Draft Of Bacteriophage Therapy Review

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Abstract. Phage therapy, which uses bacterial viruses (bacteriophages) to treat bacterial infections. In recent years, the issue of antibiotic resistance in various bacteria has become prominent, and the effectiveness of antibiotic drugs has generally declined, which has re raised people's scrutiny of bacteriophage therapy. Traditionally, bacteriophage therapy relies on the use of natural bacteriophages to infect and dissolve bacteria at the site of infection. Current research on the use of bacteriophages and their lytic proteins to combat multidrug-resistant bacterial infections suggests that bacteriophage therapy may serve as a substitute or supplement to antibiotic therapy.

Keywords: Phage therapy; Brief history of phage therapy; Application of phage therapy in plants; Current state of phage therapy; Development Prospects of phage therapy.

1. Introduction

The issue of antibiotic resistance (AMR) has been identified as one of the urgent issues affecting global health and development [1-2]. The selection of bacterial phenotype by antibiotics can lead to further changes in bacterial gene frequency, leading to the development of antibiotic resistance in pathogenic bacteria. Moreover, the therapeutic effect of antibiotics on bacteria is greatly weakened or even disappeared, which is not conducive to the treatment of certain diseases caused by bacteria. Faced with the significant challenge of antimicrobial resistance on a global scale, clear and effective measures need to be taken to address this issue. And one of the measures. It is to reactivate bacteriophage therapy to replace the current methods of antibiotic treatment for diseases.

Phages are abundant in nature and can effectively kill bacteria. Before the discovery of antibiotics, bacteriophages were used to treat and prevent human and animal infectious diseases. After the discovery of penicillin, bacteriophage therapy was replaced by antibiotic therapy. In the context of widespread antibiotic resistance, phage therapy has regained interest. Due to in-depth research on the genetic, structural, functional, and ecological characteristics of bacteriophages, as well as advances in genetic engineering technology, bacteriophage cocktail therapy currently has significant therapeutic effects on certain pathogens and is environmentally friendly and pollution-free. Phage therapy can be applied to multiple diseases and has the potential for continuous development [3].

2. The history of phage therapy

In 1915, British bacteriologist Twott accidentally discovered transparent spots on the colonies of Staphylococcus aureus while cultivating them, indicating that this part of the Staphylococcus had disappeared. After contacting the transparent plaque with the inoculum needle, Twot then touched another normal Staphylococcus bacteria. The touched part of this colony also showed transparent plaque. This phenomenon indicates that Staphylococcus was eliminated by a substance, but what this substance is was not clearly understood at the time [4].

In 1917, Canadian bacteriologist De Alere also discovered this peculiar phenomenon: when he was conducting liquid culture of dysentery bacteria, the culture medium became cloudy, indicating that countless dysentery bacteria had already grown and propagated inside. However, he soon discovered that the turbid culture medium had become clear and transparent again, and the dysentery bacteria he had cultivated had disappeared.

Why does dysentery bacilli disappear? Will it dissolve? De Alere believed that they must have been preyed upon by another organism smaller than bacteria. He called this tiny life that can "prey"

on bacteria "bacteriophages". This term, in Greek, means "eating bacteria". This event marked the discovery of bacteriophages [4]. Later, Twendler and Dreyer collaborated on research and ultimately confirmed that bacteriophages are a type of virus. They found that bacteriophages have the characteristics of traditional viruses, such as being able to replicate only within the host cell, possessing genetic material, and being able to be isolated through filters. A bacteriophage is actually a virus, a virus that specifically parasitizes within bacteria, so it also has another name called "bacterial virus". This virus that infects bacteria was later widely used in genetic chemistry research. This discovery made important contributions to the development of virology and laid the foundation for bacteriophage therapy.

Dreyer realized that bacteriophages may have the potential to treat bacterial infections and conducted the first human trial in Mumbai, India in 1919. Phages were first clinically used at the Paris Malaria Hospital, where they were successfully treated in 4 cases of pediatric bacterial dysentery [6]. In the early 1920s, De Herrell continued to build a production factory for bacteriophage therapy to treat dysentery, cholera, and the Black Death, and had a series of bacteriophage therapy centers and commercial bacteriophage production factories throughout Europe and India.

Along with Dreyer, several other entrepreneurs attempted to commercialize bacteriophage production in Brazil and the United States, commercializing bacteriophage preparations for Staphylococcus, Streptococcus, Escherichia coli, and other bacterial pathogens [6]. These preparations are transported to clinical doctors around the world, but the treatment results are mixed; This lack of reliability greatly deepens the preference of Western medicine for antibiotics.

However, this does not mean that bacteriophage therapy is not feasible. Many errors were made in early experiments of bacteriophage therapy, most of which can be attributed to a lack of understanding of the biological properties of bacteriophages. The overly basic purification and storage scheme leads to low titer of active phages, bacterial antigen contamination, and improper selection of methods, resulting in a lack of specificity of phages towards target pathogens. In addition, the accuracy of transporting bacteriophages to the infected site was reduced due to medical limitations at the time. For example, the role of patients' innate immune response in clearing active bacteriophages and reducing the efficacy of bacteriophage therapy was not clear at the time, and it was only recently discovered that the fate of bacteriophages in mammals is a potential hybrid physiological mechanism [7].

