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Bacteriophages as affordable solution for treatment of multidrug resistant bacteria, and their recent potential applications

Ghadah A. Alsubhi[1,2*]

[1]Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; [2]Immunology Unit, King Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia

[*]Corresponding author E-mail: gkhameesalsubhi@stu.kau.edu.sa

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Abstract

The impact of bacteriophages as antibacterial agents was known after their first discovery in 1915, and they were then employed to treat the bacterial infections. However, the discovery of penicillin in 1928 quickly overshadowed bacteriophage therapy, and paved the way for its large-scale production in the 1940s to enter the era of antibiotics. In recent years, resistant bacteria have contributed to increasing the studies on bacteriophages. A remarkable difference between phages and antibiotics is the remarkable phage's specificity to infect certain types of bacteria, which makes them excellent alternatives for treatment of the bacterial infections. Moreover, bacteriophages have different life cycles; knowing the differences between each cycle is essential to exploit the benefits of phages to humans. This review aimed to highlight the history of discovering the bacteriophages and their characteristics; discusses the numerous phages applications including phage therapy, and the limitations of their use.

Keywords: Bacteriophages; Phage therapy; Phage therapy limitations

1. Introduction

The term bacteriophage or phages refers to viral bacterial eaters, since viruses typically attack and eventually destroy the bacteria (Rastogi *et al*., 2016). Bacteriophages are considered as the most abundant microorganisms on earth. It is pre-estimated that the planet has almost 10^{31} bacteriophages (Hatfull, 2015). They were first discovered in 1915 by Frederick Twort, a British bacteriologist; where he reported a phenomenon that appeared in his bacterial colonies. He called this phenomenon as a glassy transformation. Twort did not complete the subsequent works for this discovery, due to the lack of funding and the start of World War I. Later, in 1917, Félix d'Hérelle discovered an agent that caused the glassy

transformation. He noticed the disappearance of *Shigella* colony when he filtered sewage water on this colony. Félix d'Hérelle originated the term bacteriophage, and published this finding in the form of an article entitled "An invisible antagonistic microbe of the dysentery *bacillus*" (D'Herelle, 1917; Letarov, 2020).

Phages have nucleic acid, which may be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). This nucleic acid can exist in the form of double or single strand. This molecule is enveloped by a capsid protein coating the phage tail, which is associated to the capsid functions by binding to the host receptor sites (Kurtböke, 2012). Phages usually attach themselves to their host bacteria and then infect them (Dion *et al*., 2020). Most phages that have been successfully classified morphologically are members of the order Caudovirales; where members of this order possess double-stranded DNA genomes. Within this order, three families can be distinguished based on the tail's appearance and morphological features. They include the *Myoviridae* family, which possess long contractile tails; the *Siphoviridae* family that is recognized by its longer, thinner and non-contractile tails, and the *Podoviridae* family which has short tails (Kurtböke, 2012; Dion *et al*., 2020).

2. Bacteriophage life cycle

According to the previous study conducted by Dowah and Clokie, (2018), the bacteriophages have two different life cycles: lytic and lysogenic life cycles. In both types of cycles, physical attachment of the phage to the bacteria is essential for phage infection. The lytic cycle begins with attachment of the bacteriophages to a single or multiple receptors on the bacterial host surface. Most phages adhere to the cell wall of the bacteria; however, some of them adhere to the pili or the flagella. Bertozzi Silva *et al*., (2016) reported that viral specificity of the phages ensures that only particular strains of the phages can attach to certain strains of their hosts. This specificity of adhesion is controlled by receptor-binding proteins (RBPs) of the phage, which recognize receptors in the host. Hyman, (2019) revealed that the second step involves penetration of the phage into the bacteria. When the phage successfully attaches itself to the surface of the bacteria; injection of the viral DNA takes place successfully via an enzymatic cleavage. During this step, enzymes from the bacteriophage make a hole in the bacterial cell wall. Some phages contract a sheath, which pushes a hollow tube into the bacteria. In cases where phages have adsorbed to the bacterial pili or flagella; their genomes enter through these parts. In both cases, there is no uncoating of the viral protein since only the bacteriophage's DNA enters the host cell. The third step is called replication, where the phage enzymes shut down the bacterial synthesis of DNA, RNA and protein synthesis of the different macromolecules. Consequently, the phage copies its genome by utilizing the metabolism of the host bacteria for synthesis of more phage enzymes, and phage structural components including the sheath and the head. The maturation phase then follows; involving assembly of the phage components around the genome. After that, the phage codes a gene that is responsible for disintegrating the peptidoglycans in the host bacterial cell wall. Disintegration of the cell wall leads to the lysis of the bacteria. Assembled and intact bacteriophages are then released. Finally, the last stage involves reinfection; where almost 50-200 phages may be produced from each infected bacterium. The infecting phage kills the host bacterium in the lytic cycle (Batinovic *et al*., 2019).

