Chapter

Therapeutic Efficacy of Bacteriophages

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Abstract

Bacteriophages are bacterial cell-borne viruses that act as natural bacteria killers and they have been identified as therapeutic antibacterial agents. Bacteriophage therapy is a bacterial disease medication that is given to humans after a diagnosis of the disease to prevent and manage a number of bacterial infections. The ability of phage to invade and destroy their target bacterial host cells determines the efficacy of bacteriophage therapy. Bacteriophage therapy, which can be specific or nonspecific and can include a single phage or a cocktail of phages, is a safe treatment choice for antibiotic-resistant and recurrent bacterial infections after antibiotics have failed. A therapy is a cure for health problems, which is administered after the diagnosis of the diseases in the patient. Such non-antibiotic treatment approaches for drug-resistant bacteria are thought to be a promising new alternative to antibiotic therapy and vaccination. The occurrence, biology, morphology, infectivity, lysogenic and lytic behaviours, efficacy, and mechanisms of bacteriophages' therapeutic potentials for control and treatment of multidrug-resistant/sensitive bacterial infections are discussed. Isolation, long-term storage and recovery of lytic bacteriophages, bioassays, *in vivo* and *in vitro* experiments, and bacteriophage therapy validation are all identified. Holins, endolysins, ectolysins, and bacteriocins are bacteriophage antibacterial enzymes that are specific. Endolysins cause the target bacterium to lyse instantly, and hence their therapeutic potential has been explored in "Endolysin therapy." Endolysins have a high degree of biochemical variability, with certain lysins having a wider bactericidal function than antibiotics, while their bactericidal activities are far narrower. Bacteriophage recombinant lysins (chimeric streptococcal–staphylococcal constructs) have high specificity for a single bacterial species, killing only that species (lysin (CF-301) is focused to kill methicillin resistant *Staphylococcus aureus* (MRSA)), while other lysins have a broader lytic activity, killing several different bacterial species and hence the range of bactericidal activity. New advances in medicine, food safety, agriculture, and biotechnology demonstrate molecular engineering, such as the optimization of endolysins for particular applications. Small molecule antibiotics are replaced by lysins. The chapter discusses the occurrences of lytic phage in pathogenic bacteria in animals and humans, as well as the possible therapeutic effects of endolysins-bacteriophage therapy *in vivo* and *in vitro*, demonstrating the utility and efficacy of the therapy. Further developments in the bacteriophage assay, unique molecular-phage therapy, or a cocktail of phage for the control of a broad range of drug-resistant bacteria-host systems can promote non-antibiotic treatment methods as a viable alternative to conventional antibiotic therapy.

Keywords: bacteriophages, bacteria, endolysins, therapy, therapeutic effects, cocktails, antibiotic resistance, multidrug resistant bacteria, *in vivo*, *in vitro*, experiments, control

1. Introduction

Bacteriophages (phage) are bacterial viruses that are also known as 'natural killer phages' may take over their bacterial host and use it to grow and multiply. The phage may recognise, infect, and kill specific bacteria or groups of bacteria, as well as their host cells of unrelated bacteria. As a result, they play an important role in bacterial population regulation. Bacteriophages are used to (a) identify specific pathogens to help in pathogen detection and (b) destroy bacterial infections in a process known as lysogeny, in which one bacterium kills another through phage particles [1–4]. Since he first discovered bacteriophages in 1917, and later in 1919, a phage treatment was offered to cure a child suffering from dysentery, and the child was cured of the illness after a single dose of phage administration, D'Herelle is widely regarded as the father of bacteriophages. Since then, the phage cocktail's protection has been verified by administering it to a number of other healthy people [3, 4]. He also noted in 1919 that bacteriophages provided between chickens effectively reduce the mortality of chickens suffering from *Salmonella* infections, indicating that phage therapy experiments against bacterial infections were extremely successful [3–5]. D'Herelle published a comprehensive account of bacteriophages and founded "An International Bacteriophage Institute" in Tbilisi, Georgia, in 1923, which is now known as "the George Eliava Institute of Bacteriophages, Microbiology, and Virology" [3–5]. The Institute is engaged in the production and distribution of therapeutic bacteriophages for the treatment of a variety of bacterial infections. Bacteriophages have been successfully used to treat skin and diarrhoeal infections caused by *Staphylococcus aureus* and *Shigella dysenteriae* [6–8]. However, phage treatment has been poor since the discovery of antibiotics, large-scale development and availability, and widespread clinical use [9–13]. Furthermore, there was a chance of endotoxin contamination since most phage therapy trials lacked random and placebo controls [5]. Overuse and misuse of antibacterial drugs have been recorded since the dawn of the antibiotic era, resulting in intolerable antibiotic resistance with an approximate global intake of 100,000–200,000 tonnes of antibiotics per year [14, 15]. Antibiotic resistance in bacteria has arisen from such indiscriminate prophylactic use of multiple antibiotics, affecting all aspects of life and public health [2, 14–18]. Antimicrobial resistance is becoming a global threat, with the World Health Organization predicting that it could kill at least 50 million people every year by 2050 [19]. As antibiotic resistance rises, researchers are looking for new ways to detect and manage drug-resistant bacterial infections [1, 2, 4, 5]. Antimicrobial-resistant bacteria have evolved from bacteria with intrinsically drug-sensitive genes to bacteria with drug-resistant genes: Multidrug-resistant bacteria are classified as bacteria that are resistant to at least one antimicrobial agent out of three or more, while drug-resistant bacteria are defined as bacteria that are resistant to all antimicrobial stages. The advent and distribution of antimicrobials has increased rapidly due to widespread use of antibiotics as a supplement in animal husbandry, misuse of various antibiotics in clinics [2, 9–11, 13]. Antimicrobials' proliferation and dissemination have accelerated in tandem with international mobility. Existing antibacterial agents were unable to destroy bacteria immune to antibiotics, ushering in the "post-antibiotic" period [9, 14–18, 20–22]. Because of their specific antimicrobial activity as an alternative to antibiotics, bacteriophage treatment is gaining popularity as a means of ensuring future development. When

antibiotics are ineffective against bacterial infections, phage therapy may help eradicate such complicated problems as a reliable treatment choice. In recent years, bacteriophages have been used to biocontrol bacterial numbers in agriculture, veterinary science, aquaculture, and the food industry [2, 10–13]. Bacteriophages have been used in agriculture to combat plant bacterial infections such as *Xanthomonas citri,* which would otherwise be treated with antibiotics. Holins, endolysin, ectolysin, and bacteriocins are bacteriophage antibacterial enzymes. Since endolysin targets induce immediate bacterial lysis, "endolysin therapy" has been developed to exploit their therapeutic potential [23]. Endolysin/recombinant endolysin has a lot of biochemical multiplication, and certain endolysins have a lot of bactericidal activity. Commercial applications have benefited from the use of endolysin enzymes or holins. The development of new drugs, creative methods, and the reduction of the risk of infectious agents and potential factors are all essential components of future bacterial disease control. Phage therapy reduces the development and replication of a wide variety of pathogenic bacteria, enhancing human and animal health and longevity. For particular groups of bacteria, however, the production of specific phage therapy cocktails is desirable. Phage therapy is a great way to treat microbial infections that are different depending on the operating system. Phage therapy is a fascinating rediscovered area of study that has many applications in science, agriculture, veterinary medicine, and medicine, including the potential prevention of antibiotic-resistant pathogens. The ability to combine antibiotic and phage therapy, the use of phage cocktails, and previously unexplored phage protein products are the most promising areas for the effective treatment of drug-resistant bacterial infections. Phage therapy is the subject of global research due to its wide range of applications and uses. This chapter addresses various aspects of phage therapy and how it can be used. After closely studying the protection and efficacy of phage, promising findings indicate that phage therapy against pathogenic bacteria could be the potential solution to pathogens that affect humans and animals.

1.1 Market potential of therapeutic bacteriophages

Bacteriophages are found all over the world, have many uses, and have contributed significantly to medicine, biotechnology, and molecular biology. Traditional antibiotic treatments are often replaced or supplemented by bacteriophage therapies and such alternative therapies has had a significant effect on revenues. In 2017, the global bacteriophage market was worth $567.9 million, and it is projected to grow at a 3.9% annual rate annual rate from 2018 to 2026. Globally, 600 million people are believed to be affected by foodborne diseases, with 420,000 people dying each year. In contrast, foodborne disease is said to affect 40% of children, resulting in 125,000 deaths per year [24]. The fastest-growing market will be for clinical applications of bacteriophages in phage therapy, diagnostics, drug development and manufacturing, phage display technology, antibacterial, vaccines, and biocontrol agents. Food and beverages currently hold the largest share of the global bacteriophage industry. Lytic bacteriophages are commonly used to control the spread of harmful infectious agents in foods such as fruits, vegetables, dairy products, and meals. Increased use of bacteriophages in such safe and healthy food items increased market potential. As a result, bacteriophages are being accepted for use in food safety applications in greater numbers. Companies are developing bacteriophage platforms and phagebanks (The Israeli Phage Bank (IPB) is a member of a global network of phage banks that provides a large assortment of purified bacteriophages) to treat multidrug-resistant bacteria in emergency situations [24]. Microgen, Amplify Bioscience Corporation, Ambiotics, and Phage Biotech Ltd. are

some of the leading players in the bacteriophage industry. According to Amplify Biosciences Corporation, clinical trials for phage therapy against *Pseudomonas aeruginosa* infection in cystic fibrosis have begun in the United States [24].

