Enterobacteriaceae therapy using Bacteriophages: A Review

Abstract

Enterobacteriaceae are a family of bacteria, including many familiar pathogens that causesigns of diarrhoea in humans and animals, such as *Escherichia coli, Salmonella, Shigella, and Klebsiella*. *Enterobacteriaceae*,the first Gram-negative bacteria,are bacilli (rod-shaped) facultative anaerobes. They ferment sugars to produce lactic acid and other end products. They are usually about 1-5μm in length. Most are motile because of many flagella; however, a few genera are non-motile. They do not form spores. Most *Enterobacteriaceae* members have fimbriae necessary for the adhesion of the bacterial cells to their hosts. They are economically significant and thus a considerable concern because they cause the deaths of millions of people each year, resulting in a significantsituation to curb their infection. Due to the emergence of drug-resistant bacteria, there is an urgency to search for replacement therapies against bacteria in the Enterobacteriaceae family. To find a solution to this traumatic problem, studies have been launched in the areas of bacteriophages and their therapeutic application as a significant replacement for antibiotics. Bacteriophage therapy utilizes a different mechanism in destroying bacteria; hence, it is a better alternative to antibiotics. This review sheds light on *Enterobacteriaceae* and bacteriophage therapy, as well as the history of bacteriophage therapy and its antibacterial mechanisms.

Keywords: *Enterobacteriaceae, Bacteriophage, Therapy, Phage Therapy, Review*

1. Introduction

Bacteriophages, also known as phages, are a broad group of viruses that infect bacteria and are easily manipulated for use in biotechnology, research, and therapeutics. Phage therapy is recorded to be practised for time immemorial, for example, in France, since 1919, when d'Herelle treated children suffering from severe dysentery using phage therapyat the Hospital des Enfants Malades in Paris [1]. However, early uses of phage therapy were often tricky because of the tedious processes involved and, most importantly, the loss of interest in the use and study of phage therapy as a result of the production of penicillin, which was successfully purified in 1942, and by 1945, became publicly available in pharmacies in the United States and Europe. Just at the dawn of these antibiotics' discovery, they worked incredibly well. They cured many ailments, thereby saving many human lives. Nevertheless, the story changed almost a century ago, and scientists are gradually losing the fight again. We

fear a time when bacterial infections like septicaemia and ventilator-associated pneumonia will not be manageable using antibiotics [2].

The world is currently in the middle of a severe problem due to resistance by microbial pathogens. Studies have shown that the core reason for antibiotic resistance is widespread abuse of antibiotics, which could be their misuse or overuse. Current research also reveals that some bacterial bodies have become resistant to antibiotics, especially those produced explicitly. The resistance has occurred in many ways and is noted in different pathogens. Important examples are the worldwide spread of Methicillin-Resistant *Staphylococcus aureus* (MRSA), infection and Vancomycin-Resistant Enterococci (VRE) [3].

It is now imperative for us to agree that we are at a critical point in treating infections, and bacteria are growing resistant with great speed. Right from 1996, bacteriophage has shown outstanding reliability as an antimicrobial agent. From critical observation, different phages (even antibiotics) can be combined to deliver a more effective result. Phages can also act as vehicles for vaccines (both DNA and protein) which can help study pathogenic strains of bacteria and detect changes in many different proteins and antibodies. More so in agriculture and the petroleum industry, usinga phage as a bioagent can be very strategic. In human health, phages can be very effective in many diverse ways. Golkar *et al.*, 2014 gather some vital information about phages. Firstly, it can be used independently to combat infections by exploiting the phage to lyse the bacterial cell due to its natural ability to do so. Secondly, it can be used as a mixture of more than one phage (a cocktail). This method combats many drug-resistant bacterial infections that refuse to respond to treatment with the latest generations of antibiotics. Thirdly, the versatile nature of phages also enhances the use of antibodies produced against the bacteria on the phage surface. Bacteriophage has a broad scope of application scaling from disease diagnosing (through phage typing), its prevention (phage vaccines) to treatment (phage therapy) [4]. Therefore, this review sheds light on *Enterobacteriaceae* and bacteriophage therapy, as well as the history of bacteriophage therapy and its antibacterial mechanisms.

