are being done to use phages to treat bacterial infections either alone or in supplement with antibiotics. Though phage therapy is not approved yet, it tends to be more successful as compared to antibiotics, as the phages are very specific, with reduced chances of opportunistic infections, and also replicate *in vivo*, so fewer doses is needed. Extreme specificity of phage is also a problem sometimes, as it will infect and kill only bacteria with matching strain [7]. This problem can be overcome by using a mixture of phages (cocktails) instead of a single phage. This is called phage cocktail strategy. Over the last decades several significant works is carried out to evaluate the phage cocktails to treat burn injuries infected by *Escherichia coli* and *Pseudomonas aeruginosa*. There are still many problems not solved yet regarding commercial use of bacteriophages to treat bacterial infection in humans, that's why, is not approved yet by the regulatory authorities. In this review, evaluated the different research papers from NCBI and pubmed and research activities unpublished in any journals

present on the internet relating to bacteriophages as an alternative therapy for bacterial infections and specifically bacteriophage cocktails strategy.

Highlights

- Bacterial resistance to antibiotics treatment has become one of the most serious threats to global health these days.
- Several features of bacteriophages like high specificity harmless to humans, plants or animals make them an attractive therapeutic agent.
- Of all the clinical trials done so far, very few trials are randomized and controlled and that too result in partially negative.
- Problems to prepare the formulations for standardized and clinical use in bacterial control, limit the risk of emergence of bacterial resistance needed to be solved.
- The cocktail preparations for different bacterial infections must be optimized and also immune responses to different phage therapy must also be monitored closely and overcome so that phage therapy can be introduced into clinical practice.
- Seeing the encouraging data obtained so far and the advancement of biotechnology, we can expect that within a decade, bacteriophages either alone or in combination with antibiotics will be used in clinical practice globally to treat all the chronic infections that will be either completely resistant to all available antibiotics.

LITERATURE REVIEW

Methodology

Research work done on Bacteriophages as an alternative therapy for bacterial infections was reviewed by independent reviewers (SMA) using electronic databases. Studied some of the important research articles published in pubmed and google scholar and some unpublished data on the internet related to the topic. Search terms used for this purpose included Bacteriophages, alternatives to antibiotics, phage therapy, phage cocktail, bacteriophages as an alternative to antibiotics.

Exclusion Criteria

The search included all published literature with the abstract available in english and some unpublished data also. No further exclusion selection criteria were applied.

Study Selection

A total of 68 studies were initially identified and considered potentially relevant. Of these, 39 studies did not meet the inclusion criteria. Twenty nine studies were evaluated in detail. Seventeen studies meeting the selection criteria were considered for the current review and analysis [8].

Phage Therapy

Phage therapy is the therapeutic use of bacteriophages to treat infections caused by pathogenic bacteria. This

phenomenon was first observed by Twort and d'Herelle in 1915 and 1917. Bacteriophages are viruses and like all other viruses are very simple in structure, contain DNA surrounded by a protective capsid coat (Figure 2).

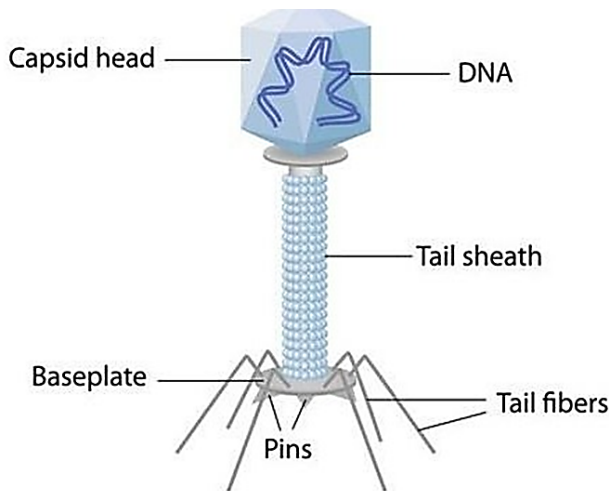


Figure 2: Structure of bacteriophage and illustration of its part.