1.2 Isolation and identification of bacteriophages

Seclusion, identification and propagation of the patient's infecting bacterial strain are critical for successful phage treatment. In medical practice, once a patient is suspected of having a contagious incurable infection, effective bacteriophages should be isolated, identified and purified from isolates of pathogenic bacteria occurring in the samples of urine, blood, and chronic wounds of patients. The bacterial colonies shall be picked up from selective Agar/LB agar plates according to their colony morphology, size, and pigmentation variability. The isolates are subjected to the staining procedures, biochemical and molecular tests and are cultured in various media to identify the genus and species of bacteria with the help of *Bergey's Manual of Determinative Bacteriology* and Bergey's manual of systematic bacteriology [25, 26]. Each of the bacterial isolates shall be transferred to LB broth at 37°C for 18 hours and then be stored at −20°C after the addition of 20% glycerol for further studies. With the development of diagnostic techniques, nonculture-methods such as 16S rRNA, PCR, RT-PCR, microarray, DNA/RNA sequencing, proteomics, ELISA and immunological methods and MALDI-TOP MS are used in clinical laboratories for microbial testing, identification and classification [1, 26–29]. If patients are opting for phage treatment, the foremost step is to isolate the disease-causing pathogenic bacteria using traditional methods. Subsequently the pathogens can be identified by using non-traditional methods. Bacteriophages uninfected host cells of bacteria multiply in Nutrient/LB agar plate to form a confluent film of bacterial growth over the surface of the plate at 37°C. In contrast, bacteriophages of infected cells of pathogenic bacterium if occur, bursts of such cells take place and release offspring bacteriophages. A visible, circular area of clearing zone in the confluent bacterial growth is known as a plaque, occurring after 8-10 hours of incubation and halos, zones of secondary lysis around plaques, can be identified after 24 hours. A suspension consisting of incubated samples of phage and cells of bacterial isolates shall be poured on to an appropriate LB/Nutrient agar medium to form a thin 'top layer'. Lastly, sensitivity and specificity of phage to the pathogenic bacteria is tested therapeutically. A key option in the treatment of infection is to use standard antibacterial drug therapy based on an anti-bacterial profile and / or physician experience [27]. Therefore, phage therapy will only be recommended if antibacterial drugs are unsuccessful and/or as soon as the infection is triggered by multidrug-resistant or pandrug-resistant bacteria.

2. Phage library and sensitivity

Availability of a library with a range of therapeutic bacteriophages is the foundation for the success of phage therapy. Bacteriophages are observed to show off a narrow to broad host specificity [30–32]. The lytic bacteriophages ought to have the capacity to kill strange bacterial species even as a range of bacteriophages can kill the identical bacterial strain (**Figures 1–4**) [33]. For a safe medical use of the phage, genes of toxins, antibiotic-resistance, and multidrug-resistant genes should not be present in the genome of the phage', whilst the lytic phage must have the potential to kill the multidrug-resistant bacteria such as *Acinetobacter baumannii, Enterococcus, Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* [34–38]. Confirmation of phage-sensitive bacteria is the prerequisite for

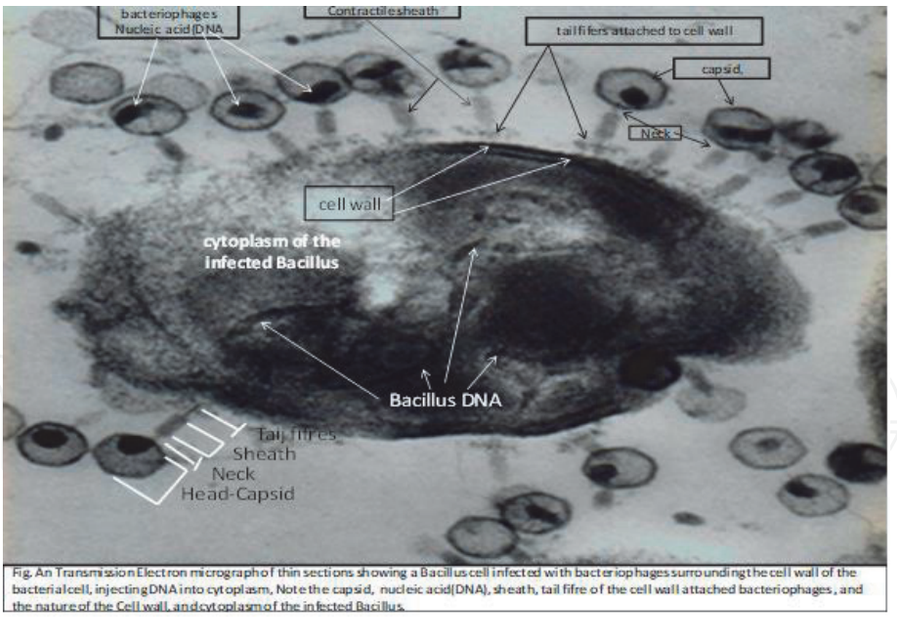

Figure 1.
Transmission electron micrograph (TEM) bacteriophages infected Bacillus. Note the capsid, nucleic acid, tail fibers [2].

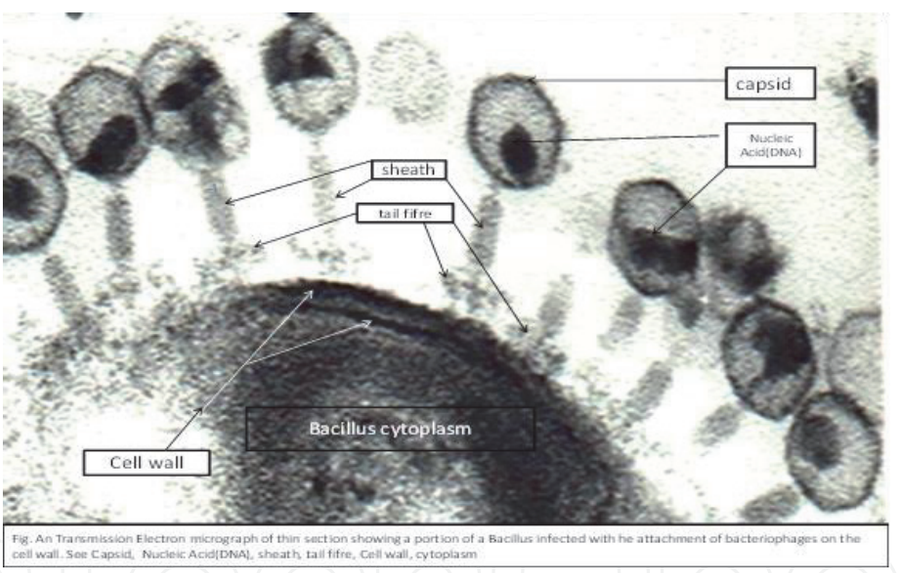

Figure 2.
A magnified portion of a Bacillus infected with bacteriophages is seen in this Transmission electron micrograph (TEM). The capsid, nucleic acid, tail fibers should all be noted [2].

initiation of antibacterial therapy. First, the bacterium inflicting the infection in the patient has to be received and identified; second, phage in the library that are effective against the pathogenic bacterium need to be screened, and selected for therapeutic use. If there are specific phage or cocktails of phage in the library that kill the identical bacterial strain, are preferred for the therapy [39, 40]. Reports have shown that phage cocktail preparations would possibly decorate bactericidal efficacy and additionally limit the chance of the emergence of phage-resistant isolates for the duration of the therapy [30–32]. Bacteriophages (phage) have a unique sorting mechanism for their target bacteria since they have a number of necessary characteristics such as inherent natural specificity, ease of use of cell signalling and receptor molecules, and simple phage or phage-derived product processing. These characteristics make bacteriophages more suitable for use as bacterial detectors and

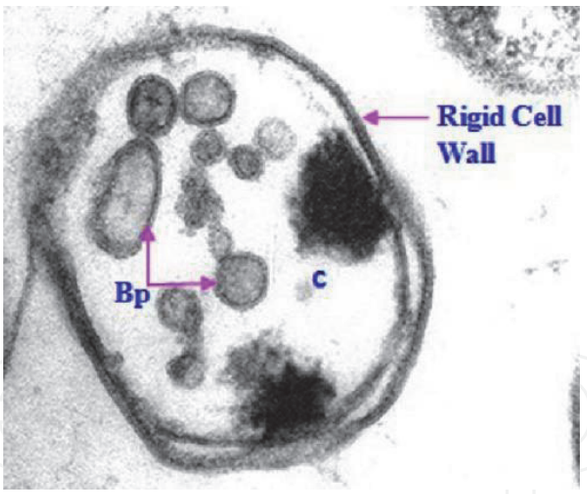

Figure 3.
TEM showing V. vulnificus *(VV-1) bacteriophage particles (Bp) within the cytolyzed cytoplasm (c) of the host cell bacterium* Vibrio *sp. Note the presence of phage within the cytoplasm [10].*

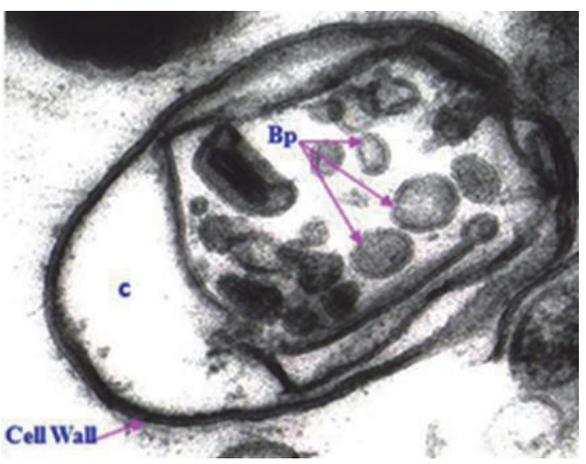

Figure 4.
Electron micrograph showing V. vulnificus *(VV-2) bacteriophage (Bp) particles within the lysed cytoplasm (c) of the host cell bacterium* Vibrio *sp [10].*

as aids in the detection of human pathogens. Phage-based systems are currently being used to diagnose *Staphylococcus aureus, Mycobacterium tuberculosis, Bacillus anthracis*, and *Yersinia pestis* in the clinical setting.