During World War II, bacteriophage therapy was widely used in the Soviet military. During the war, Soviet soldiers successfully cured many bacterial infections through bacteriophage therapy. In the 1920s, bacteriophage therapy gradually became widely used in European countries, especially in the Soviet Union. Soviet doctors achieved good results in using bacteriophages to treat bacterial infections. After the introduction of drug antibiotics in the 1940s, bacteriophage therapy was gradually weakened. In addition to the former Soviet Union and some countries in Eastern Europe, such as Poland and Georgia, bacteriophage therapy was still widely used to treat antibiotic resistant infections caused by a series of infectious bacteria such as Staphylococcus, Pseudomonas, Klebsiella, and Escherichia coli [8,9]. The results showed that bacteriophages can effectively treat dysentery and other bacterial infections.

In recent years, due to the intensification of bacterial resistance, bacteriophage therapy has begun to attract people's attention. Some countries, such as the United States, Canada, and the United Kingdom, have also begun to research and apply bacteriophage therapy. At present, bacteriophage therapy has been proven to be a safe and effective method for treating bacterial infections, with relatively few side effects. More and more studies have shown that bacteriophage therapy can be an effective alternative method for treating bacterial infections [9]. Currently, bacteriophage therapy has been approved and used for clinical treatment in some countries. In addition, with the development of gene editing technology, scientists are still exploring how to use bacteriophages to treat some chronic diseases, such as cancer and inflammatory bowel disease.

In summary, bacteriophage therapy has been widely used in history and has achieved good therapeutic effects. With the development of modern technology and the increasing demand for human health, bacteriophage therapy is expected to be more widely used in the future.

3. The principles of bactriophge therapy

A bacteriophage is a virus composed of a protein capsid and DNA or RNA. Phages cannot reproduce independently and ultimately rely on bacterial hosts for survival and reproduction. Phages typically bind to specific bacterial cell receptor surfaces, inject their genetic material into the host cell, and then integrate their own genetic material into the host bacterial genome to replicate with the host bacterial DNA (mild phages), or break down the host cell to release new phages (strong phages). When the critical mass of phage progeny is reached, there can be over 1000 viral particles depending on environmental factors. The lysed protein becomes active and hydrolyzes the peptidoglycan cell wall to release new phages and restart the lysis cycle [10,11].

The growth mechanism of bacteriophages refers to the process of replication and reproduction by utilizing the metabolic system of host cells after infection. Specifically, the growth mechanism of bacteriophages can be divided into several stages, including adsorption, genome injection, synthesis, assembly, and release. Firstly, in the adsorption stage, bacteriophages bind to specific receptors on the surface of the host cell through specific receptors, and then fix the bacteriophage on the surface of the host cell through anchored proteins and other molecules. Adsorption is one of the key steps in the life cycle of bacteriophages, requiring specific recognition of host surface proteins, lipopolysaccharides, or other molecules (wall acids, pili, flagella) on the bacterial envelope. After successful recognition, bacteriophages can then adsorb on the bacterial surface. Next is the injection genome stage, where the phage injects its genome into the host cell through a syringe like tail. This process involves peptidoglycan lysozyme located at the tail of the bacteriophage [62]. The bacteriophage genome generally includes DNA or RNA encoding proteins required for bacteriophage replication and assembly. After genome injection, based on the type of bacteriophage and the physiological condition of bacterial cells, the bacteriophage genome is integrated into the host genome in the form of a plasmid, and then the metabolic system of the host cell is used to start synthesizing the proteins and nucleic acids required by the bacteriophage. These molecules form new bacteriophage particles. Taking the T4 bacteriophage as an example, T4 assembles its shell by forming a DNA free protein precursor, called a prophyll or prophyll. Enhancing the affinity of host RNA polymerase for early transcription through GPALT protein ADP riboprotein, leading to preferential transcription of early T4 genes [65]

Next is the assembly stage: the newly synthesized bacteriophage particles begin to assemble into complete bacteriophage particles, including structures such as the head, tail, and syringe. Finally, in the release stage, the assembled bacteriophage particles will cause the host cell to rupture and release. This process is called lysis, and the newly formed phage particles can infect other host cells again and undergo growth and replication [12,13].

The life cycle of bacteriophages includes lysis, lysogen, and pseudo lysogen cycles. During the lysis cycle, bacteriophages immediately begin to produce new viral offspring after infection and release them by lysis of the host. In the lysogenic cycle, the phage genome replicates synergistically with the host DNA, either integrating into the host chromosome or forming a long-term stable coexistence with the host in a free plasmid like state [63]. Pseudolysosomes are a non-classical phage life cycle, in which phages neither cleave the host nor integrate into the genome to establish long-term stable relationships [64]. Pseudolysogenicity is related to the hunger stress of bacterial hosts and occurs in both soluble and temperate phages. After entering the host cell, the bacteriophage genetic material remains inactive in the form of circular fragments, and the development cycle pauses until environmental conditions improve.

In summary, the growth mechanism of bacteriophages is a complex process that requires interaction and regulation between bacteriophages and host cells. Further research on the growth

mechanism of bacteriophages can help to better understand their biological characteristics and provide theoretical basis for the development of related applications. Deletion: Delete the author and affiliation lines for the extra authors