They infect in the similar way like other viruses, attach to the receptors on the bacterial cell surface, inject its DNA which replicate inside the bacteria and finally cause the lysis of bacteria and release to infect other similar bacteria in the surrounding and stop the infection shown below in Figure 3. The lysis of bacterial cell occur either soon after the replication of viral DNA inside bacterial cell (lytic cycle) or take time in lysing and become integrated into the bacterial chromosome and replicate with it and pass on to the progeny without causing any harm, but at some stage when the bacterial cell deteriorate probably because of depletion of nutrients, the lytic cycle will become active [9,10].

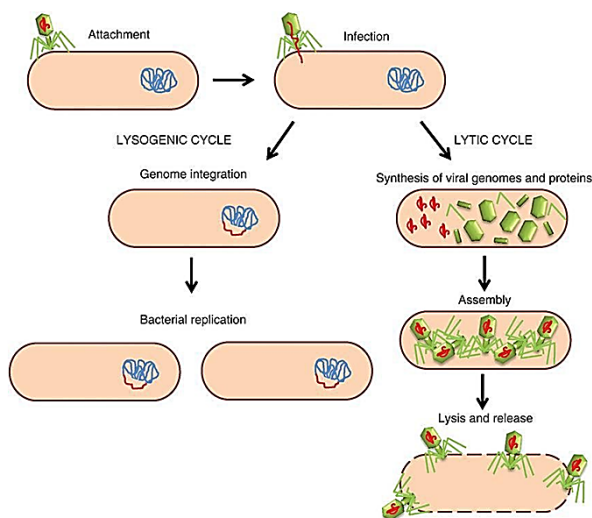


Figure 3: Bacteriophage life cycle.

Bacteriophage Life Cycle

Lytic phages attach and infect a bacterial cell which results in the reproduction of phages and lysis of the cell host and this lysogenic cycle results in the integration of a phage genome into the bacterial genome. Phage therapy was licensed to be used in the early 1930's [11]. Eli Lilly Company then produced seven phage products and phage therapy was used in the early 1940's commercially [12]. But after the start of antibiotics, the phage therapy use came to a halt in most parts of the World and only practiced in Russia and Poland on a small scale [13]. Some of the important studies using different bacteriophages and using phage cocktail therapy, carried out by different researchers are discussed briefly hereby:

DISCUSSION

Studies Using Animals

One of the most important studies to evaluate the effectiveness of bacteriophage to treat an infection was carried out by Smith et al. back in the 1980's. He evaluated the efficacy of specific *E.coli* bacteriophages in acute diarrhea in the mice. The results were very interesting and encouraging. He found that six strains of pathogenic *E.coli* can be degraded by single dose of pool of six bacteriophages specific for pathogenic *E.coli* [14]. The same researchers after one year evaluated the efficacy of specific phage therapy in treating the diarrheal infection of experimental *E.coli* in calves, piglets and lambs and obtained similar results as they obtained in case of treating acute diarrhea in mice [15,16]. One of the advantages of using phage therapy as known by one of the studies performed in 1991 and 1992, phage therapy does not require repeated dose of administration as that is needed for antibiotics for several days [17,18]. Many scientists then analyzed the success of this experiment and this encourages them to further experiments towards use of bacteriophages in bacterial infections. A study carried out in 1992 showed that induced infection in mice by experimental *Acinetobacter baumannii*, *P. aeruginosa*, and *S. aureus* pathogens can be controlled by specific bacteriophages [19]. Two years later in another study carried out by Soothill, et al. it showed that use of bacteriophage can be effective in guinea pigs after skin graft that are susceptible to infection by *Pseudomonas aeruginosa* [20]. Many recent studies have also shown that many food producing animals after treatment with bacteriophages are less likely to be susceptible to the contamination of food by bacteria during processing. Among these studies included the research of who worked on the use of phage therapy to reduce *Campylobacter* spread in humans from chickens. Many attempts to treat respiratory infections of animals have also been made using bacteriophages. For example, Hawkins, et al. in 2010 investigated the treatment of chronic, refractory *P. aeruginosa* otitis media in ten dogs using specific bacteriophages. He instilled a single dose of pool of each of 6 bacteriophages, specific for these pathogens, directly into the auditory canal of one ear. He then assessed the conditions of the ear by evaluation of a clinical score