3. Production and purification of bacteriophages

Appropriate cultural media used for the growth, proliferation, and fermentation process of cells of bacterial hosts of bacteriophages for therapeutic applications. The fundamental processing of bacteriophages consists of several stages of purification (**Table 1**). These are broth specifications with low-speed centrifugation or filtering, cell removal, and cellular debris. Chloroform will be added to the lysate to form lysis and release the phage from non-lytic cells. Specified bacteriophage lysate can be used in many applications but clinical applications require additional purification of the lysate to eliminate endotoxins, metabolites, hydrophilic O-specific polysaccharide, phosphorylated oligosaccharides, phage, bacterial cells, and other wastes. Impure preparations of bacteriophages should not be used for injection [41, 47]. Occurrences of such as endotoxins in the bacteriophage preparations may aggravate

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages	Methods of isolation and purification	Therapeutic effects	Reference
1	Bacteriophages of *Pseudomonas, Klebsiella,* Serratia	Bacterial infection of *Pseudomonas, Klebsiella, Serratia*	A single production run can produce up to 64,000 treatment doses at 10^9 PFUs	Protocol used an aggregate of modified traditional techniques, membrane filtration processes, and no organic solvents to yield on average 23 mL of 10^{11} plaque-forming devices (PFUs)/ milliliter for *Pseudomonas, Klebsiella,* and *Serratia* phages tested	The protocol is beneficial for large scale standardized cultivation, purification, and manufacturing of bacteriophages. The approach emphases' eliminating endotoxins by up to 106-fold in phage preparations.	2020 Tiffany Luong [41]
2	T4-like coliphages or a commercial Russian coliphage	*E. coli* diarrhea Bacterial diarrhea	3.6×10^8 PFU of T4-like coliphage cocktail (T)	1 gm stool in 5 ml TS (8.5 g NaCl, 1 g tryptone/l), centrifuged at 14,500 g for 15 minutes. Filtered in a Millex AP20 prefilter and a Minisart filter. The presence of phages was determined in E. coli strains WG5 and K803, a K-12.	Fecal coliphage increased in treated over control children, however the titers did no longer show substantial intestinal phage replication. Lack of clinical efficacy of oral phages. No adverse events are attributable to oral phage application.	2016 Shafiqul Alam Sarker [42]
3	1. Bϕ-R2096 2.YMC 13/03/ R2096 ABA BP (phage Bϕ-R2096) lytic phages family Myoviridae	carbapenem-resistant *Acinetobacte baumannii* (CRAB) Serious nosocomial infection	Treated with concentrated phage Bϕ-R2096(1×10^{10} PFU) at two MOIs (MOI 100 and10)30 min after infection with CRAB (1×10^8 CFU).	Carbapenem-resistant *A. baumannii (*CRAB) lytic phages isolated from sewage samples, purified, concentrated, treated NaCl (1M), (PEG) 8000 incubated at 4°C for 24 h, filtered using 0.22 μm membranes and centrifuged at 12,000 × g for 1h at 4°C, resuspended in sodium chloride-magnesium sulfate (SM) buffer.	Bacteria-only-infection group died rapidly. A sizable reduction in mortality in both *G. mellonella* larval and the mouse acute pneumonia models; Bϕ-R2096 improved the survival rates of each G. mellonella larvae and the mice *in vitro* and *in vivo*. No mortality or serious side effects in phage-treated experimental animal groups.	2019 Jongsoo Jeon, [43]
4	Lytic bacteriophage, phage 1513	Multidrug Resistance *Klebsiella pneumoniae* Pneumonia	Intranasal administration of a single dose of 2×10^9 PFU/mouse 2h after KP 1513 inoculation	Centrifuged sewage sample, supernatant was supplemented with CaCl2. 10 mL supernatant, 10 mL 2 LB broth, and 2 mL bacteria solution [*K. pneumoniae* (KP 1513)] were incubated. By applying NaCl and (PEG8000), phages were precipitated,	Mice were protected from lethal pneumonia. When compared to untreated controls, phage-treated mice had a lower K. pneumoniae burden in the lungs. The phage KP 1513 has a significant antibacterial effect in vitro and in vivo, indicating that it could be	2014 Fang Cao [44]

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages	Methods of isolation and purification	Therapeutic effects	Reference
				ultracentrifuged, and passed via a Detoxi-Gel endotoxin removing gel. The purified phages were held at 4°C before they were used.	used instead of antibiotics to treat pneumonia caused by multidrug-resistant K. pneumoniae.	
4	A. baumannii phages	*Acinetobacter baumannii* Wound Infections	10^6 PFU. For cocktail synergy studies- 10^8 PFU per well for an MOI of 100	After growing AB5075 or AB5075P to exponential phase, 1 ml of each strain was added to 100-ml aliquots of the TSB-sewage mixture, inoculated with *A. baumannii,* incubated. 1 ml of infected TSB-sewage was centrifuged, the supernatant was filtered in a 0.22-m Spin-X centrifuge tube filter, and the centrifuged at 6,000 g. purified by using cesium chloride density centrifugation and filtered via a 0.22-m filter. Stocks of phages were held at 4° C.	The phages cocktail reduces bioburden in the wound, prevents infection and necrosis from spreading to adjacent tissue, and reduces infection-related morbidity.	2016 James M. Regeimbal [45]
5	CM8-1 and SJT-2 Bacteriophage	*Klebsiella pneumoniae* mastitis in dairy cattle	Bacteriophages bMECs were treated with or without K. pneumoniae (MOI, a 10:1 ratio of K. pneumoniae to bMECs), bacteriophages CM8-1, or SJT-2 (MOI, ratio of bacteriophage to K. pneumoniae was 1:10)	Bacteriophages CM8-1 and SJT-2 were isolated from dairy wastewater and mixed with a mid-log phase bacterial solution before being spread over a double-layer agar plate. Sodium magnesium (SM) buffer was applied after the bacteriophage had spread across the entire plate. A 0.22 m filter was used to filter the bacteriophage SM solution. PEG8000 (10%) was applied to the bacteriophage stock solution and stored at 4 o C overnight before being centrifuged for 10 minutes at 10,000 g.	Bacteriophages bMECs decreased bacterial adhesion, invasion, and cytotoxicity. The bacteriophage significantly reduced morphological damage and decreased TNF- and IL-1 concentrations, which were visible 4 to 8 hours after infection with *K. pneumoniae.*	2021 Yuxiang Shi, [46]

Table 1.
Methods of isolation and purification of therapeutic bacteriophages.

the immune system responses viz. fever, leucocytosis, leukopenia, fatal endotoxin shock, can open up macrophages, and release inflammatory mediators such as TNF-α, IL-6, and IL-1 and cause serious side effects. The final limit of endotoxins recommended for intravenous administration is 5 Endotoxin Unit (100 pg) (EU)/kg. The elimination of endotoxin from bacteriophages is a multidisciplinary procedure. Two-Phase fluid extraction processes viz. LPS affinity resins, ultrafiltration, and chromatographic methods for removal of well-charged endotoxin proteins. Ion exchange, size exclusion chromatography, interaction with histidine or polymyxin B, and anion-exchange chromatographic exchange were methods used for further phage purification. Diafiltration was used to exchange phage particles from lysate media with a suitable buffer. Cesium chloride density gradient centrifugation, ultracentrifugation, PEG precipitation, and ultrafiltration used for the removal of endotoxins and purification of phages. Bacteriophage CM8-1/SJT-2 stock solution mixed with bacterial culture in the mid-log phase spread on a double-layer agar Petri plate, Sodium magnesium (SM) buffer added after the bacteriophage had grown of the entire Petri plate and placed on a shaker at 120 rpm/min for 2 hours. The SM-bacteriophage lysate solution was filtered through a 0.22 μm filter, PEG8000 (10%) was once added to the bacteriophage stock solution, left the solution overnight at 4°C, and centrifuged at 10,000 × g for 10 min to obtain bacteriophage precipitation [46]. By contrast, T4 bacteriophages had prepared by using a stepwise gradient of anion-exchange quaternary amine (QA) CIM column and NaCl elution buffer [48, 49]. Purified bacteriophages of *Mycobacterium smegmatis* and *S. aureus* were prepared by using columns such as QA CIM and diethylamine (DEAE) while QA and DEAE CIM columns, were employed to remove endotoxins from pre-purified phage preparations by using the Endotrap HD column (Cambrex BioScience, EndoTraptm Blue) [50]. Enterococcal bacteriophages, viz. ENB6 and C33 were prepared from the raw wastewater by using caesium chloride density gradient centrifugation and stored at 4°C. Thus, there was a great deal of variation in the elution conditions between the different phages. A common operating procedure for varied phage preparations, storage, and transport is lacking [51]. Standard operating procedure (s) for large scale-bacteriophage cultivation, isolation, titration, and purification and to produce sufficient plaque-forming units of bacteriophages (PFUs) per milliliter of *Pseudomonas, Klebsiella, and Serratia* were established [41, 52]. Such a universal process and production of the final phage preparations for use could reduce endotoxins, might be pivotal in alleviating fears and the phage therapy shall be readily accepted all the world over.