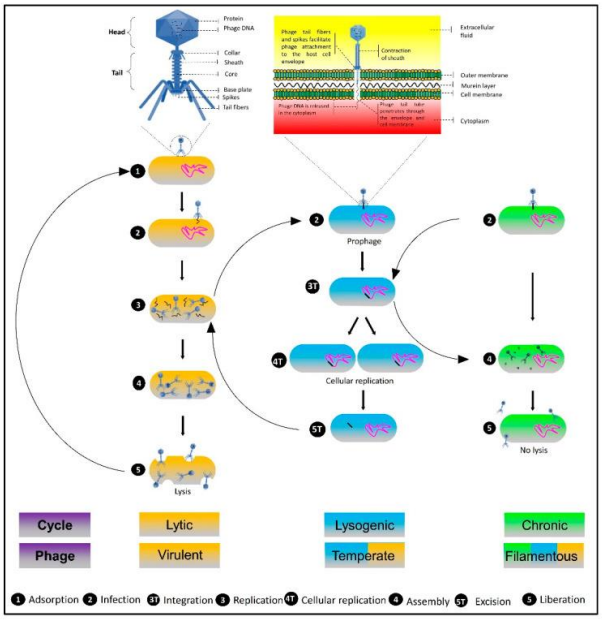

Fig 1. Three life cycles of bacteriophages [66]

4. The mechanism of bacterial resistance to phage infection

Most bacteriophages only infect bacteria with complementary receptors on their surface, and the type of complementary receptors on the bacterial surface determines the host range of the bacteriophage [14]. In order to resist the infection of bacteriophages, bacteria have evolved many mechanisms.

The first step in resisting phage infection is to resist phage adsorption. Firstly, bacteria can resist the adsorption of bacteriophages by altering their surface structure, thereby avoiding infection. For example, some bacteria may express special glycoproteins on the surface of cells, which are incompatible with receptors on the structure of bacteriophages, making them unable to bind to cells; Some bacteria can secrete special enzymes that break down the outer shell of bacteriophages, thereby avoiding infection. Bacteria can resist phage adsorption by altering their genome; Some bacteria have special deoxyribonucleic acid (DNA) modifying enzymes that can modify bacterial DNA, making it difficult for bacteriophages to recognize and bind to cells; Some bacteria can also resist the adsorption of bacteriophages through other means, such as regulating the permeability of cell walls and changing the acidity and alkalinity of cells.

For blocking the penetration of bacteriophage DNA, bacteria also have a super infection elimination system to block the penetration of bacteriophage DNA into cells. In order to degrade bacteriophage DNA, bacteria have also evolved restriction modification (R-M) systems and CRISPR Cas systems to resist the invasion of bacteriophages. For example, bacteria can integrate bacteriophage DNA into clustered regular interval palindrome repeat sequences or CRISPR related systems (CRISPR/Cas) to disrupt the reproductive process of bacteriophages and degrade them. In addition, bacteria can also synthesize various homologous proteins to cause sudden infection of bacteriophages. Unlike the previous anti phage systems, the bacterial setback infection system (Abi) plays a crucial role in the replication, transcription, or translation of phage infections, leading to the death of infected cells [15-18]. And bacteriophages have also evolved various mechanisms to break down these resistance mechanisms of bacteria against bacteriophages. Phages can obtain new parasitic strategies and adversarial mechanisms through mutation or gene recombination, such as identifying new or altered receptors and mutating anti CRISPR genes, in order to avoid being

recognized and destroyed by the bacterial restrictive modification system or CRISPR-Cas system, leading to their generation of new structures or functions [19]. In addition, bacteriophages can also utilize mechanisms such as incorporation into the host genome or production of inhibitory substances to interfere with bacterial resistance mechanisms, inhibit host modification systems, and avoid being attacked by bacterial defense mechanisms. Phages can also utilize bacterial mechanisms, such as parasitism using bacterial outer membrane proteins or flagella [20]. There are many unpredictable interactions between various systems within the same bacterium, and the collective effects and evolutionary effects of bacteriophage populations are often overlooked. The mutual resistance between different bacteriophages is also not taken seriously. The most significant drawback of bacteriophage therapy is that it cannot treat bacterial infections that are resistant to bacteriophages, and it may also lead to the emergence of phage resistant strains. Therefore, clarifying the resistance mechanism of bacteria to bacteriophages, as well as the co evolution mechanism between bacteriophages and bacteria, provides a reliable basis for future research on bacteriophage therapy targets and the treatment of phage resistant bacterial infections, and prevents the recurrence of resistance problems caused by antibiotic abuse. [21-23]

5. Current reaserch status of bacteriophage therpyacknowledgments

Phage therapy first requires screening to obtain appropriate phages, which need to be collected from the environment or patients for screening to ensure the selection of phages with high specificity for the target bacteria. Then, phage propagation and amplification can be carried out. Based on the characteristics of the selected phage, phage propagation and amplification can be carried out on an appropriate culture medium to prepare a sufficient number of phages for treatment. Next, it is necessary to determine the treatment dose. Based on the patient's condition and degree of infection, the doctor will determine the appropriate treatment dose. Finally, there is the method of administration. Phage therapy can be administered through various channels, such as oral, intravenous, or topical, and the specific method needs to be selected based on the patient's condition and treatment purpose. Monitoring and evaluation of treatment effectiveness are also necessary, and patients need to be monitored and evaluated during the treatment process to ensure treatment effectiveness and safety. If necessary, dosage adjustments can be made or phage varieties can be changed [24-28].

The commonly used therapy for bacteriophages is cocktail therapy. Cocktail therapy, originally referred to "highly effective antiretroviral therapy" (HAART), was proposed by Chinese American scientist He Dayi in 1996. It is a combination of three or more antiviral drugs to treat AIDS [49]. The application of this therapy can reduce the resistance caused by a single medication, maximize the inhibition of virus replication, partially or even completely restore the damaged immune function of the body, thereby delaying the progression of the disease, prolonging the patient's life, and improving the quality of life. The therapy mixes protease inhibitors with a variety of antiviral drugs, so that AIDS can be effectively controlled [49].

In the treatment of bacteriophages, HAART therapy usually refers to the use of multiple bacteriophages with different bactericidal mechanisms in combination to improve treatment effectiveness and reduce the risk of bacterial resistance to a single bacteriophage.