Temperate phage also known as non-virulent phage has a lysogenic life cycle, which doesn't include lysis of the bacterial cell (Batinovic *et al*., 2019). This temperate phage utilizes the host as a refuge for its dormant state. In the lysogenic cycle, the injected genome of the bacteriophage will be integrated and inserted into the bacterial genome by using the integrase enzyme. This condition is known as prophage (Fig. 1). A previous study of Howard-Varona *et al*., (2017) highlighted that the temperate phage will enter the lytic cycle if the bacteria come across challenging conditions such as ultraviolet radiation or antibiotic treatment. When the bacteria

encounter these conditions, induction of the temperate phage occurs. The phage begins the lytic cycle, which terminates in lysis of the bacterial cell and release of the viral progeny.

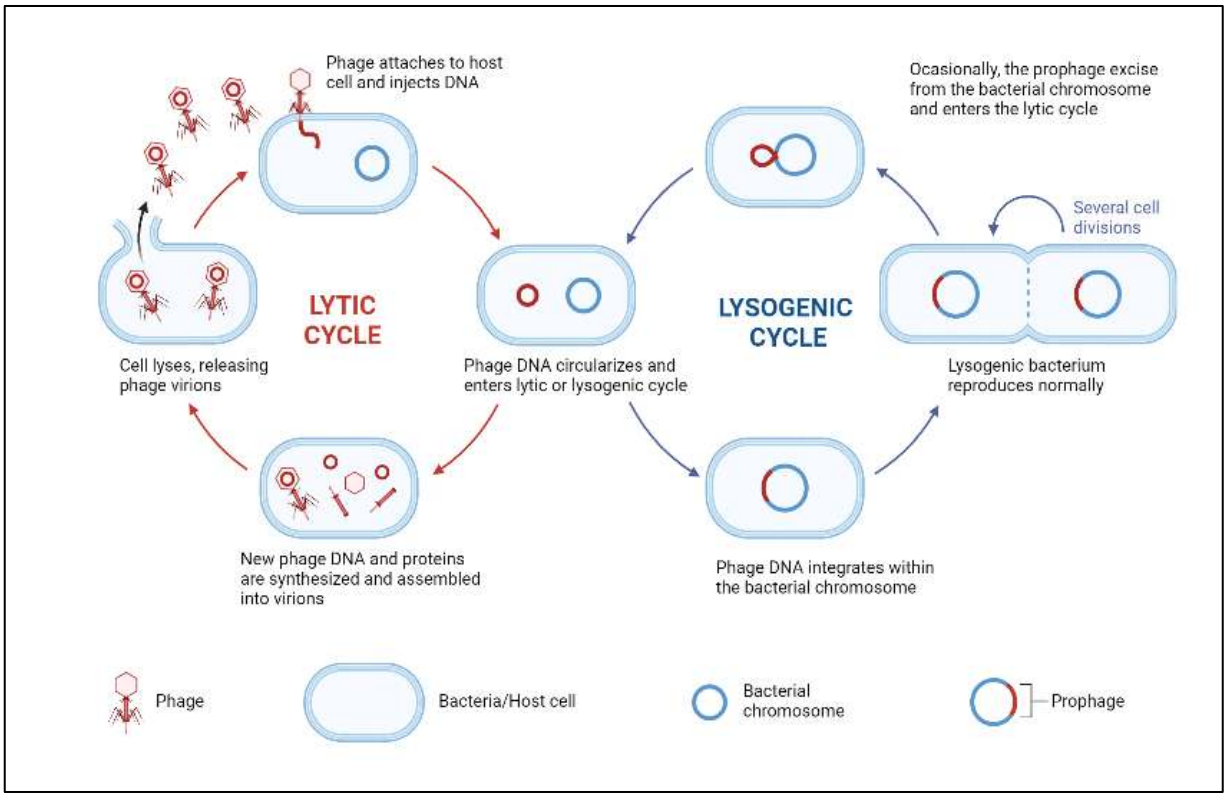

Fig. 1: Diagram of the bacteriophages life cycle

Both of the lytic and lysogenic life cycles involve phage attachment to the host bacterial cell. Then the phage DNA selects to enter the bacterial cell either through the lytic cycle or the lysogenic cycle. In the lytic cycle, consequent multiplication and release of mature viruses follow the bacterial lysis. The lysogenic cycle involves integration of the bacteriophage's genome into the genome of the host bacteria; in this condition it is known as a prophage. This figure has been designed using BioRender (https://app.biorender.com/).

3. Bacteriophages host range

Host range refers to the limitation of a particular bacteriophage strain to a specific strain of the host bacteria. Some phages can infect different bacterial species, while other phages are limited to a single strain. It is believed that almost all bacteriophages can infect a narrow range of bacterial strains, due to the host phage-binding specificity (RBPs), as revealed by Hyman and Adedon, (2010). The host range of phages is determined via different types of assays, including the plaque assay; by testing the phage's ability to form a plaque in a specific species of bacteria. The spot test is another assay used to determine the host range of phages. In this test, a small drop of phage is placed on a growing lawn of bacteria to test the ability of the phage to infect the bacteria. Some phages have a broad host range, which is defined as the ability of the phage to infect various bacterial species and various strains of the same species (Ross *et al*., 2016). For example, a recent study conducted by Hyman, (2019) reported that coliphages such as Mu phage can infect two hosts groups, since they have two sets of tail fibers.