4. Storage of phages

Phage preparations for clinical use ought to be (i) endotoxin-free, (ii) phage must be intact with high titers [53–55]. (iii) Suitable storage and transport are crucial. (iv) protected from high temperature, extremely acidic, or alkaline conditions [56], and (v) phage stock should not be refrozen and rethawed [57]. The usefulness of preparation of phage lysate, modified treatment methods evolved and accepted for long-term storage of phage was elucidated. In a study that demonstrated the infectivity of the phages remained unaffected with chloroform and DMSO treatments and storage for 30 days to a year at 4°C − 40°C [10, 58–60]. Infectivity of long-term stored bacteriophages at −80°C can be increased by adding 15%-25% glycerol to phage lysate preparations, and by rapid freezing and storage of phage infected bacteria at −70°C. Similarly, phage was shown to remain highly stable underneath normal storage conditions or also stable in NaCl and $MgSO_4$ due to its stabilizing effect. Considerable numbers of viable phage have been described

to occur even after storage in distilled water. Phage isolates were found to remain stable upon storage at 4°C, or a rapid loss of phage infectivity was encountered with repeated freezing and thawing at −70°C. Phage infectivity could not be inhibited with trypsin, protease, ribonuclease treatments, or chloroform whilst the infectivity over the phage was inhibited together with lysozyme and SDS treatments [10, 59, 60]. The enzymatic treatments and inhibition of phage infectivity of several bacteriophages had been reported. Similarly, *Mycoplasma arthritidis* virulent 1 (MAV1) phage infectivity was reported to be unaffected by treatment with Triton X-100 and used to be resistant to non-ionic detergents [55, 61]. Phage survived a hundred percent at pH 7 and exhibited infectivity, whilst none of the phage survived at extreme pH conditions (pH 3 and pH 12) [10]. At a temperature below 37°C, phage JSF9 was shown to be stable whereas, at 50°C, the phage had been rapidly inactivated. Phage (VPP97) of *V. parahaemolyticus* have been shown to be stable up to 65°C and were totally inactivated at 70°C [10, 61–64]. Bacteriophages were detected to survive extremes over 95°C [52]. Bacteriophages such as T-ϕD0, T-ϕD2S, T-ϕHSIC, and T-ϕD1B exhibited a latent period ranging beyond 90°C [64]. The effects of temperature on the survival and infectivity of bacteriophages have clearly shown that the physicochemical parameters are very important for the survival and infectivity of phage [55, 58, 59]. Bacteriophages can be resilient to low/high temperatures, salinity, pH, and ions. They can tolerate extreme environments. New data on these along with therapeutic phage survivability, methods of their preservation and transport shall be useful.

5. Phage therapy

A bacteriophage therapy is a treatment for a patient's bacterial disease illness that is provided after the patient has been diagnosed. Bacteriophages are the most valuable and ubiquitous (10^{31}) organisms in the world, and are known to infect >140 bacterial genera. Description of phages and their antibacterial activity has initially been set up [6]. Bacteriophage therapy exhibits precise antibacterial lytic activities that have turned out to be a really useful concept to kill even an intracellular pathogenic bacterium and guarantee future development and consequently the therapeutic phages are re-emerging. As a substitute to antibiotics, experimental bacteriophage therapy might replace them when they fail to treat chronic infections, and such successful eradication of drug-resistant bacteria has been properly identified and demonstrated [65–72]. A single dose of phage has been shown to be more effective treatment than many doses of antibiotics such as amphetamines, tetracycline but chloramphenicol [73]. Moreover, careful phage collection, propagation, and purification requires complete experimental conditions. Such a focus ought to assist in the improvement of medical phage therapy utilized to a variety of systems, which is viewed an attribute on an emerging choice to antibiotic therapy and vaccination. The consequences of phage therapy are dependent on the plan of preparations and rout of administration of bacteriophage. The best possible administration route for phage preparations which should facilitate sufficient phages coming into direct contact with the bacteria. Routes of phage administration vary from oral, intravenous to multiple topical applications. There are different types of bacteriophage preparations developed to facilitate direct contact of the phage with the pathogenic bacterium for special bacterial infections and they are: (i) a phage powder, phage-containing lotion or dry gauze layer containing phages could be used for skin infections [74]. (ii) bacteriophages that have been sprayed dry become phages that can be inhaled as powder [75–77]. (iii) aerosolized phage preparations may be chosen for respiratory tract infections [76–78]. (iv) cream of phage

preparations for skin infections. (v) injectable types of phage formulations [41, 47, 79]. (vi) phage infusion preparations may be considered for bloodstream infection [80, 81]. (vii) capsules containing phages (encapsulation/micro-encapsulation) that can protect particles from stomach acid inactivation should be preferred for gastrointestinal infections [75, 82]. An improved understanding of how synergic interactions of bacteriophages, cocktails with antibiotics impact bacterial infection is needed to stop unintentional inhibition of phage replication. Aerophages and IV phages each rescued 50% of animals from severe MRSA pneumonia. A mixture of aerophages and IV phages rescued 91% of animals, which was higher than either monotherapy or cocktail phage therapy [12]. Phage alone or a mixture of phages with antibiotics were treated against several bacterial infections in skin, blood, lung, and chronic otitis [36, 66, 80]. In contrast, other clinical reports have shown that some phages do not work due to constant infection and ETEC (Enterotoxic *Escherichia coli*) -complex diarrhoea [42, 83]. However, the prevalence of MDR bacteria is increasing, and our port drug portfolio is obsolete. The evolution of antibiotic resistance bacteria has thus become a major world health care problem. Clinical threats include MRSA, *Mycobacterium tuberculosis* and Vancomycin-Resistant *Enterococcus* (VRE) [84–86]. MDR bacterial infection is challenging and expensive to treat because of the increased resistance to all the antibiotics in practice. According to the Centres for Disease Control and Prevention (CDC), two million people are infected with antibiotic-resistant bacteria, and 23,000 people die each year in the USA from antibiotic-resistant bacterial infections. Prescribing antibiotics for the treatment of only standardized bacterial infections may slow down the process, but will not slow down the overall trend. Frequent use of antibiotics against diseases in humans and other organisms contaminates the environment and its cumulative effect on the development of antibiotic-resistant bacteria. As the number of antibiotic-resistant bacteria increases, alternative methods must be developed to effectively control them. Therefore, the use of antibiotics is a danger. Bacteriophage therapy with specific phages or a cocktail of phages signify an exciting alternative development to antibiotic therapy and vaccination. The progress of bacteriophage assays, biosensor tools, and bio-nano-targeted drug delivery system against drug-resistant bacteria elucidated. Bacteriophages are highly specific to target bacteria, and hence its usage is targeted toward a specific bacterial species and significantly minimizes off-targets effects on microbiome or human patient, as bacteriophages do not directly affect human cells [87]. Thus, phage treatment has been re-emphasized as the severity of drug-resistant bacteria has increased [66]. Therapeutic bacteriophages, units and outcome of the treatment of some antibiotic resistant bacterial infections are presented in **Tables 1** and **2**.

5.1 Personalized therapeutic phage

The term "personalised phage therapy" refers to the preparation and precise targeting of phage(s) against bacteria isolated from infected patients. Phage therapy has made extensive use of such a precise approach [80, 96], (**Table 3**). The patient's conditions need to be observed regularly to evaluate whether or not they are improving, and clinical samples from bacterial infection sites should be assessed in a timely manner to evaluate therapeutic efficacy, the emergence of phage-resistant strains and efficient phage titers. Phage should be replaced once a particular phage-resistant bacterium emerges [96]. If there are no phage in the library that kill a phage-resistant bacterial strain, the bacterial strain can be further used as a host bacterium to screen various types of samples (e.g., soils, faeces, urine) to isolate new effective phage. Such new bacteriophages can be added continuously to enrich the phage library if they meet the criteria. Phage therapy can be considered as an

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
1.	Two novel bacteriophages, PBAB08 and PBAB25	(MDR) *Acinetobacter baumannii* Nasal infection	1×10^9 PFU of phage cocktail, intranasally injected	Mice treated with the phage cocktail showed a 2.3-fold higher survival rate than those untreated in 7 days post infection. 1/100 reduction of the number of *A. baumannii* in the lung of the mice treated with the phage cocktail.	2018 Kyoungeun Cha [88]
2.	PP1131 -phage cocktail	*Pseudomonas aeruginosa* Endocarditis	10^{10} PFU	Single-dose phage therapy was enough to control *P. aeruginosa* EE infections and act synergistically with ciprofloxacin. Phage-resistant mutants had impaired infectivity of *P. aeruginosa.*	2016 Frank Oechslin [89]
3.	Caudovirales phage strains, MPK1 and MPK6	Pseudomonas aeruginosa Peritonitis-sepsis caused by intraperitoneal (i.p.) infection	Mouse-2×10^6 or 2×10^7 PFU, *Drosophila melanogaster* - 5×10^7 PFU	Mice treated with phage had lower bacterial burdens in their livers, lungs, and spleens. Both phages significantly delayed the PAO1-induced killing of *D. melanogaster* ($P < 0.001$), although MPK1 persisted longer than MPK6 in uninfected D. melanogaster tissue samples. Infection is valid for evaluating the antibacterial efficacy of phage therapy against *P. aeruginosa* infections.	2009 Yun-Jeong Heo [90]
4.	Bacteriophage (MSa)	*Staphylococcus aureus*	Bacteriophage (MSa) (10^8 PFU)	All mice in the control group and the group treated with the lowest phage dose 107 PFU / mouse, died within 4 days (10/10 mice). mice treated with an intermediate dose 108 PFU/mouse were incompletely protected (2/5 mice survived). mice	2007 Rosanna Capparelli [91]