based on 5 indicators, and measured bacteriophage and *P. aeruginosa* concentrations. Results revealed that bacteriophage therapy was very effective and safe, as pathogens mean counts decreased by 67%, accompanied by a significant increase in bacteriophage counts, showing significant viral replication. Furthermore this therapy did not cause any other adverse effects. Moreover, use of bacteriophages with an aerosol spray also reduced the mortality of broiler chickens with *E. coli* respiratory infection and limited the mice infection by *P. aeruginosa*, *Klebsiella pneumoniae* or *Burkholderia cenocepacia*. Over the past few years, research focusing on using phage cocktails (simultaneous use of more than one phage type) also termed as polyphagia therapy tends to increase as the results of using single phage preparation is not quite good in some cases. The aim of using cocktail therapy is not to replace single phage preparation but instead to broaden the spectrum of treatment of bacterial infection. In a study carried out by Kelly, et al. developed a cocktail therapy against previously resistant strains of *S. aureus*, based on *Staphylococcus* phage K. Cocktail was prepared by using six of the most potent phage derivatives, along with the original phage K. This cocktail result in broadening the spectrum of activity compared with *Staphylococcus* phage K used as a single phage preparation. Like Kelly also developed a phage cocktail preparation, consisting of three phages, tested for efficacy against pathogenic *Klebsiella pneumoniae* infection in mice. A single dose of cocktail preparation administered intraperitoneally 1 h after *Klebsiella pneumoniae* inoculum resulted in 100% recovery.

Studies Using Human

Extensive research is ongoing to investigate the efficacy of bacteriophages to treat bacterial infections in humans; most of this research is focused on topical use of bacteriophages to treat skin infections. Different scientists have shown the beneficial effects of bacteriophages on topical applications in case of burns, wounds, diabetic foot ulcer during and after the second world war. Reported in the 1990's that infection caused by multidrug resistant *P. aeruginosa* in burns can be cured by the use of specific bacteriophages, and 18 out of 30 adult patients in whom he used this therapy allowed successful skin grafting. Recent works done by researchers like from 2009 to 2018 also confirmed the beneficial effects of bacteriophages in the treatment of local topical infections for instance treatment of numbers of diabetic foot infections, which previously show resistance to antibiotic therapy. But all these research on topical infections were not randomized. The only randomized clinical trials on bacteriophage efficacy in topical infections were carried out in 2018. He used a cocktail preparation of 12 phages but unfortunately his results were very disappointing that sulfadiazine cream showed more efficacy than the cocktail preparation. In 1993 proposed that use of bacteriophages orally is effective in treating cholera, but yet no randomized, placebo controlled trials have been done in patients with this disease. Similarly, the studied carried out proposed that the use of a two phage cocktail is effective in the treatment of diarrheal infection caused by

E. coli, but the results of later randomized and placebo controlled clinical trials carried out were disappointing, where the two phage cocktail preparation did not show very positive results. Some *in vitro* studies also suggested that use of bacteriophage cocktail therapy can be effective in treating patients with cystic fibrosis. But yet no randomized and placebo controlled clinical trials done on patients enrolling with cystic fibrosis. Though some cases have shown the efficacy of bacteriophages when administered with aerosol, but we cannot draw a definitive conclusion about the relevance of bacteriophage with cystic fibrosis treatment. The Figure 4 given below demonstrates the timeline of some of the important events that occur during the studies of Phage therapy and antibiotics.