Phages are highly specific to the bacterial receptors. This specificity can be used to increase the effectiveness of the bacteriophage therapy. However, testing for the presence of inducible prophages is essential for the clinically isolated bacteria; as temperate phages are not desirable for bacteriophage therapy. Therefore, when choosing an isolation strain; it is advisable not to use isolation hosts, which could be induced to release non-virulent phages (Abedon, 2020).

4. Phage applications

Since time immemorial, phages have been used in several industries, ranging from healthcare to the food industry; with incredible success. An example of phage applications is its use in the detection of bacteria. Bacteriophages are highly specific and infect only a limited range of host bacteria. This trait is essential and can differentiate among different strains of bacteria within the same species, which were previously indistinguishable through serological testing's (Hassan *et al*., 2021). Furthermore, phages can be engineered to show bioluminescence on infecting specific bacteria. This type of engineered phage has been used to detect various bacteria such as; *Staphylococcus aureus* (Šuster *et al*., 2017), and *Salmonella* spp. (Kim *et al*., 2014).

Another new application is the phage display, which is a new technique that uses genetically modified phages as platforms to deliver anticancer drugs (Garg, 2019), antibodies (Sioud, 2019), in addition to antigens as vaccine vectors (Hess and Jewell, 2020). This technique is achieved by inserting the DNA that needs to be delivered into the phage capsid gene. After that, the gene of interest will be encoded during the phage replication (Pires *et al*., 2016).

Phages are also used as indicators, to indicate the microbiological quality of water. For example, the presence of coliphages and enterococci bacteriophages in wastewater is considered as indicator of faecal contamination with *E. coli* and *Enterococcus* spp. Somatic coliphages also provide more information than bacterial indicators in certain freshwaters (Barrios *et al*., 2018). Moreover, bacteriophages are used in food safety; as they act as novel biocontrol agents in food production and preservation. In a previous study, Capita and Alonso-Calleja, (2013) documented that phages have been used as direct spray to cause lysis of different pathogens including; *Listeria* spp., *Salmonella* spp., *Campylobacter* spp., and *E. coli* on fruits, vegetables and meat. Recently, Brives and Pourraz, (2020) revealed the previous use of phages in treatment of bacterial infections. With the rising incidences of antibiotic resistance; phage therapy is once again a valid treatment option.

5. Phage therapy

Phage therapy is the use of phages to treat bacterial infections. Bacteriophages are viruses that are capable of infecting and killing bacteria. Meanwhile, there are no harmful impacts on human or animal cells as a result of this bacterial damage.