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
				treated with the highest dose 10^9 PFU/mouse were all protected from the infection of *S. aureus*. The phage MSa inhibited abscess development.	
5.	A range of phages	*Pseudomonas aeruginosa* Chronic bilateral otitis externa	Approximately 400 PFU of phage (in 0.2 ml saline) were instilled into the right auditory canal.	No adverse effects were observed. *P. aeruginosa* was isolated from the ears after treatment, there were recurrent cycles of improvement and deterioration in the condition of the ears but they were better than before phage treatment	2006 J.A. Sivera Marza [92]
6.	Phage WSa	*Vibrio vulnificus*. Local and Systemic Disease	10^8 PFU	Infected mice with *V. vulnificus* may be treated to avoid local and systemic illness, as well as death. Phage therapy is a viable treatment choice for bacterial infections.	2002 Karen E. Cerveny [93]
7.	Enterococcus phages ENB6 and C33	Vancomycin-Resistant *Enterococcus faecium*. Gastrointestinal tract infection-VRE bacteremia and endocarditis	3×10^8 PFU of the phage strain	ENB6 phage formed plaques on 57% of the VRE clinical isolates and inhibited the bacterial growth of an additional 22% of the strains, thus exhibited an antibacterial effect against 79% of the strains. At higher doses of phage, 100% of the animals survived with minimal signs of illness such as mild lethargy in the first 24 hours	2001 Biswajit Biswas [94]
8.	Cocktail of four phages provided by Texas A&M and the San Diego-based biotech company AmpliPhi	Multidrug-Resistant Bacterial Infection. *Acinetobacter baumannii*	Phage cocktail is normally applied topically or taken orally. Phages were injected intravenously and into the abdominal cavity through catheters.	The bacteria gradually gained resistance to the phages, but the team compensated by constantly tweaking treatment with new phage strains and antibiotics, some of	2017 Scott LaFee and Heather Buschman [95]

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
				which they had obtained from sewage. The road to recovery has not been without bumps. There have been setbacks that have nothing to do with the phages.	

Table 2.
Efficacy of therapeutic bacteriophages treatment of antibiotic resistant bacterial infections.

example of personalized medicine for bacterial infections [80]. Phage resistance may also be accompanied by changes in antibiotic resistance [99]. Therefore, the antibiotic resistance profile of phage-resistant strains should be simultaneously tested. The synergistic bactericidal activity of combining phage and antibiotics in the clinical cases should be considered [100] and further treatment strategies using phage alone and/or in combination with antibacterial drugs should be considered based on the results. The development of phage-sensitive and -resistant strains should be monitored regularly during phage therapy to see if phage therapy is a viable choice for successfully dealing with this issue. A clinical trial demonstrating the therapy's beneficial effects is critical in verifying its medical importance.

5.2 Gangrene wounds

Gangrene is the death of body tissue due to bacterial infection or lack of blood flow. Gas gangrene is caused by infection with a bacterium called *Clostridium perfringens* which in turn produces toxins that release gas causing tissue death [91]. A concoction of bacteriophages has been used to cure gangrene which is lively towards *Staphylococcus* spp., *Streptococcus* spp. and *Clostridium* [36, 50, 56]. Therapeutic efficacy of the phage has been improved, with the utility of "Pyophage" (a poly-specific cocktail of phage), achieved after detection of the particular etiologic agents and application of mono-specific lytic phage. The sequence of phage therapy treatments consisted of washings of the wound with a phage preparation, followed by subcutaneous injections of phage(s) as soon as to 4 instances per day. The utility of phage therapy has led to the removal of 69% Staphylococcal and 50% Streptococcal infections. Poly-specific (Pyophage, Sekstaphage) and mono-specific therapeutic phage cocktails developed have been used. Bacteriophages had been administered locally, via subcutaneous injections, and orally. Notably, phage therapy used to be carried out as a monotherapy, or complex treatment, which covered phage(s) and antibiotics administration. The investigations revealed that complicated treatment diminished the healing time by way of 1.2–2.5 times compared with antibiotic treatment. Even application of bacteriophages unique to one of the infectious agents in a wound expanded restoration and prompted quicker recuperation and purification. Importantly, it has been proved that a single utility of a bacteriophage would now not be adequate to stop infectious lesion problems. However, the investigators could not be concluding that the utility of bacteriophages barring antibiotics is better, as they had been unsuccessfully handled with antibiotics. They cautioned that the use of phage preparations supplied a fantastic impact on mono-infection, whilst complicated treatment, consisting of bacteriophages and

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
1.	Φ2 (KpJH46Φ2)	*Klebsiella pneumoniae*. Prosthetic joint infection (PJI)	The patient received daily infusions of 6.3×10^{10} phages in 50 mL of normal saline each weekday for a total of 40doses.	Local symptoms, signs of infection, and recovery were all improved with phage therapy. The patient had no medication-related side effects and was asymptomatic 34 weeks after finishing treatment.	2020 Edison J Cano [81]
2.	Bacteriophage OMKO1	*Pseudomonas aeruginosa*. Prosthetic vascular graft infections	1,000 PFU phage OMKO1 (10e7 PFU/ml) in 10 ml phage OMKO1	The infection tended to resolve after a single treatment of phage OMKO1 and ceftazidime, with no signs of recurrence.	2018 Chan, B. K. [97]
3.	Cocktail of 2 bacteriophages	Multidrug-resistant *Pseudomonas aeruginosa*, Bacteremia/sepsis after the ASD/ VSD closures	Dose of 3.5×10^{5} PFU every 6 hours.	When the patient resumed bacteriophage therapy, blood cultures that had reverted to positive for many days surprisingly, reproducibly reverted to sterile, which coincided with clinical progress.	2018 C. Duplessis [98]
4.	*A baumanii* bacteriophages	Multidrug-Resistant *Acinetobacter baumannii*. Craniectomy Site Infection	2×10^{10} PFU/mL, with an endotoxin level of 3.5×10^{5} endotoxin units (eu)/ mL. The phage dose given was 2.14×10^{7} PFU/mL	While the craniotomy site and skin flap healed well, fevers and leukocytosis continued. After surgical debridement, there were no more signs of infection at the craniotomy site, and no purulence to send for a repeat culture.	2018 Stephanie LaVergne [34]

Table 3.
Therapeutic bacteriophages for personalized treatments.

antibiotics, was once required for combined bacterial infections [101]. The use of distinctive bacteriophages was once greater than therapy with unique poly cocktails [102]. The most effective of this kind of custom-made phage therapy can be accelerated by using the specificity and virulence of phage to host strains. However, modified phage preparations require certain planning due to the fact they can incorporate temperate bacteriophages produced with the aid of a kind of scientific bacterium which has been used for adaptation.

5.3 Burn wounds

Burn wounds of patients have risks of bacterial infections. The floor of burn wound areas of sufferers may exhibit sepsis, lymphopenia, and intoxication. The

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
1.	Lytic anti- *P aeruginosa* bacteriophages	*P. aeruginosa/ E. coli* Burn infections	PP1131; 1×10^6 PFU per mL	At very low concentrations, PP1131 decreased bacterial burden in burn wounds than standard of care	2017 Patrick Jault [83]
2.	*P. aeruginosa* phages 14/1 (Myoviridae) and PNM (Podoviridae) and *S. aureus* phage ISP (Myoviridae)	*Pseudomonas aeruginosa*, *Staphylococcus aureus*. Burn wound infection	10^9 PFU/ml of each phage	No adverse events, clinical abnormalities or changes in laboratory test results that could be related to the application of phages were observed.	2014 Thomas Rose [106]
3.	P. aeruginosa phages	P*seudomonas aeruginosa* Burn Wound	10^8 PFU/100 μl inoculum of each of the following phages: Pa1 (ATCC 12175-B1); Pa2 (ATCC 14203-B1), and Pa11 (ATCC 14205-B1) (ATCC catalogue of bacteria and bacteriophages,	All the thermally injured mice that were not infected with PAO1Rif but administered the phage cocktail survived. The phage cocktail was not toxic to traumatized mice	2007 Catherine S. McVay [108]

Table 4.
Therapeutic bacteriophages for antibiotic-resistant burn infections.

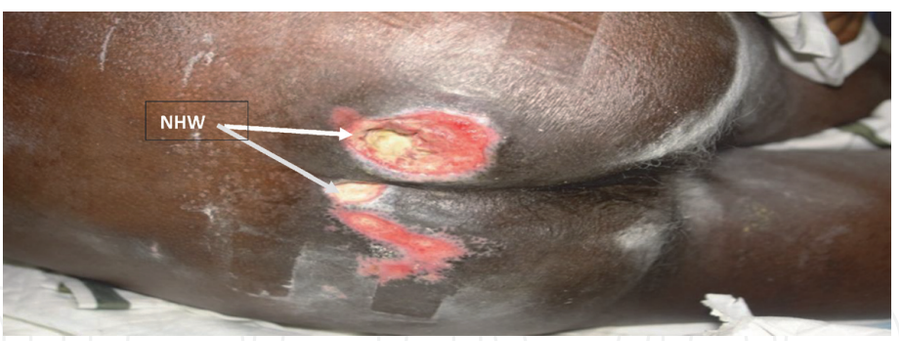

Figure 5.
Diabetic chronic non-healing wounds (NHW).

use of phage therapy was shown to be superb in eradication of pneumonia, the drug-resistant (MDR) *P. aeruginosa* infections in the burn wounds, and stopping the formation of sepsis [92, 103–107]. Therapeutic bacteriophages used for treatment of antibiotic-resistant burn infections are detailed in **Table 4**. In a complicated remedy comprising bacteriophages per OS and antibiotics, the use of bacteriophages has proven higher medical consequence in sufferers with contaminated burns (29% complicated instances of wounds) than in sufferers dealt with antibiotics (12.6% of cases) [103]. The volume of therapeutic phage particles ($\geq 10^6$ PFU/ml) used in the remedy is proven to be very extensive and the high-quality result of cure varied relying on the phage titer, routes of phage administration, sensitivity, specificity, and accessibility of bacterial host to the phage, length of phage therapy progression. A single dose (10^3 PFU/ml) of the phage BS24 has been confirmed to provide a

wonderful impact and in contrast, no encouraging wound restoration response has been determined when the phage cocktail BFC-1 10^9 PFU/ml has been utilized at the wound floor [51, 92, 106, 109]. Dosage, remedy procedure, safety, efficacy, and pharmacodynamics of two phage cocktails, suggestions to deal with *E. coli*, and *P. aeruginosa* contaminated burn wounds are described [51].