Some studies have shown that HAART therapy can significantly improve the effectiveness of bacteriophage therapy in treating bacterial infections. For example, a study published in Frontiers in Microbiology found that in a mouse model treated with multiple phages in combination for Klebsiella pneumoniae infection, HAART therapy significantly improved treatment efficacy, reduced bacterial resistance to phages, and no significant toxic side effects were observed. [50]

Although HAART therapy has great prospects in bacteriophage therapy, designing a bacteriophage cocktail is much more complex than designing a combination antibiotic treatment plan due to the vast diversity of environmental bacteriophages. The composition of bacteriophage cocktails is crucial for the success of bacteriophage therapy [29], and whether to use standardized or customized cocktails for bacteriophage therapy is currently a challenge. Customizing bacteriophage cocktails for each

infection is time-consuming and costly, and multiple other factors need to be considered, such as the incidental effects of bacteriophages on local microbiota, the lifecycle of bacteriophages, etc. These aspects have not been fully explored [30-31]. More research is still needed to determine the optimal combination strategy, dosage, and treatment plan for bacteriophage therapy. In addition, the application of HAART therapy in bacteriophage therapy also needs to consider issues such as safety and stability, which require strengthened research and exploration.

SBS (Step by Step Method) is a common method for developing bacteriophage cocktails that can combat multidrug-resistant bacterial pathogens. SBS first isolated wild-type lysis strains and wild-type phage resistant mutants to develop bacteriophages for bacteriophage cocktails. If wild-type bacteria are not sensitive to the first phage, it can lead to the production of phage resistant mutants. Subsequently, the phage resistant mutant can be used to further isolate the second phage. The third bacteriophage can also be isolated by utilizing the resistance of bacterial mutants to the second bacteriophage. Finally, combining all phages into a cocktail can suppress phage resistant bacteria [51]. For example, phage mixtures (GH-K1, GH-K2, and GH-K3) established through the SBS method have good therapeutic effects on single phage resistant Klebsiella pneumoniae and reduce the frequency of mutations that trigger drug resistance [52].

6. Research On Phage Therapy In Plant Model.

At present, bacteriophage cocktail therapy has been studied and applied in combating plant bacterial pathogens and thus curing plant diseases. Bel é n Á lvarez et al. discovered three new lysis bacteriophages, vRsoP-WF2, vRsoP-WM2, and vRsoP-WR2, which can effectively control plant bacterial wilt caused by Ralstonia solanacearum. They used a single bacteriophage and its pairwise combination to conduct biological control experiments on susceptible tomato plants to bacterial wilt and simulated field conditions for cultivation. The disease rate of the control group was about 80%, while the disease rate of the experimental group treated with a single bacteriophage decreased to between 20-6% (different types of bacteriophages have different effects). When using pairwise bacteriophage combinations and three bacteriophage combinations, it was observed that the percentage of tomato plants suffering from bacterial wilt decreased to 3-0%, and the incidence rate significantly decreased. The data shows that vRsoP-WF2, vRsoP-WM2, and vRsoP-WR2 have a preventive effect on bacterial wilt disease, and mixed bacteriophages are more effective than single bacteriophages [54]. Fructobacilli are one of the pathogens of onion soft rot, and the lack of effective control measures for these pathogens has led to serious crop losses. Maja A. Zaczek Moczyd ł Oska et al. conducted three years of field experiments and demonstrated for the first time that a mixture of Siphoviridae and Podoviridae bacteriophages can protect onions from soft rot under field conditions. This provides a new approach to eradicate onion soft rot disease and further optimizes the formulation of bacteriophage cocktails through laboratory and field testing [55]. A type of bacteriophage consisting of three members from the Muscoviridae family and Podoviridae ϕ Ea2345-6 ϕ The phage cocktails of Ea1337-26 and Eh21-5 can be used to prevent and control the fire blight of apples and pears, as well as the phage cocktails composed of four myoviridae bacteriophages Eram2, Eram26, Eram24, and Eram45, which can be used to prevent and control the fire blight of pears. Phage cocktails are being vigorously applied in effectively controlling amyloidobacteria [53]. From the above research, we can see that bacteriophage therapy has significant therapeutic effects on certain bacterial diseases, and compared to the use of a single bacteriophage, the bacteriophage cocktail formed by mixing multiple bacteriophages has a more significant therapeutic effect on diseases. This may indicate that synergy can be established between these bacteriophages in cocktails, or that one bacteriophage can enhance the characteristics of another bacteriophage, and the cooperative effect of multiple bacteriophages improves the adsorption ability of a single bacteriophage on bacteria and the efficiency of pathogen lysis.

Phage therapy not only has the function of treating diseases, but also has the function of disease prevention and control. Mayukh Das et al. found that a mixture of multivalent bacteriophages can

reduce pathogens in grape vines γ - The level of Proteus and its preventive and therapeutic effects on Pierce's disease. They divided the grape vines into two groups. One group was pre treated with bacteriophage cocktails, and the other group was not treated. Then, pathogens were inoculated three weeks after pretreatment. At weeks 8 and 12, it was found that the grape vines treated with preventive treatment did not show PD symptoms, while the untreated grape vines had higher levels of pathogens, reaching a maximum of~103 CFU/gt at week 8 and~106 CFU/gt at week 12. This is the first study to apply bacteriophage based biological control strategies to control PD, and provides a solution that can replace broad-spectrum insecticides to control vector borne plant diseases caused by Trichoderma species [56].