2. Phage Therapy History

In discussing phage therapy, the initial point would be the discovery of the entity phages itself. We give credit to Hankin, who happened to be the first person who made observations about bacteriophages. His statement was that of a presence of antibacterial activity against *Vibro cholerae* far back in 1896 [5]. Credit also goes to Gamelaya, who observed a similar process as he worked with *Bacillus subtilis* [6]. The subsequent discovery was the presence of plagues on *Staphylococcus aureus* cultures prepared by Twort. After much research in 1915, Twort concluded it was a viral infection that led to the formation of those plagues [7]. Three years along the research line, d'Herelle became the first to demonstrate treatment using phages. His research successfully treated Shigella strains isolated from sick patients suffering from dysentery. When recording his results, he observed small clear zones on the plates. He decided to name them 'bacteriophage'. He did this by merging two words; bacteria and phagein [1]. The second most significant stride he made was when he firmly stood his ground to the opinion that phages were live viruses and not some sort of 'enzymes' as suggested by many others in the field then.

That stride was the beginning of research in the line of phage therapy. People started using phages to treat infections. For instance, d'Herelle's anti-dysentery phage therapy was used to cure a boy and some patients responded very well. Unfortunately, these findings were not adequately documented. The first documentation came in 1921 when Bruynoghe and Masin used a phage to cure a staphylococcal skin infection [8]. After many repeated animal and human tests, many firms like the Parke-Davis Company and Eli Lilly & Company began commercial production of phages against numerous bacterial pathogens. This, however, was after the East European Scientific researchers had recognized a standard dosage of these phages in 1932. The therapy was birthed in China in 1955 when Si *et al.* used bacteriophages to treat *Shigella dysenteriae* [9]. Sadly, phage therapy still has challenges and limitations, especially in narrow host range, less purity, and inconsistency or instability. [10].

3. The Bacteriolytic Mechanism

Bacteriophage therapy involves applying phages therapeutically to destroy infectious pathogens of bacteria. When these phages attach to a bacteria, bacteriolysis (the processing of lysing bacterial cells) begins; this happens in two different host lysis mechanisms, notwithstanding whether they are in line with an endolysin requirement or not. Hence they are two types of bacterial lytic agents. The first mechanism depends on the phage, producing

lysozymes with their dsDNA to lyse bacteria, while the second mechanism does not. Examples of lysozyme-dependent phages include phage K and T4, while that independent of lysozyme include phage φX174 with ssDNA [11,12].

a. The Lysozyme-Independent Lysis System

The lysozyme-independent lysis system targets the host cell wall synthesis. Bacteriophages in the system lack genes that can encode lysozymes. They lyse the host strains by synthesizing proteins that can hinder the biosynthesis of the host cell wall. When achieved, the host cell will break up (lysis) during cell growth. For instance, *E. coli* ssRNA phage Qβ produces protein A2 and binds to protein MurA, a catalytic enzyme in cell wall formation. This binding hinders catalysis by blocking phosphoenolpyruvate from accessing the active site [13]. Another example is where the ssDNA phage φX174 encodes a protein E (a membrane protein). This membrane truncates the activities of MraY enzymes (an enzyme that fastens the initial step for synthesising peptidoglycan precursor, an essential component of the cell wall), thereby resulting in host cell lysis [14]. After the lysis of the cell wall, there is always a dump of large cell debris, leaving the small lesions formed by the host's cell walls [11].

b. Lysozyme-Dependent Lysis System

In this system, the bacteriophages possess dsDNA, which helps encode lysozymes that lyse host cell walls. These bacteriophages are from the order *Candovirals*. They currently comprise about 95% of all the bacteriophages studied [15].

4. *Enterobacteriaceae*

The class of *Enterobacteriaceae* consists of gram-negative bacteria. They are facultatively anaerobic in nature and rod-like in shape. *Enterobacteriaceae* has been implicated in many diseases and infections, cutting through humans and animals such as poultry and fish. These diseases lead to the death of millions of people in the world every year. They include bacteremia, septic arthritis, lower respiratory tract infections, urinary tract infections, and intra-abdominal and ophthalmic infections. Some drugs have shown efficacy against them [12].