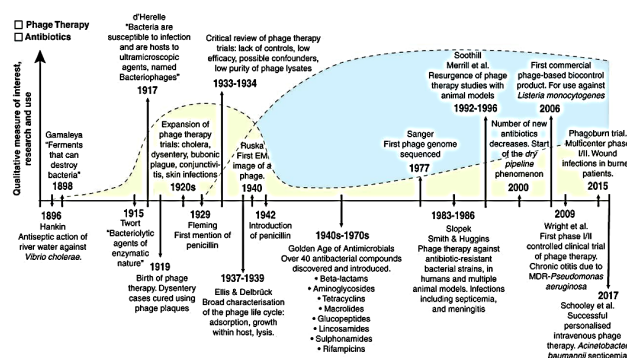


Figure 4: Timeline of some of the important events that occur during the studies of phage therapy and antibiotics.

The curves in the figure represent a qualitative measure of the overall interest, research, and use of phage therapy and antibiotics, showing how the introduction of antibiotics and the critical review of the early phage therapy studies coincided to bring phage therapy research and development to an almost complete standstill around the 1940's.

CONCLUSION

In conclusion, several features of bacteriophages like high specificity toward infecting and lysing pathogenic bacteria only without causing any harm to humans, plants or animals make them an attractive therapeutic agent. The recent research also indicated that it is rational to think phage therapy against pathogenic bacterial infections as an alternative to conventional antibiotics treatment. However, the data obtained from the recent research is not enough to use bacteriophage therapy in humans. Of all the clinical trials done so far, very few trials are randomized and controlled and that too result in partially negative. Also the problem to prepare the formulations for standardized and clinical use in bacterial control, limiting the risk of emergence of bacterial resistance is not solved. In view of global health problems by increasing antibiotics resistance, desperate need of alternative strategies to treat bacterial infection and research data collected so far regarding phage therapy, further studies need to be carried out to solve the above mentioned problems that limit the use of bacteriophage therapy.

Future Perspective

Keeping in view the useful and encouraging data obtained so far by the researchers regarding phage therapy and the fact that bacterial resistance to antibiotics treatment has become a serious threat to global health, there is a need to start over all the clinical trials of bacteriophage therapy using modern biotechnology. This time these clinical trials must be randomized and placebo controlled. Currently, phage therapy is used only in Poland and Russia on a very small scale so it is much needed that the scientists and pharmaceutical companies in the western world must carry out more controlled research and must overcome all problems before finally using this for humans in the Western side and in America. The cocktail preparations for different bacterial infections must be optimized and also immune responses to different phage therapy must also be monitored closely and overcome so that phage therapy can be introduced into clinical practice. Seeing the encouraging data obtained so far and the advancement of biotechnology, we can expect that within a decade, bacteriophages either alone or in combination with antibiotics will be used in clinical practice globally to treat all the chronic infections that will be either completely resistant to all available antibiotics.

Limitations with the use of Bacteriophages

Despite rigorous research on bacteriophages as an alternative to conventional antibiotic therapy in bacterial infection, still it is not approved by the FDA, because we don't have enough data regarding use of bacteriophage therapy in humans and also all the research done so far is not randomized or placebo controlled. Also, the preparations of bacteriophages for clinical use is difficult, and many problems related to the biology of the bacteriophages need to be solved first.

Some of the factors that limit the development of new phage preparations includes:

- Absence of specific activity in bacteriophage against certain bacterial strains is one of the limitations in the use of phage therapy.
- The genome of bacteriophage must be sequenced and free from integrate genes, antibiotic resistant genes, genes for phage-encoded toxins or genes for other bacterial virulence factors for the development of new phage preparations. Difficulty in the production of sequenced genomes of bacteriophage is another limiting factor.
- Another limiting factor in the development of new phage preparations is that formulation and stabilization of pharmaceutical preparations for clinical use undergo some problems, hence stabilization strategies should be optimized for each bacteriophage separately which can result in very costly clinical trials and is discouraging for pharmaceutical companies to carry out preparations for humans.
- Bacterial cell can adopt several mechanisms to prevent viral infections probably by change or loss of receptor, by inhibiting phage DNA injecting in the cell, or by blocking phage DNA replication and cell lysis. Because of these

mechanisms, it is quite possible that bacteria can develop resistance against specific phage. This is another important factor that limits the use phage therapy. However this problem can be overcome by using phage cocktails that is a mixture of different phages to kill pathogenic bacteria.