Complex phage cocktails containing multivalent bacteriophages may also affect the plant microbiome. After using bacteriophage cocktails on plants, the addition of multivalent bacteriophages in bacteriophages can directly affect the evolution and population size of the plant's microbial community, or indirectly affect the competition between microbial species within the plant to affect the composition of the plant microbiome. This also indicates that bacteriophages may have an impact on non targeted bacterial communities while killing pathogenic bacteria, and may even kill beneficial endophytic bacteria [57]. In addition, if bacteriophage cocktails have antagonistic effects on plant pathogens, the plant microbiome may be resistant to the effects of bacteriophage cocktails. Therefore, in field experimental studies, we can explore the impact of bacteriophage cocktail application on plant microbiome by using molecular quantitative methods. Further explore the relationship between bacteriophage bacteria plant interactions.

The use of bacteriophage cocktail therapy to prevent and treat plant diseases is an eco-friendly method. Unlike chemical fungicides, bacteriophages naturally exist in the environment, and as long as the host exists, they will reproduce in large quantities [58]. Chemical fungicides such as copper based insecticides do not naturally exist in the environment, and their widespread use may cause abnormal accumulation in the soil and cause certain damage to the ecological environment [59]. At present, there is a growing demand among market consumers for food that does not contain chemical fungicides and preservatives, and they require that there be no chemical residues in crop production and processing [60]. As a biopesticide, bacteriophages are more in line with consumer demand trends.

7. Application Of Bacteriophage Therpy In Animal Models

Phage therapy has also been experimentally studied on some animal models. Majkowska Skrobek G et al. validated the effectiveness of using bacteriophages to treat intestinal sepsis caused by Pseudomonas aeruginosa in a mouse model and found that bacteriophage therapy has potential therapeutic effects in reducing the number of Pseudomonas aeruginosa in the intestine and blood, improving mouse survival, and lowering inflammatory response indicators. When intestinal sepsis caused by Pseudomonas aeruginosa occurs, oral phages can save 66.7% of the mortality rate in mice, while the control group mice all die [32]. Adams MH conducted a series of mouse experiments in the laboratory, treating mouse infections by injecting different types of bacteriophages and evaluating their therapeutic effects. The results showed that under certain conditions, phage therapy can effectively treat mouse infections and has certain advantages compared to antibiotic therapy. [33]. Phage therapy also has significant therapeutic effects on multidrug-resistant Escherichia coli O25: H4-ST131. D ą Browska et al. used a mouse model of sepsis and meningitis caused by the O25b: H4-ST131 Escherichia coli strain producing CTX-M-15. They injected mice with three different cocktails of phages targeting this strain. Researchers observed that compared to the control group, the injection of bacteriophages effectively reduced the number of bacteria in the blood and brain and improved the survival rate of mice. [34]., Phages can also restore antibiotic sensitivity of drug-resistant bacteria. Chan et al. tested the ability of a group of phages with specificity for multidrug-resistant Pseudomonas aeruginosa strains to restore antibiotic sensitivity in vitro. This study found that using this phage therapy can reduce the minimum inhibitory concentration (MIC) of antibiotics against

multidrug-resistant Pseudomonas aeruginosa strains. This indicates that the use of bacteriophages may restore the sensitivity of multidrug-resistant Pseudomonas aeruginosa to antibiotics. [35].

8. Summary

The research methods of bacteriophage therapy in the field of treating plant bacterial diseases are currently relatively mature, and have made some progress in the treatment of animal diseases. The application scope is constantly expanding, mainly applied in surgical procedures, skin infections, and other fields. In the future, it may expand to more fields, such as respiratory infections, urinary tract infections, etc. [45-46].

On May 3, 2022, researchers from Harvard University School of Medicine and the University of Pittsburgh found that phage therapy, combined with antibiotics and surgery, has been proven to be effective in treating multidrug-resistant Mycobacterium chelonae infection in immunocompromised patients. This is the first report on the successful treatment of Mycobacterium chelonianum infection with bacteriophage therapy, and describes the observed clinical efficacy [47].

Next, bacteriophage therapy may continue to explore the interaction mechanism between bacteriophages and bacteria, as well as the impact of bacteriophages on the microbiome in the target environment. By studying the interaction mechanism between bacteriophages and bacteria, we can understand the degree and speed of coevolution between bacteriophages and bacteria, identify the targets of bacteriophage therapy for bacteria, and evaluate the possibility and speed of bacterial resistance to bacteriophages to continue improving bacteriophage therapy and reducing the possibility of bacteriophage therapy repeating the mistakes of antibiotic therapy. By studying the impact of bacteriophages on the microbiome in the target environment, we can further reduce the impact and interference of bacteriophage therapy on the environment, reduce the "false killing rate" of bacteriophages on probiotics, and enhance the precise treatment effect of bacteriophage therapy on corresponding pathogenic bacteria. At the same time, collaborative research on relevant environmental microorganisms and bacteriophages may also find that bacteriophage and environmental microorganisms can work together to kill probiotics, Thus, "adapting to local conditions" can achieve twice the result with half the effort and further enhance the therapeutic ability of bacteriophage therapy.