According to Torres-Barceló and Hochberg, (2016), phages can be used alone or in combination with conventional antibiotic therapy to treat bacterial infections. Earlier, Felix d'Herelle's study (d'Herelle, 1926) provided essential knowledge and framework for treatment with bacteriophages. The first large-scale use of this treatment method was in the Soviet Union; where patients afflicted with various bacterial diseases were treated, and this treatment proved successful. However, due to the difficulty to understand the Russian language of these early publications, they did not go to the west (Chanishvili, 2016). After the 2nd world war; antibiotics were proved easier and cheaper to use. Gibson *et al*., (2019) reported that antibiotic therapy caused the lack of enthusiasm with which phage therapy was viewed in the west. Phage therapy provides an avenue for the application of novel treatment procedures against bacterial infections (Golkar *et al*., 2014). The increased use of

antibiotics especially in the third-world countries; has contributed to the increased bacterial pathogens resistance against antibiotics. Due to this defect; treatment of bacterial diseases has grown more complex especially against Gram-negative bacteria, thus few new antibiotics have been developed (Ghosh *et al*., 2019).

A previous study conducted by Gibson *et al*., (2019) reported that more information is required before a phage strain can be used in the treatment of a bacterial infection. These include isolation of the host and phage; growth properties, analysis of sequence, determination of the host range and virulence properties, which are presented in several contemporary clinical isolates. Establishment of this information is vital before adding a phage to the phage libraries for its potential use. According to Malik *et al*., (2017), the plaque assay is used to test for the isolation of *E. coli* and cloaca phages from the environment. On the other hand, the titration protocols are used to determine the phage host range and virulence.

6. Gut phages and their therapy

Numerous bacteriophages exist naturally within the human skin, genital tract, nasal cavity, oral cavity and the gut (Barr, 2017; Łusiak-Szelachowska *et al*., 2020). Most of the phages in the human gut are non-virulent bacteriophages; therefore, most bacteria in the human body host prophages. Under stressful conditions, when induction occurs in these prophages; it can imbalance the various microflora. This induction may be caused by diet and by different therapies such as chemotherapies and\or the lifestyle. Knowledge of these prophage stimulators is essential to control the microbial activity in case of dysbiosis, which could lead to more advances and further implementation of phage therapy, as highlighted recently by Ganeshan and Hosseinidoust, (2019); Royer *et al*., (2021).

It is essential to know how lytic phages used in human therapy interact with prophages in the gut. When these prophages are induced by the action of lytic phages directly or indirectly, they may lead to disturbance of the gut microbiota. They may also lead to more complications of the environment where the phage used for therapy is supposed to function (Ganeshan and Hosseinidoust, 2019; Royer *et al*., 2021).

A previous work conducted by Sutton and Hill, (2019) reported that the goal of using the bacteriophages in microbiome therapy is to modify the human microbiome; as a therapeutic method to fight many of the chronic or degenerative illnesses thought to be related with dysbiosis in the gut microbiota. Bacteriophages express highly specific targeting on the bacteria. When virulent or temperate phages are utilized in gut therapy, they enable the manipulation and engineering of the gut microbiota to achieve positive effects, which could lead to more healthier and balanced microflora (Paule *et al*., 2018).

Different previous scholarly groups have stated contrasting views on the effects of bacteriophages on the gut. Some have postulated that bacteriophages lead to minimal alterations in the phylogenetic constitution of the gut (Hibbing *et al*., 2010). On the contrary, other groups stated that these phages lead to significant changes in the phylogenetic composition of the gut. These inconsistent reports may be attributed to the difference in microbiota and gnotobiotic models used (De Sordi *et al*., 2017). There are other differences related to the used host systems during the study. For example, particular phages persist for weeks or even months in the gut, while others take just a few hours. External factors including the presence of various ions and acidity may also affect the successful administration efforts and persistence of the phages in the gut microbiome. Moreover, the acidic gastrointestinal conditions cause a significant reduction in the phage titer and stability. This fluctuation in the acidity levels is witnessed in a fasting human whose median gastric pH is 1.7, compared to the other regions of the digestive tract that have a pH more than 6

(Dąbrowska, 2019). These considerations affect the efficacy of phage therapy in the gut. However, antacids could be administered orally concurrently with phage therapy. Furthermore, another solution may be suggested is encapsulation of the therapeutic phages in protective matrices (Ganeshan and Hosseinidoust, 2019).