5.4 Psoriasis

Psoriasis is a common chronic skin disease causing red and itchy scaly patches on the scalp, knees, elbows, and trunk. The "Phagoburn project" aims to reduce bacterial growth and reduce the incidence of psoriasis in patients with severe inflammation and infection. "Phagobon" has been used in the treatment of phage cures to treat *E. coli* and *Pseudomonas aeruginosa* diseases. Phase I/II clinical trials were established in France, Belgium, and Switzerland. Although this project is a breakthrough in phage medical studies, *in vitro* trials and clinics are still needed to gain widespread acceptance in the use of the therapeutic phage to treat people with pathogenic diseases or MDR. A biodegradable polymer wound dressing called, "PhagoBioDerm" is impregnated with numerous antimicrobial elements containing the phage cocktail Pyophage, and the dressing exhibited a slow degradation and presentation of the antimicrobial, and the release of phage particles for a long time had been demonstrated, exhibiting higher healing of infected venous leg ulcers [36–38, 96, 110]. The use of the PhagoBioDerm is promising for each remedy and prevention of microbial infections in wounds [36, 111]. Therapeutic bacteriophages, method of isolation, purification, and storage, units of phage, outcome of the bacteriophage therapy and reference are listed in **Table 1**.

5.5 Diabetes ulcers

Exposed non-healing wounds on the feet are considered "chronic ulcers". Chronic ulcers show up in sufferers with diabetes, atherosclerosis, and varicosity of the limbs (**Figure 5**). The healing processes of such chronic diabetic foot ulcers (DFU) depends on the coexisting infection of aerobic and anaerobic microorganisms' viz. *Staphylococcus* spp., *S. aureus*, *Proteobacteria*, and anaerobes *Anaerococcus*, *Bacteroides, Clostridium*, *Peptonihilus*, and *P. aeruginosa* [30, 31, 35–38, 110]. Antibacterial cure of ulcers infected with a variety of microbial organisms shall be difficult [32, 33, 35]. Long-term administration of antibiotics for healing the ulcers in diabetes mellitus sufferers may additionally be complicated and ineffective. In such complicated instances of infected diabetic foot ulcers, phage therapy could be an alternative or a supplementary treatment to antibiotics treatments. Phage therapy used to be the most incredible in ulcers with one bacterial agent (100%), however, a personalized phage therapeutic strategy can also lead to the removal of pathogens in instances with combined infections. There are quite a few studies that have described the efficacy and well-being of phage treatment of infected ulcers in humans. Previous antibiotic treatment was unsuccessful with a mixture of microbial infections of DFU unlike the results of the Phage therapy treatment of patients [38, 96]. The fundamental challenge in treating such infected wounds was once the inability to rapidly select phages towards all recognized bacterial diseases. Patients with DFU infected with methicillin-resistant and methicillin-sensitive *S. aureus* strains were effectively treated and cured with *Staphylococcus* phage Sb-1 [37, 111]. Commercially available phage cocktails can be chosen in every case following their specificity to particular infectious agents in an ulcer. When no such precise phage cocktail was once commercially available, a custom-made phage preparation can be prepared. Commercially available bacteriophage solution has been used against

infections of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* occurring in Chronic Venous leg ulcers (VLU). No adverse events were reported for the study product and no significant differences were determined between the testing and control groups for the frequency of adverse events, the healing rate, or the frequency of healing [110].

5.6 Urinary tract infections

Acinetobacter baumannii is a Gram-negative nosocomial pathogen involved in human bacterial, meningitis, and respiratory infections (**Table 5**). A 68-year-old man with diabetes developed necrotizing pancreatitis, a complication of a pancreatic pseudocyst infected by the multi-drug resistant strain of *A. baumannii* [80]. Despite antibiotic treatment, the patient's condition deteriorated rapidly. Bacteriophage treatment has been initiated as part of an urgent new drug protocol. Commercially available Pyo bacteriophage solution (prophages; 20 mL) was used to enhance the treatment effect in the urinary tract infections in patients undergoing intravenous bacteriophage therapy TURP [112]. At very low concentrations of bacteriophage PP1131, the burden of the bacterium *P. aeruginosa* in burn wounds was less than the standard of care [83]. In another case of treatment, a solution consisting of 10^7–10^9 PFU/mL of the bacteriophages was introduced 2 times per 24 hours i.e., 8.00, 20.00 for 7 days, soon after surgery [113]. The patients were requested to hold the solution in the bladder for 30–60 min to control *Staphylococcus aureus, E. coli, Streptococcus* spp. (Streptococci group D renamed as *Enterococcus* spp.), *Pseudomonas aeruginosa*, *Proteus* spp. of urological infections of urinary tract infections after transurethral resection of the prostate. After treatment, four patients presented no significant bacterial growth while *E. coli* and *Enterococcus* spp. were still detected in the urine culture of four and one patient, individually. Bacterial counts decreased in six out of nine patients (67%), after the phage therapy treatment. No bacteriophage-associated adverse events have been detected. In one of the patients, (cephalosporin was given on day 3 after the development of fever (>38.0°C), the symptoms disappeared within 48 hours. Urine culture showed *P. aeruginosa* [113]. Intravascular bacteriophage therapy is no less than standard-protective care of antibiotic treatment, but it is no better than placebo bladder irrigation in terms of efficacy or safety in treating UTIs in patients with eruption. The data indicated that infection of the six lytic bacteriophages, each at a titre of 10 PFU mL − 1.20 mL ($\sim 2 \times 10^7$ p.f.u.) Pyo-phages were self-sustaining and self-limiting, with the phages decreasing in number along with the viable target organisms in which they replicated [114].

5.7 Pneumoniae

Bacterial pneumonia is an infection of *Streptococcus pneumoniae, Klebsiella pneumonia*, and *Mycoplasma pneumoniae* in each lung inflicting irritation in the alveoli or air sacs stuffed with fluid or pus, making it is hard to breathe. Pneumonia Phage (Φ2 (KpJH46Φ2)), *Klebsiella pneumoniae* examined for K Joint affected person against prostatic infection [43, 44], (**Tables 1** and **3**). The affected person received 6.3×10^{10} phages in 50 ml of normal saline solution and forty, doses every week. As a result of the treatment of phage, the local characteristics and *K. pneumoniae* infection symptoms had been resolved, the overall performance also had been restored. The affected person did now not experience any adverse effects related to treatment and remained asymptomatic within 34 weeks of completion of phage therapy when receiving minocycline. Intravenous injection of a single dose of 2×10^9 PFU of lytic bacteriophage of multidrug resistance *Klebsiella pneumoniae* KP

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
1.	Pyo bacteriophage cocktail	Urinary tract infections	Intravesical Pyo bacteriophage (Pyophage; 20 mL) or/intravesical placebo solution (20 mL) twice daily for 7 days in a double-blind fashion	Intravesical bacteriophage therapy was found to be comparable to regular antibiotic therapy. In terms of eptitude and protection in treating UTIs in patients undergoing TURP, however, it was not superior to placebo bladder irrigation.	2021 Lorenz Leitner et al., [112]
2.	Pyo bacteriophage	*Staphylococcus aureus, E. coli, Streptococcus* spp. (Streptococci group D renamed as *Enterococcus* spp.), *Pseudomonas aeruginosa*, *Proteus* spp. urinary tract infections after transurethral resection of the prostate	The bacteriophages have 10^7–10^9 PFU/ mL. The solution was given twice every 24 hours, at 8 a.m. and 20 a.m., for seven days, beginning the day after surgery. The patients were advised to keep the solution for 30–60 minutes in their bladders.	Four patients had no noticeable bacterial growth after treatment, while *E. coli* and *Enterococcus* spp. were still contained in the urinary cultures of four and one patient, respectively. After phage therapy, bacterial counts decreased in six out of nine patients (67 percent). There have been no reported side effects. The symptoms in one of the patients vanished within 48 hours after receiving cephalosporin on day three after developing a fever (>38.0°C). *P. aeruginosa* was discovered in urine culture.	2018 Aleksandre Ujmajuridze [113]
3.	*A. baumannii*-specific lytic bacteriophage cocktails PC, IV, and IVB, PC AC4, C1P12, C2P21, C2P24	*A. baumannii*. Necrotizing pancreatitis complicated by an MDR *A. baumannii* infection	Average endotoxin levels of bacteriophage cocktails PC, IV, and IVB were $2.4x10^3$ EU/ml, $5.89x10^3$ EU/ml, and $1.64x10^3$ EU/ ml, respectively 4 $x10^9$ PFU of bacteriophages, $5x10^9$ PFU of bacteriophages PC AC4, C1P12, C2P21,	The administration of bacteriophages intravenously and percutaneously into the abscess cavities was linked to the patient's clinical course being reversed, clearance of the *A. baumannii* infection, and a returning to health.	2017 Robert T. Schooley [80]

S. no	Therapeutic bacteriophages	Type of bacteria/ disease	Units of phages used	Therapeutic effects	Reference
			C2P24, Intracavitary, Intravenous 2		
4.	Lytic bacteriophages	*Pseudomonas aeruginosa*, Urinary tract infection	Six lytic bacteriophages, each at a titre of 10^6 PFU ml − 1 ... .20 ml ($\sim 2 \times 10^7$ p.f.u.) Pyophage	The bacteriophage infection was self-sustaining and self-limiting, with the phage's number declining in tandem with the number of viable target species in which it replicated.	2011A. Khawaldeh [114]

Table 5.
Therapeutic bacteriophages for antibiotic-resistant Urinary tract bacterial infections.