Phage therapy can not only directly use intact phages to kill viruses through the infection mechanism of phages, but also treat bacterial diseases by isolating phage encoded lyases, which are functionally similar to eukaryotic lysozymes. The genes of these enzymes are expressed by bacterial hosts during the lysis cycle and assist phages in releasing viral progeny by hydrolyzing cell walls. This indicates that we can directly destroy the bacterial cell wall to achieve the effect of killing bacteria, and avoid the subsequent infection release stage of bacteriophages, greatly reducing the risk of bacteriophage therapy and a series of other problems caused by bacteriophage infection, such as the interaction between bacteriophages and the microbial environment and interference with the interaction between bacteriophages and bacteria. The discovery and analysis of these proteins in the field of bacteriophage lyases have opened up possibilities for the development of new bacteriophage based drugs. Moreover, the lyase is easy to purify, easy to directional operate, has high specificity, and does not damage normal bacterial communities, among other advantages. The purified lysase has good antibacterial activity, and the phage lysase of Enterococcus faecalis can effectively kill Enterococcus faecalis, Enterococcus faecalis, and Streptococcus pyogenes [48].

Currently, there are no approved bacteriophage therapy products for human use in the European Union or the United States. However, in the food industry, there are already several commercial bacteriophage preparations used for biological control of bacterial pathogens, which can be used for the treatment of Salmonella, Listeria monocytogenes, MRSA, Escherichia coli O157: H7, Mycobacterium tuberculosis, Campylobacter, and Pseudomonas syringae [36-39]. Phages also have potential value for pathogen detection, such as using bioluminescent reports to detect Bacillus anthracis. The use of bacteriophages in the food industry indicates that bacteriophage biocontrol can

effectively improve food safety in many stages of meat production and processing, and may also reduce bacterial contamination in fruits, vegetables, and dairy products [38]. These studies on bacteriophage biocontrol in food production have demonstrated the safety of oral bacteriophage administration, And gradually began to fill the knowledge gap in the safety of bacteriophage therapy [40-43]. Through further research on bacteriophage therapy, the safety of bacteriophage therapy has gradually been confirmed. Previous studies have shown that bacteriophage therapy can safely and effectively treat chronic otitis media caused by antibiotic resistant Pseudomonas aeruginosa [44].

References

[1] Mendelson M, Matsoso MP. The World Health Organization Global Action Plan for antimicrobial resistance[J]. S Afr Med J, 2015, 105(5):325.

[2] Sulakvelidze, A., Alavidze, Z., & Morris Jr, J. G. (2001). Bacteriophage therapy. Antimicrobial agents and chemotherapy, 45(3), 649-659.

[3] Wright A, Hawkins CH, Anggård EE, et al. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy[J]. Clin Otolaryngol, 2009, 34(4):349-357.

[4] Kutter, E., De Vos, D., Gvasalia, G., Alavidze, Z., Gogokhia, L., & Kuhl, S. (2010). Phage therapy in clinical practice: treatment of human infections. Current pharmaceutical biotechnology, 11(1), 69-86.

[5] Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches. Adv Virus Res 2012; 83: 3-40 [PMID: 22748807 DOI: 10.1016/B978-0-12-394438-2.00001-3]

[6] Sulakvelidze A, Alavidze Z, Morris JG. Bacteriophage therapy. Antimicrob Agents Chemother 2001; 45: 649-659 [PMID: 11181338 DOI: 10.1128/AAC.45.3.649-659.2001]

[7] Hodyra-Stefaniak K, Miernikiewicz P, Drapała J, Drab M, Jończyk-Matysiak E, Lecion D, Kaźmierczak Z, Beta W, Majewska J, Harhala M, Bubak B, Kłopot A, Górski A, Dąbrowska K. Mammalian Host-Versus-Phage immune response determines phage fate in vivo. Sci Rep 2015; 5: 14802 [PMID: 26440922 DOI: 10.1038/srep14802]

[8] Carlton RM. Phage therapy: past history and future prospects. Arch Immunol Ther Exp (Warsz) 1999; 47: 267-274 [PMID: 10604231]

[9] Weber-Dabrowska B, Mulczyk M, Górski A. Bacteriophage therapy of bacterial infections: an update of our institute's experience. Arch Immunol Ther Exp (Warsz) 2000; 48: 547-551 [PMID: 11197610]

[10] Delbrück M. The growth of bacteriophage and lysis of the host. J Gen Physiol 1940; 23: 643-660 [PMID: 19873180]

[11] Weinbauer MG. Ecology of prokaryotic viruses. FEMS Microbiol Rev 2004; 28: 127-181 [PMID: 15109783 DOI: 10.1016/j.femsre. 2003.08.001]

[12] Suttle, C. A. (2005). Viruses in the sea. Nature, 437(7057), 356-361. doi: 10.1038/nature04160

[13] Catalão, M. J., Gil, F., Moniz-Pereira, J., & Pimentel, M. (2013). Diversity in bacterial lysis systems: bacteriophages show the way. FEMS Microbiology Reviews, 37(3), 554-571. doi: 10.1111/1574-6976.12018

[14] Rakhuba DV, Kolomiets EI, Dey ES, Novik GI. Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. Pol J Microbiol 2010; 59: 145-155 [PMID: 21033576]

[15] Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. Nature Reviews Microbiology, 8(4), 317-327.

[16] Abedon, S. T. (2017). Bacteriophage resistance mechanisms: new approaches to combat antimicrobial resistance. Journal of Basic Microbiology, 57(8), 640-656.

[17] Wang, X., Kim, Y., Ma, Q., Hong, S. H., Pokusaeva, K., Sturino, J. M., ... & Wood, T. K. (2010). Cryptic prophages help bacteria cope with adverse environments. Nature Communications, 1(1), 1-8.

[18] Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., ... & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709-1712.

[19] Koskella B, Brockhurst MA. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS Microbiol Rev 2014; 38: 916-931 [PMID: 24617569 DOI: 10.1111/1574-6976.12072

[20] Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. Nat Rev Microbiol 2010; 8: 317-327 [PMID: 20348932 DOI: 10.1038/nrmicro2315]

[21] Koskella, B., & Brockhurst, M. A. (2014). Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS microbiology reviews, 38(5), 916-931.