Some of the bacteria in this class and their implication include *Klebsiella pneumonia* (implicated pneumonia), *Salmonella enterica*and *Salmonella bongori* (involved with gastroenteritis), and *Shigella* strains (implicated with shigellosis) in addition to *Escherichia coli*[16-18]

5. Bacteriophage Therapy targeted against *Enterobacteriaceae*

The treatment of infections by microorganisms, especially bacteria, is done with experimented drugs; however, most bacteria have developed radical resistance to some of the produced medicines [19].

a. Escherichia coli

E. coli is one of the *Enterobacteriaceae* that is causing many diseases. More than any other bacterial species, it has a wide range of conditions [20]. Because of its high infection rate, it is responsible for many diseases and infections in children. According to WHO, acute diarrhoea has killed up to five million children worldwide [12].

Treating *E. coli* has not been resolved yet. No specific drug or treatment procedure has been secured yet for its diseases and infections. But, then, oral rehydration played a significant role as a treatment route [21] and even helped save lives. Nevertheless, all the simple measure has not addressed the natural course of the diseases nor enlightened on the underlying potential of anti-bacteria. Moreover, due to widespread resistance, people now have less trust in antibiotics [22].

Recently, phages have been used to treat *E. coli* infections. It has been reported that phages are safer in usage in tackling *E. coli* infections through murine and human tests [23, 24].Denou *et al.*,2009) used a T4 coliphage to treat *E. coli* diarrhoea, utilising both in vitro and in vivo tests. This treatment proves zero adverse effect but shows a significant therapeutic effect with no anti-T4 antibodies triggered after one month of observing treatment. Smith *et al.* 1983used phage combination to tackle diarrhoea in young animals, precisely calves, piglets, and lambs [25].

b. Salmonella enterica

Another case to be looked at is *S. enterica. A*ccording to Paterson, [26], this species is the cause of Salmonellosis in humans, and it inhibits the intestinal tracts of some birds and mammalians [26]. The most available transmission mode in humans is egesting food contaminated with animal faeces. Phage therapy has also been found to work on *Enterobacteriaceae* based on prior research [27-29].

c. Klebsiella pneumoniae

K. pneumoniae, a member of this family, is an opportunistic pathogen implicated with intra-abdominal infections, urinary tract, and, popularly, pneumonia [18].Bacteremia caused by *K.*

pneumoniae usually results in significant morbidity and even death among the general population [30]. With the emergence of antibiotic resistance, the treatment of *K. pneumoniae* strains infections has become even more challenging [18].

Specific phage attacks on K. pneumonia cells have been observed to control its infection [18]. In an article by Malik *et al*., [31],he used bacteriophage KØ1 in treating third-degree burns wo Leverentz unds of mice administered with a lethal dose of *K. pneumonia* [31]. After treatment, a remarkable decrease in bacterial load was observed in mice's peritoneal lavage, blood, and lung tissue compared to the control experiment groups. This fall in the microbial count was notable through subcutaneous or intraperitoneal bacteriophage therapy. In curtailing the occurrence of phage-resistant bacteria variants, Gu *et al*. [32]established a systematic approach by making a phage cocktail that consisted of three phagesshown for *K. pneumoniae* [32]. The phage cocktail significantly reduced the mutation rate of *K. pneumonia* compared to when used with any single phage and efficiently salvaged *K. pneumonia* bacteremia. Besides, the phage mixture's nominal protective dose was significantly smaller than a single monophage and could protect bacteremic mice from lethal *K. pneumoniae* K7 infection. Also, Hung *et al*. [33] treated *K. pneumoniae*-induced liver infection using an isolated phage φNK5. Their results indicated that a single dose of lower than 2×10^8 PFU phages was effective. Through intraperitoneal or intragastric treatment, the mice showed that *K. pneumoniae* was significantly eliminated from the blood and liver tissues compared to the control experiments. Their work suggested that the low dose of the phage, φNK5, was an efficient therapeutic agent against *K. pneumoniae*-induced liver infection [33]. Also, the administration of phage showed recommendable protection in infected mice in a short time. The phage was appropriate to rescue *K. pneumoniae*-mediated respiratory infections in the same study. However, the phage treatment was ineffective after a six-hour phage administration delay following the infection induction.

It is relevant to pay attention to time during phage therapy. This is because it affects the result and its success. Although there are a few phage therapies for human *K. pneumonia* infection, the studies suggest that bacteriophages or bacteriophage mixtures can modulate the disease caused by *K. pneumonia* [12].

d. Shigella strains

Shigella, a gram-negative rod, is non-motile and lacking capsule. It causes 'shilgellosis' in humans, posing a severe health challenge, especially in developing countries, and even death [34, 17]. As reported still by Phalipon and Sansonetti, [35], the infectious dose can sometimes

be as minute as just 100 bacterial cells to cause infection; taking its contamination through the fecal-oral route, direct person to person contact, via fomites, water, food, or insects.