1513/mouse protected animals from sublethal pneumonia. The severity of pneumonia has been shown to be low. Compared with the untreated control, Phage-treated mice are more unlikely to develop *Klebsiella pneumoniae* in the lungs. Phage KP 1513, has a significant antibacterial impact *in vitro* and *in vivo*, and its host *K. pneumoniae* is multi-drug-resistant. Phage KP 1513 can be used as choice to antibiotic treatment for pneumonia caused by *Klebsiella pneumoniae* [44]. Aerophages/ Intravenous injection of bacteriophages saved 50% of animals from severe MRSA pneumonia compared to placebo controls. In contrast, administration of bacteriophages by both the aerophages and IV phages rescued 91% of animals, which used to be greater than either monotherapy. Standard-of-care antibiotic linezolid saved 38% of animals [79]. The natural phages belonging to Caudovirales including order Siphoviridae, Myoviridae, and Podoviridae had been separated from the clinical strains of multidrug-resistant *K. pneumoniae*. *In vitro* lytic activity of phages on isolated bacteria revealed 70% coverage of 33 isolated antibiotic-resistant strains, of which 50% targeted multiple phages. Overall, these results suggest the possibility of phage detection by strong action against antibiotic-resistant KP strains and may furnish a new therapeutic approach to the treatment of ESBL and CRKP infections [115].

5.8 Diarrhoea

Bacterial diarrhea occurs in humans if infected with bacteria such as *Salmonella* and *E. coli*. Symptoms of diarrhea appears if the lining of the intestine is unable to absorb fluid, or secretes fluid, and bowel activities become loose or watery 3 or more times a day. Loss of fluid and electrolytes were encountered as a result of diarrhea [42]. In a placebo-controlled clinical trial, oral administration of Coliphage 10^9 PFU against *Escherichia coli* 3 times/day/4 days showed no significant clinical benefit between the control and test group (**Table 1**) [51, 83]. Fifteen healthy volunteers with *Escherichia coli* diarrhea received *Escherichia coli* phage T4 dose (10^3 PFU/ml), high-phage dose (10^5 PFU/ml), and fifteen healthy adult volunteers received low dose *Escherichia coli* phage (PG4). Volunteers receiving high-dose (10^5 PFU/ml), high-dose phage showed stool phage 1 day after exposure. This prevalence is only 50% in those receiving low-dose bacteriophages. One week after the 2-day course of oral phage application, no faecal phage was detected. Oral phage

application did not reduce the total stool *E. coli* count. In addition, no significant phage T4 replication was found in the early *E. coli* population. The study described the production of phage cocktails for use in clinical trials and Phage preparations are already entering clinical trials [51, 83]. Phage therapy has recently been re-emphasized due to the severity of drug-resistant bacterial infections [9]. Antibiotics alone or with antibiotics have been used successfully to treat a variety of bacterial infections, including atherosclerosis, lung and lung infections, chronic otitis, skin burn infections and enteric infections [65, 68, 80, 92, 116]. In contrast, other clinical reports have shown that bacteriophages are less effective than expected due to inadequacy or coverage for topical bacterial infections and ETEC (Enterotoxigenic *Escherichia coli*) [42, 83]. In addition, published reports show no side effects in clinical trials or no adversative actions associated to phage application [51, 117].

5.9 Tuberculosis

Tuberculosis (TB) is a lung infection caused by the endogenous bacterium *Mycobacterium tuberculosis*. First-line TB drugs drugs like rifampicin and isoniazid are resistant to certain types of multidrug-resistant tuberculosis (MDR-TB). In 2018, 484,000 new TB patients failed to respond to rifampicin, according to the World Health Organization (WHO). Seventy-eight percent of these patients have tuberculosis with multidrug resistance (MDR-TB) [118]. Mycobacteria exist in over 170 distinct species, each with its own pathogen development in humans [119]. *Mycobacterium ulcerans* and *M. leprae*, in addition to tuberculosis, M*ycobacterium* ulceration and M*ycobacterium* leprosy, respectively, cause Barley ulcers and leprosy [120]. Alternative therapies for MDR-TB are important for disease control, particularly as newer approaches to mycobacteriophage therapy emerge. To date, 11,282 mycobacteriophages have been discovered [121]. *M. smegmatis*, a non-pathogenic vector, can transport phages to the same intracellular compartments as *M. tuberculosis* [122]. M. mycobacteriophage D29's antimicrobial value was doubled after it was given twice in a 24-hour period to treat tuberculosis H37RV [123]. Aerosolized bacteriophage D29 treatment reduced TB cases in the lungs and vaccinated mice against tuberculosis [124]. Tuberculosis-prone health workers can benefit from aerosolized mycobacteriophages. D29 was used to treat burly ulcers caused by Mycobacterium ulcers in the Marine Footpad model [125]. As the disease progresses, infected patients experience necrosis of the skin, subcutaneous tissue, and bone, necessitating surgical skin rupture. Mycobacterial and pathological counts were decreased after D29 was injected subcutaneously. In footballs and lymph nodes, it causes the development of water-borne cytokines. This approach was used to deliver the lytic mycobacteriophage TM4 to *M. tuberculosis*-infected RAW264.7 macrophages, which decreased bacterial counts. On the other hand, the phage was found to be inactive on its own. *M. smegmatis*-TM4 complex substantially reduced bacterial counts in *M. avium*-infected mice's spleens, while TM4 or *M. smegmatis* alone had no effect [126]. Phage cocktails may be used to overcome phage resistance tuberculosis.

5.9.1 Endolysin therapy

Endolysin therapy is a major part of phage therapy. Endolysins are considered protein-based antibiotics or antimicrobials. The purified endolysin is a powerful antibacterial agent for curing bacterial infections in human beings and animals. The efficacy of endolysin enzyme, host bacteria, bacterial disease and the therapuetic use in experimental animal models is listed in **Table 6**. Endolysin is an enzyme used by the bacteriophages to degrade the bacterial host's peptidoglycan from the inside,

S. no	Enzyme	Sources/bacteriophages	Bacteria/disease	Unit of enzyme	Therapuetic effects	Reference
1.	Depolymerase KP34p57	Lytic phage KP34	*K. pneumoniae*	KP34p57 depolymerase was evaluated at four different concentrations: 7.5 ng/ml, 75 ng/ml, 750 ng/ml, and 7500 ng/ml.	After 2 hours of incubation with the enzyme, none of the concentrations examined showed major improvements in colony count.	2020 Latka, A., [127]
2.	Two capsule depolymerases (Dpo42 and Dpo43)	Phage IME205 isolated from a raw sewage	Carbapenem resistant *Klebsiella pneumoniae* (CRKP)	Dpo42 or Dpo43 (20 ng)	Both Dpo42 and Dpo43 depolymerases rendered the host bacteria prone to serum complement killing. Anti-virulent capsule depolymerases show promise in the battle against CRKP infections.	2020 Liu, Y., [128]
3.	LysSS	LysSS recombinant protein was purified from the host cells of *E. coli* BL21 Star® (DE3)	*Salmonella* spp., *A. baumannii*, *E. coli*, *P. aeruginosa*, *E. faecium*, *S. aureus*, *K. pneumoniae*	MHB was used to incorporate freshly grown bacteria (104 CFU/well) and purify them. LysSS was added at different concentrations (20–200 g/well) along with 200 L of 1 MHB per well in each well. 1.25 mg/mL LysSS was applied at the end.	Without pre-treatment with an outer membrane permeabiliser, LysSS demonstrated activity against MDR *A. baumannii,* MDR *E. coli,* MDR *K. pneumoniae*, MDR *P. aeruginosa*, and *Salmonella* sp. LysSS stopped methicillin-resistant *S. aureus* from growing (MRSA).	2020 Kim, S., [129]
4.	Tripleacting staphylolytic peptidoglycan hydrolases (Lysostaphin and LysK-)	Recombinant phage lysin proteins	*Staphylococcus aureus*	Lysostaphin 0.77 g/ml (27 nM); LysK, 47 g/ml (840 nM); Lysostaphin and LysK (L + K) in combination 0.2 g/ml (7 nM and 3 nM, respectively); triple fusion K-L, 7 g/ml (97 nM); triple fusion L-K, 7.8 g/ml (107 nM); triple fusion L-	Nasal colonisation was decreased by 87 percent when 200 g lysostaphin was used. In cultured mammary epithelial cells and a mouse model of *S. mastitis*, *S. aureus* demonstrated biofilm eradication and the ability to destroy intracellular *S. aureus*	2016 Becker, S. C., [130]
5.	PlyC holoenzyme, mediated by PlyCB subunit	C1 bacteriophage	*Streptococcus pyogenes* strain D471	Treatment with 50 µg/ml WT PlyC	Lower concentrations showed a dose response and reduced intracellular colonisation (CFUs) by 95% within 1 hour. The endolysins	2016 Shen, Y., [131]