[22] Chaturongakul, S., & Ounjai, P. (2014). Phage–host interplay: examples from tailed phages and Gram-negative bacterial pathogens. Frontiers in microbiology, 5, 442.

[23] Samson, J. E., & Moineau, S. (2013). Bacteriophages in food fermentations: new frontiers in a continuous arms race. Annual review of food science and technology, 4, 347-368.

[24] Sulakvelidze A, Alavidze Z, Morris Jr JG. Bacteriophage therapy. Antimicrobial agents and chemotherapy. 2001 Apr;45(3):649-59.

[25] Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy. Future microbiology. 2013 Feb;8(6):769-83.

[26] Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. Phage therapy in clinical practice: treatment of human infections. Current Pharmaceutical Biotechnology. 2010 Jan 1;11(1):69-86.

[27] Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, Resch G. Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. The Journal of Infectious Diseases. 2017 May 15;215(10):703-12.

[28] Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. Antimicrobial agents and chemotherapy. 2017 Sep 22;61(10):e00954-17

[29] Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy. Future Microbiol 2013; 8: 769-783 [PMID: 23701332 DOI: 10.2217/fmb.13.47]

[30] Mai V, Ukhanova M, Reinhard MK, Li M, Sulakvelidze A. Bacteriophage administration significantly reduces Shigella colonization and shedding by Shigella-challenged mice without deleterious side effects and distortions in the gut microbiota. Bacteriophage 2015; 5: e1088124 [PMID: 26909243 DOI: 10.1080/21597081.2015.1088124]

[31] Galtier M, De Sordi L, Maura D, Arachchi H, Volant S, Dillies MA, Debarbieux L. Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition. Environ Microbiol 2016; 18: 2237-2245 [PMID: 26971586 DOI: 10.1111/1462-2920.13284]

[32] Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, Sumiyama Y, Uchiyama J, Sakurai S, Matsuzaki S, Imai S, Yamaguchi K. Efficacy of bacteriophage therapy against gut-derived sepsis caused by Pseudomonas aeruginosa in mice. Antimicrob Agents Chemother 2007; 51: 446-452 [PMID: 17116686 DOI: 10.1128/AAC.00635-06]

[33] Soothill JS. Treatment of experimental infections of mice with bacteriophages. J Med Microbiol 1992; 37: 258-261 [PMID: 1404324 DOI: 10.1099/00222615-37-4-258]

[34] Pouillot F, Chomton M, Blois H, Courroux C, Noelig J, Bidet P, Bingen E, Bonacorsi S. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b: H4-ST131 Escherichia coli strain producing CTX-M-15. Antimicrob Agents Chemother 2012; 56: 3568-3575 [PMID: 22491690 DOI: 10.1128/AAC.06330-11]

[35] Chan BK, Sistrom M, Wertz JE, Kortright KE, Narayan D, Turner PE. Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. Sci Rep 2016; 6: 26717 [PMID: 27225966 DOI: 10.1038/srep26717]

[36] Monk AB, Rees CD, Barrow P, Hagens S, Harper DR. Bacteriophage applications: where are we now? Lett Appl Microbiol 2010; 51: 363-369 [PMID: 20796209 DOI: 10.1111/j.1472-765X.2010.02916.x]

[37] Nannapaneni R, Soni KA. Use of Bacteriophages to Remove Biofilms of Listeria monocytogenes and other Foodborne Bacterial Pathogens in the Food Environment. Biofilms in the Food Environment, Second Edition. USA: John Wiley & Sons, Ltd., 2015: 131-144 [DOI: 10.1002/ 9781118864036.ch5]

[38] Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. Phage therapy in the food industry. Annu Rev Food Sci Technol 2014; 5: 327-349 [PMID: 24422588 DOI: 10.1146/annurev-food-030713-092415]

[39] Schofield DA, Sharp NJ, Vandamm J, Molineux IJ, Spreng KA, Rajanna C, Westwater C, Stewart GC. Bacillus anthracis diagnostic detection and rapid antibiotic susceptibility determination using 'bioluminescent' reporter phage. J Microbiol Methods 2013; 95: 156-161 [PMID: 23994352 DOI: 10.1016/j.mimet.2013.08.013]

[40] Bruttin A, Brüssow H. Human volunteers receiving Escherichia coli phage T4 orally: a safety test of phage therapy. Antimicrob Agents Chemother 2005; 49: 2874-2878 [PMID: 15980363 DOI: 10.1128/AAC.49.7.2874-2878.2005]

[41] Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 2009; 4: e4944 [PMID: 19300511 DOI: 10.1371/journal.pone.0004944]

[42] McCallin S, Alam Sarker S, Barretto C, Sultana S, Berger B, Huq S, Krause L, Bibiloni R, Schmitt B, Reuteler G, Brüssow H. Safety analysis of a Russian phage cocktail: from metagenomic analysis to oral application in healthy human subjects. Virology 2013; 443: 187-196 [PMID: 23755967 DOI: 10.1016/j.virol.2013.05.022]

[43] Sarker SA, Sultana S, Reuteler G, Moine D, Descombes P, Charton F, Bourdin G, McCallin S, Ngom-Bru C, Neville T, Akter M, Huq S, Qadri F, Talukdar K, Kassam M, Delley M, Loiseau C, Deng Y, El Aidy S, Berger B, Brüssow H. Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. EBioMedicine 2016; 4: 124-137 [PMID: 26981577 DOI: 10.1016/j.ebiom.2015.12.023]

[44] Wright A, Hawkins CH, Anggård EE, Harper DR. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clin Otolaryngol 2009; 34: 349-357 [PMID: 19673983 DOI: 10.1111/j.1749-4486.2009.01973.x]

[45] Abedon, S.T. (2017). Phage Therapy: Past, Present and Future. Springer International Publishing.