Four species of shigella can cause human disease: *S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei*[35].

The legendary d'Herelle was the first person who attempted the treatment of *Shigella* with phages in 1917. He used phages to split *Shigella* strains isolated from several soldier patients with hemorrhagic dysentery [36].

Exciting research was conducted in Tbilisi, Georgia, around 1963 and 1964 to see how effective therapeutic phages can be in treating bacterial dysentery [37]. Youqiang *et al.* 2015 explain the outcome of this research; thus, a total of 30,769 children between ages six months to seven years were covered in the study. Out of the total number, 17,044 received *Shigella* phages orally, while the rest of the children were not given. The final results showed that the incidence of dysentery was 3.8-fold higher in the group without phage treatment than that of the phage-treated group, indicating the efficiency of phage therapy against *Shigella* strains.

e. Serratia marcescens

S. marcescens is a bacteria with a close attraction to the central nervous system; meningoencephalitis or a brain abscess implicated with this pathogen has a severe neurologic projection [38]. Also, newly born babies can likely be infected with S. marcescens when they have immunocompromised systems and low birth weight [39]. Recently we have seen a new development of drug-resistant strains of *S. marcescens* in pediatrics, and this has made the prevention of this bacterium difficult with antibiotics [40].

Research has again proven how effective phage therapy can be in treating *S. marcescens.* One of these research was conducted as far back as 1967 by Iino and Mitani and another recent one in 2009 [41].

In 1967, Iino *et al.* used a phage with a broad host range, phage χ, to lyse 20 of *S. marcescens* strains. However, this phage was only able to affect the strains with flagella which indicated that the possible binding sites (receptors) of phages were somewhere on the flagella [42]

In 2009, isolated two phages, KSP90 and KSP100, from environmental water related to the T4-type phage and phiEco32 phage, respectively [41]. They extensively studied the biological features, DNA features, virion proteins, and phylogenic relationships of these two

phages. Their study showed the therapeutic potential of the phages to control *S. marcescens* infection.

6. Therapy for other strains of *Enterobacteriaceae* family

Bacteriophage therapy research is ongoing on other members of the *Enterobacteriaceae* family. Some include *Edwardsiella* [43], *Proteus* [44] *Erwinia* [45], and *Citrobacter* [46]. These works point to the workability of therapeutic candidates for bacteriophages.

7. Usefulness of Bacteriophage Therapy

There is a need to revisit bacteriophage therapy as an alternative to controlling *Enterobacteriaceae-related* infections [12]. Also, an excellent level of awareness must be done among people, mostly the health workers, if virologists must help reduce the rate at which multi-drug-resistant bacterial strains are growing. Education must be put in place for the general population and health personnel on the coherent and balanced use of antibiotics, regulated sales of over-the-counter antibiotics, and an intentional assessment of the public health system structure.

Secondly, an additional way to solve the multi-resistance problem is to find alternative remedies against drug-resistant pathogens, a pressing challenge to contemporary medicine. Both scientists and clinicians alike are looking to find alternative treatments in the form of phage therapy [19].

The curiosity about using phages as therapy has been revitalized in Western countries due to the ever-increase in antibiotic-resistant bacteria. Also, very significantly after the US National Institute of Allergy and Infectious Diseases enlisted phage therapy as one of seven approaches to tackle antibiotic resistance. This therapy is projected to be one of the best alternative treatment plans to control and treat *Enterobacteriaceae* and other bacterial infections in humans and animals. The application will also reduce food contamination for safe consumption [47].

8. Phage Formulations

Today we have an increasing number of articles discussing phage therapy, yet there is a lack of concentration on the formulation types and the effectiveness/effects. Developing Phage formulations can help widen the scope of applications suitable for phage therapy. By bringing up different kinds of formulations, the mode of delivery can be broadened to suit more

specific bacterial infections. It is also essential to create long-term studies on the stability of these formulations to avoid detrimental reactions in the treatment process. The above area needs to be attended to for us to fully engage the full potential of phage therapy[48].