7. Successful cases of phage therapy

Various cases showed high efficiency of phage therapy. In one case reported by Fish *et al*., (2016), about 9 patients were suffering from diabetes and their toe ulcers had poor perfusion. Tests confirmed the presence of *S. aureus* infection in the soft tissue and the bone. The patients had failed to respond positively to the antibiotic therapy, and the only alternative therapy in these cases was amputation. On the other hand, phage application was made topically once a week, which varied based on the area and volume of the wound. The phage therapy was successful since all the ulcers had a positive response and healed within an average of 7 weeks. However, one extreme case required treatment for 18 weeks due to poor vascularization of the toe.

Another successful case recorded in Egypt involved Patterson; a Psychiatry professor, who was infected with a multi-drug resistant *Acinetobacter humanii*. Antibiotic therapy using colistin, tigecycline and meropenem was the only effective treatment. However, an accidental slip caused his drain to spill the pouched bacterial load into the bloodstream and the gut. Patterson went into a septic shock and was unresponsive. Within 3 days of application of a novel phage treatment, Patterson awoke from his coma and regained most of his body functions on his way to recovery, as recorded by LaFee and Buschman, (2017). These two case studies showed the immense success that has been experienced with phage therapy in cases where antibiotic therapy has failed. Personalization of the dosage and treatment regimen in these two case studies was beneficial.

8. Limitations of phage therapy

Despite its attractiveness, phage therapy also has several limitations. One obvious limitation that may defeat the process is that bacteria may develop resistance to phage therapy similar to their development of antibiotic resistance. However, this problem may be solved by using a wide range of a specific phage. Another way to mitigate bacterial resistance would be using cocktails of phages (Torres-Barceló, 2018). Patterson's case cited earlier employed this form of therapy (LaFee and Buschman, 2017). Another limitation is the difficulty of identifying a suitable bacteriophage for treatment. Before a phage may be considered as a possible therapeutic agent, it must be proven to be specific for a particular bacterial strain. This problem may be hard to solve since the proof of a phage's lytic capacity varies depending on the interrelationships between the phage and the bacteria; how they change over time, and the virus dosage used in the test. Sequencing of the phage genome must be carried out to determine the absence of bacterial virulence genes, phage-encoded toxin genes, antibiotic resistance genes and integrase genes. Pharmaceutical preparation of the phage cocktails for use in therapy is also problematic, as stability of the preparations depends on the bacteriophage. Therefore, methods used to optimize stabilization of the bacteriophage may be different for each bacteriophage (Principi *et al*., 2019).

The lysogenic phages integrate themselves into the host's DNA, although their contribution to antibiotic resistance has not been established. This integration may inadvertently lead to the exchange of antibiotic-resistance genes; as the phage moves to infect a different host. Accordingly; development of more resistant bacteria or new microbes are possible due to transduction (Ghannad and Mohammadi, 2012). The uncertainty regarding the role played by the bacteriophages necessitated more research, to determine the frequency of phages encoding for the antibiotic resistance genes. Most of the current studies have established that viruses act as reservoirs

of the antibiotic resistance genes. Such genes lead to resistance to several antibiotics including; tetracyclines, sulphonamides, macrolides, fluoroquinolones and β-lactams (Subirats *et al*., 2016).

Moreover, bacteriophages may induce an immune response due to their nature as non-self-antigens. These immune responses may obstruct the efficacy of phage therapy; irrespective of the means of administration. Previous studies recorded immune responses to phage therapy in humans and animals (Górski *et al*., 2019). However, the nature of these responses varies with prior exposure; route of administration and the phage strain. Dąbrowska, (2019) highlighted that the other limitations of phage therapy include the non-specific nature of the used doses; quantity of the phages, the indefinite length of treatment, and the fact that some types of phages may not work as well as others in treating the various bacterial infections.

Conclusion

In light of the current problem of the arising antimicrobial resistance, scientists must quickly establish if phage therapy is a viable option for successfully combating these antibiotic resistant bacteria. Clinical trials demonstrating benefits of the phage therapy are crucial in confirming its medicinal usefulness. In this review; limitations of the phage therapy were discussed in depth to overcome them in the future studies.

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Conflict of interest

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Ethical approval

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