[46] Chhibber, S., & Kaur, S. (2019). Challenges and future prospects for the delivery of bacteriophages in vivo. Journal of Medical Microbiology, 68(1), 1-6.

[47] Jessica S. Little 1,2,3,8✉, Rebekah M. Dedric, Bacteriophage treatment of disseminated,2022 cutaneous Mycobacterium chelonae infection.

[48] 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 [PMID: 19114881 DOI: 10.1097/CCM.0b013e31819586a6

[49] Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998;338(13):853-860.

[50] Mohamed et al,Bacteriophage cocktail significantly reduces Escherichia coli O18:K1:H7 recovery from lung in a mouse pneumonia model,Frontiers in Microbiology,2018

[51] Kering K.K., Kibii B.J., Wei H. Biocontrol of phytobacteria with bacteriophage cocktails. Pest Manag. Sci. 2019;75:1775–1781. doi: 10.1002/ps.5324.

[52] Gu J., Liu X., Li Y., Han W., Lei L., Yang Y., Zhao H., Gao Y., Song J., Lu R. A method for generation phage cocktail with great therapeutic potential. PLoS ONE. 2012;7:e31698. doi: 10.1371/journal.pone.0031698.

[53] Sabri M., Benkirane R., Habbadi K., Sadik S., Ou-Zine M., Diouri M., Achbani E.H. Phages as a potential biocontrol of phytobacteria. Arch. Phytopathol. Plant Prot. 2021;54:1277–1291. doi: 10.1080/03235408.2021.1902033.

[54] Álvarez B., López M.M., Biosca E.G. Biocontrol of the major plant pathogen Ralstonia solanacearum in irrigation water and host plants by novel waterborne lytic bacteriophages. Front. Microbiol. 2019;10:2813. doi: 10.3389/fmicb.2019.02813.

[55] Zaczek-Moczydłowska M.A., Young G.K., Trudgett J., Fleming C.C., Campbell K., O'Hanlon R. Genomic characterization, formulation and efficacy in planta of a siphoviridae and podoviridae protection cocktail against the bacterial plant pathogens Pectobacterium spp. Viruses. 2020;12:150. doi: 10.3390/v12020150

[56] Das M., Bhowmick T.S., Ahern S.J., Young R., Gonzalez C.F. Control of Pierce's disease by phage. PLoS ONE. 2015;10:e0128902. doi: 10.1371/journal.pone.0128902.

[57] Koskella B., Taylor T.B. Multifaceted impacts of bacteriophages in the plant microbiome. Annu. Rev. Phytopathol. 2018;56:361–380. doi: 10.1146/annurev-phyto-080417-045858.

[58] Iriarte F. B., Obradović A., Wernsing M. H., Jackson L. E., Balogh B., Hong J. A., et al. (2012). Soil-based systemic delivery and phyllosphere in vivo propagation of bacteriophages: two possible strategies for improving bacteriophage persistence for plant disease control. Bacteriophage 2 215–224. 10.4161/bact.23530

[59] Hirst J. M., Riche H. H., Bascomb C. L. (1961). Copper accumulation in the soils of apple orchards near Wisbech. Plant Pathol. 10 105–108. 10.1111/j.1365-3059.1961.tb00127.x

[60] Hirst J. M., Riche H. H., Bascomb C. L. (1961). Copper accumulation in the soils of apple orchards near Wisbech. Plant Pathol. 10 105–108. 10.1111/j.1365-3059.1961.tb00127.x

[61] Rakhuba, D. V.; Kolomiets, E. I.; Dey, E. S.; Novik, G. I. Bacteriophage Receptors, Mechanisms of Phage Adsorption and Penetration into Host Cell. Polish J. Microbiol. / Pol. Tow. Mikrobiol. = Polish Soc. Microbiol.2010, 59(3), 145–155

[62] Osserman, E. F.; Canfield, R. E.; Beychok, S. Lysozyme; Osserman, E., Ed.; Academic Press Inc., 1974

[63] Salmond G.P., Fineran P.C. A century of the phage: Past, present and future. Nat. Rev. Microbiol. 2015;13:777–786. doi: 10.1038/nrmicro3564.

[64] Mäntynen S., Laanto E., Oksanen H.M. Black box of phage–bacterium interactions: Exploring alternative phage infection strategies. Open Biol. 2021;11:210188. doi: 10.1098/rsob.210188.

[65] Wolfram-Schauerte M, Pozhydaieva N, Viering M, Glatter T, Höfer K. Integrated Omics Reveal Time-Resolved Insights into T4 Phage Infection of E. coli on Proteome and Transcriptome Levels. Viruses. 2022 Nov 12;14(11):2502. doi: 10.3390/v14112502. PMID: 36423111; PMCID: PMC9697503.

[66] Farooq T, Hussain MD, Shakeel MT, Tariqjaveed M, Aslam MN, Naqvi SAH, Amjad R, Tang Y, She X, Her Z. Deploying Viruses against Phytobacteria: Potential Use of Phage Cocktails as a Multifaceted Approach to Combat Resistant Bacterial Plant Pathogens. Viruses. 2022 Jan 18;14(2):171. doi: 10.3390/v14020171. PMID: 35215763; PMCID: PMC8879233.