9. Factors affecting Phage Formation

Compared to storing phage lysates in the laboratory, preparing bacteriophage formulations poses a more significant challenge. Typically, phage lysates can be stored long-term in conducive conditions, but this is not the case for phage formulation as it is always subject to extreme conditions depending on the application of such phage formulation. From observation, phage formulations prepared as dried, non-liquid forms tend to be more stable over a long time. However, they can be influenced and affected by different factors, such as heat, which can cause a decline in the titer. Also, during the production of some phage formulations, bacteriophage degradation can set in, in the actual process of the production. This is evident in phage formulations' production processes, such as freeze-drying and spray-drying [49,50].These and more are essential factors one must consider when creating a phage formulation, especially when the aim is to deliver the phage to the target bacteria, creating high-level stability and improving phage survival when producing these formulations [51].

10. Methods of Phage Formulations

In producing the most common phage formulation, we must encapsulateit because most of them rely on it. Encapsulation is a term that connotes methods (which we will briefly look at) whereby bacteriophages are submerged or surrounded by agents that can improve stability, thereby shielding the phage from an external environment which may not be favourable for it. Once phages are encapsulated, they must be released from the material when required to target bacterial cells [51].

Encapsulation methods include:

a. Emulsification

The bacteriophage or host genus in this method is K *(Staphylococcus),* and the formulation is semi-solid [52]. The benefit of this method is that the material produced is ideal for cream-type treatments and promotes absorption when applied topically. However, the limitation is that it is difficult to transport/store at a large scale, is easily prone to bacterial contamination and can only be stabilized when refrigerated [51].

b. Freeze-Drying

Here the host genus is M13 *(Escherichia)* and can be formulated into a powder [53]. The final product of freeze-drying is more accessible to store/transport than emulsification. It also has high stability after production with different applications but is time-consuming and involves a costly process. Mainly, ice crystal formation can decrease phage viability [51].

c. Spray-Drying

In spray-drying, the bacteriophage used is PEV2, PEV40 *(Pseudomonas)* and can be formulated into powder-like freeze-drying [49]. Also, the final product is easy to store/transport with high post-production stability and various application modes. Still, though, its process is energy consuming, and the temperature can decrease phage viability [51].

d. Liposome Entrapment

KP01K2 *(Klebsiella)* is the organism used in preparing this type of formulation and is always formulated into a liquid form [54]. The Liposome entrapment protects phages against in vivo conditions. However, there are limitations associated with this method. Encapsulation yield of liposome phagesischallenging to control;large-scale transportation and storage are also complicated and need refrigeration to remain stable [51].

e. Electrospinning

The host genus often used is Felix O1 *(Salmonella)* and is formulated into nanofibers [55]. In electrospinning, a diverse array of materials can be produced, so fibre-encapsulated phage is quickly deposited onto other substrates. Still, the fibre-spinning process can damage phages[51].

11. Importance of Phage Formulations

When a phage is formulated, it can ensure its preservation over a long time in adverse environmental conditions, thus making its therapeutic application more effective. Also, formulations provide mass production of these phage therapies, which can be stored easily without drops in phage titer from time to time [51].

12. Conclusion

Bacteriophage therapy, undoubtedly, is full of efficacy and can be a very reasonable approach to bring back bacterial infections under control. Bacteriophage therapy has more advantages when compared to antibiotics because of the unique nature of bacteriophages. This includes the ability to multiply in numbers, specifically at the host's target site during the bacteria-killing process, and contribute to creating an established phage dose; again, it has a lower cost of agent production. The second advantage is that bacteriophages have a host-specific range and rarely divert from them. By this, they can only face their target bacteria, leaving very little or no effect on the body's normal flora. Also, phages show little or no toxicity compared to antibiotics which can be toxic at times to the flora and environment.

However, many factors can limit bacteriophage as a potential medicine. One of these is the safety problem. All contributing phages required for producing a phage cocktail or mixture need accurate dissecting or characterization before they can be used clinically for treatment. Fortunately, the intensive improved genome sequencing studies have given us an edge over this challenge. Though good in an aspect, the second troubling challenge is the fact that phages have a narrow host range. However, the newest development of joining more than one phage together to produce cocktails handles that challenge. Thirdly, the nature of phage therapeutic agents can be unstable at times; nevertheless, studies and research are still actively sorted to curb this challenge. More so, phageresistance developed by bacteria during their co-evolution with phages. There is a bone of contention about if the same result will surface again in the future, as bacteria will develop multi-phage resistance like antibiotics. This is a significant problem in which future studies should focus on.

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