- Another factor that limits the use of bacteriophage to treat bacterial infection commercially is that phages when following lysogenic infection cycle may integrate their DNA into bacterial chromosomes, causing exchange of genetic material. This transduction can result in the development of more resistant bacteria against antibiotics.
- Finally, reduced activity of bacteriophages as a result of response shown by the immune system toward bacteriophages also limits the development of new phage preparation against bacterial infection. This is because bacteriophages and their products are non-self antigens and recognized by the immune system which then act by reducing the benefits resulting from bacteriophage administration.

COMPETING INTERESTS

No significant competing interests were declared by the author.

REFERENCES

1. Foster TJ (2017) Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiol Rev.* 41(3):430-449.
2. Tarsillo B, Priefer R (2020) Proteobiotics as a new antimicrobial therapy. *Microb Pathog.* 142:104093.
3. Sharma S, Chatterjee S, Datta S, Prasad R, Dubey D, et al. (2017) Bacteriophages and its applications: an overview. *Folia Microbiol (Praha).* 62(1):17-55.
4. Duckworth DH (1976) Who discovered bacteriophage? *Bacteriol Rev.* 40(4):793-802.
5. Laemmli UK, Eiserling FA (1968) Studies on the morphogenesis of the head of phage T-even. *Mol Gen Genet.* 101(4):333-345.
6. Fischetti VA (2008) Bacteriophage lysins as effective antibacterials. *Curr Opin Microbiol.* 11(5): 393-400.
7. Hagens S, Loessner MJ (2007) Application of bacteriophages for detection and control of foodborne pathogens. *Appl Microbiol Biotechnol.* 76(3):513-519.
8. Nandhagopal N, Simpson AA, Gurnon JR, Yan X, Baker TS, et al. (2002) The structure and evolution of the major capsid protein of a large, lipid-containing DNA virus. *Proc Natl Acad Sci USA.* 99(23):14758-14763.
9. Boyd JSK (1951) Observations on the relationship of symbiotic and lytic bacteriophage. *J Pathol Bacteriol.* 63(3):445-457.
10. Boyd JSK (1951) Observations on the relationship of symbiotic and lytic bacteriophage. *J Pathol Bacteriol.* 63(3):445-457.

11. Summers WC (2012) The strange history of phage therapy. *Bacteriophage*. 2(2):130–133.
12. Sulakvelidze A, Alavidze Z, Morris JG (2001) Bacteriophage therapy. *Antimicrob Agents Chemother*. 45(3):649–659.
13. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM (2011) Phage treatment of human infections. *Bacteriophage*. 1(2):66–85.
14. Smith HW, Huggins MB (1982) Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. *Microbiol*. 128(2):307-318.
15. Smith HW, Huggins MB (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J Gen Microbiol*. 129(8):2659-2675.
16. Smith HW, Huggins MB, Shaw KM (1987) The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J Gen Microbiol*. 133(5): 1111-1126.
17. Bogovazova GG, Voroshilova NN, Bondarenko VM (1991) The efficacy of *Klebsiella pneumoniae* bacteriophage in the therapy of experimental *Klebsiella* infection. *Zh Mikrobiol Epidemiol Immunobiol*. 4:5-8.
18. Bogovazova GG, Voroshilova NN, Bondarenko VM, Gorbatkova GA, Afanas'eva EV, et al. (1992) Immunobiological properties and therapeutic effectiveness of preparations from *Klebsiella* bacteriophages. *Zh Mikrobiol Epidemiol Immunobiol*. 3:30-33.
19. Soothill JS (1992) Treatment of experimental infections of mice with bacteriophages. *J Med Microbiol*. 37(4): 258-261.
20. Soothill JS (1994) Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa*. *Burns*. 20(3): 209-211.