S. no	Enzyme	Sources/bacteriophages	Bacteria/disease	Unit of enzyme	Therapuetic effects	Reference
					from the B30 and Ply700 streptococcal phages, on the other hand, failed to reduce intracellular Spy CFUs significantly.	
6.	Chimeric lysin	Phages infecting Gram-positive bacteria	methicillin-resistant *Staphylococcus aureus*. Burn wound infected with MRSA WHS11081	ClyF doses are 25, 37.5, and 50 mg/kg, with a maximum dose of 100 mg/kg. 6×10^7 CFU WHS11081 6×10^7 CFU WHS11081 /mouse model of MRSA WHS11081 infected burn wound (1×10^7 CFU/mouse)	Chimeric lysin is an effective antibacterial against MDR *S. aureus* in a mouse burn wound.	2017 Yang, H [132]
	λSA2-E-Lyso-SH3b and λSA2-E-LysK-SH3b	Bacteriophage endolysins (peptidoglycan hydrolases)	*Staphylococcus aureus*	100 μg/ml, λSA2-E-Lyso-SH3b and λSA2-E-LysK-, infusion of 25 μg of λSA2-E-Lyso-SH3b or λSA2-E-LysK-SH3b	Compared to control, SA2-E-Lyso-SH3b and SA2-E-LysK-SH3b decreased *S. aureus* bacterial load by 3 and 1 log units within 3 h at 100 g/ml, respectively. When SA2-E-LysK-SH3b and lysostaphin (12.5 g each/gland) were tested together in mice, they resulted in a 3.36-log reduction in CFU.	2012 Schmelcher, M. [23]
7.	Chimeric Lysin (ClyS)	Staphylococcus-specific phage	Methicillin-Resistant and -Sensitive *Staphylococcus aureus* strains	Topical ClyS activity was tested in vitro by combining 1 ml of PBS with a dose of 10% ClyS in Aquaphor. The mixture was centrifuged for 10 minutes at 4,000 rpm.	ClyS killed more methicillin-susceptible bacteria (MSSA) and methicillin-resistant S. aureus (MRSA) than mupirocinl, with a 2-log reduction with mupirocinl compared to a 3-log reduction with ClyS. In vitro, the use of ClyS reduced the ability for MRSA and MSSA species to develop resistance relative to the use of mupirocin.	2011 Pastagia, M. [133]

S. no	Enzyme	Sources/bacteriophages	Bacteria/disease	Unit of enzyme	Therapuetic effects	Reference
8.	ClyS	Fusion of N-terminal catalytic domain of *S. aureus* Twort phage lysin with C-terminal cell wall-targeting domain from another *S. aureus* phage lysin (phiNM3)	Methicillin-Resistant *Staphylococcus aureus*	A single treatment with ClyS, 200 U/mg or 7.1 U/nM One intraperitoneal dose of ClyS, ClyS and oxacillin at doses (A unit of ClyS activity per millilitre was described as the reciprocal of the highest lysin dilution that reduced absorbance by 50% in 15 minutes.	In a mouse nasal decolonization model, a single treatment with ClyS decreased the viability of MRSA cells by two logs in one hour. MRSA-infected septicemia mice were given a single intraperitoneal dose of ClyS and survived. In a mouse model of MRSA septic death, ClyS, in addition to oxacillin, offered synergistic defence against septic death.	2010 Daniel, A [134]
9.	Endolysin LysH5 and nisin	Staphylococcal bacteriophage phi-SauS-IPLA88	*Staphylococcus aureus*	300 nM Lys109	When LysH5 was combined with nisin, a bacteriocin, a significant synergistic effect was observed. Nisin and LysH5 minimum inhibitory concentrations were decreased by 64 and 16 times, respectively. On cell suspensions, Nisin increased LysH5's lytic activity by an order of magnitude.	2010 García, P [135]
10.	Pneumococcal lysins	Streptococcal bacteriophage	Antibiotic-resistant S. pneumoniae/ acute otitis media (AOM), septicemia,bronchitis, meningitis	A 2,000 µg2,000-µg dose of Cpl-1, mouse	Cpl-1-treated mice survived a fatal pneumonia infection 100 percent of the time. At 24 hours after infection, treated mice recovered quickly.	2009 Witzenrath, M. [117]
11.	Lysin Ply700	*Streptococcus uberis* (ATCC 700407)	*Streptococcus uberis*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae*	Ply700 (50 µg/ml)	Activity against *E. coli, S. aureus*, or *S. agalactiae* induced a rapid, calcium-dependent lysis. *S. uberis* is killed. With an inoculating dose of 4500 cfu/ml, 31 percent killing was observed, while 81 percent killing	2008 Celia, L. K. [136]

S. no	Enzyme	Sources/bacteriophages	Bacteria/disease	Unit of enzyme	Therapuetic effects	Reference
					was observed when the inoculum was reduced to 600 cfu/ml.	
12.	PlyGBS	Streptococcal bacteriophage	Prophylactic for Group B streptococcal (GBS) vaginal colonization in pregnant women	Single dose of PlyGBS, 100 μl of purified PlyC	Single dose of PlyGBS could cause a 3 log10 reduction of the bacterial cellsnin mice that had been vaginally challenged with GBS. PlyGBS can be used as a decontaminant to eliminate GBS from new-borns. Can reduce the rate of neonatal meningitis and sepsis	2005 Cheng Q. [137]
13.	PlyV12	*E. faecalis* phage	VRE (Vancomycin-Resistant Enterococcus)	100 μl of PlyV12 at 25 U/ml.	The lysin exhibited lytic activityagainst *Staphylococcus* and Groups A, B and C streptococci, thus the lysin exhibited a broad lytic spectrum.	2004 Yoong, P. [138]
14.	PlyC lysin	Streptococcal bacteriophage C1	Groups A, C and E streptococci. Group A streptococci (GAS) S. pyogenes/ pharyngitis rheumatic fever	10 ng of PlyC, mouse	PlyC lysin successfully eliminated upper respiratory colonization of GAS in mice. None of the lysin treated mice were colonized compared to 100% of the control mice.	2001 Nelson, D. [139]

Table 6.
Endolysin therapy: therapeutic effects of Enzymes on bacterial diseases.

resulting in the release of cell lysis and offspring virions [23, 115]. *In vitro* and in mice models, the recombinant phage-derived lysins exhibit highly efficient bactericidal activity against multidrug-resistant *E. faecalis*. Endolysin LysEF-P10, EF24C, Lys168, Lys170 PlyV12 LysEF-P10, IME-EF1, and lysine CF-301 zap methicillin-resistant *Staphylococcus aureus* (MRSA). Antibiotic-resistant *S. pneumoniae*/Acute otitis media (AOM) infected mice exhibited a quick recovery from infection after 24 hours when they were treated with CPL-11 (therapeutic pneumococcal lysin streptococcal bacteriophage) at a dose of 2,000 μg [117]. The mixture of lysostaphin and the chimeric phage lysin λSA2E-LysK-SH3b synergistically kill *S. aureus in vitro* and in mouse models of bovine mastitis [140, 141]. Some lysins, such as CF-301, N-Refasin, P128, and Art-55, are at various stages of pre-clinical or clinical development and are antibacterial for the cure of multiple antibiotic drug-resistant (MDR) infections of Gram-positive, and Gram-negative pathogens. Synchronization of pneumococcal phage lysine with CPL-1 and autolysin LytA eliminates *Streptococcus pneumonia, S. pseudopunemonia,* and *S. aureus* [80, 140]. Endolysin shows synergistic action with phage lysin LySMP or antibiotics that are very specific to cell wall components and is considered an alternative to drug antimicrobial therapy because lysine kills target bacteria rather than other microorganisms. There has been a significant increase in potential applications of phage lysin which is specifically promising, kind of topically applied therapeutics, and lysin also becomes a practicable alternative to antibiotics in long-term systemic therapy [117, 142, 143]. Endolysins have been used effectively in medical applications. They exhibit specific antimicrobial activities in controlling and treatment of pathogenic bacteria such as *Streptococcus* and *Staphylococcus*. Beneficial synergistic interactions increase the efficacy of treatments and reduce the risk of resistant strain development. There was no inactivation nor adverse side effects detected *in vivo*. The creation of chimeric proteins by rearrangement of functional domains of lysins of multiple species established molecular engineering of lysins which can increase lytic activity, widen specificity, advance binding affinity, enhance solubility and reduce the chance of resistance formation, thereby optimizing lysins for specific applications. Moreover, endolysin-based antimicrobials viz. (Outer membrane permeabilizers (OMPs) and protein transduction domains (PTDs) are used to control Gram-negative and intracellular pathogens. Molecular engineering of lysins is predicted to gain momentum in the coming years and the dogma of endolysins to be effective only against Gram-positive bacteria when applied externally to decrease [144].

5.9.2 Side effects

Most of the drug resistance in bacteria studies had been carried out on the use of *in vitro* and *in vivo* experiments in animal species with a particular look upon human studies. Predicaments of the misuse of bacteriophages as medicament specialists are in many situations recognized into four classifications: (1) phage selection, (2) bacteriophage have host-range restrictions, (3) the uniqueness of phages as recommended medications, and (4) uncommonness with phage. Studies on T4 phage had recognized no massive fitness effects and pronounced negative results such as inconvenience, itching, wetness, and unattractive scent, unfavourable events, sore throat, belly pain, nausea, extended peristalsis [65, 66]. *Staphylococcus* bacteriophages proved drug intolerance and hypersensitive manifestations at the site of injury on days three to five of bacteriophage therapy, and hepatalgia was detected after several hours [145, 146]. Adverse activities occurred in six (21%) of 28 victims in the Pyophage team in distinction with 13 (41%) of 32 victims in the placebo group Urinary tract infection intravesical Pyobacteriophage (Pyophage;

20 mL) [112]. A cocktail of 12 lytic anti-*P aeruginosa* bacteriophages in the PP1131 group, twenty-three (23%) of 13 analysable individuals had adverse reactions versus seven (54%) of 13 in standard-care-group [83]. It is gratifying to understand renewed interest in bacteriophages which is nature's different tailored solution to the problem of antibiotic-resistant bacteria. Beyond the urgent problem of untreatable infections, detailed research of bacteriophages have the possibility of finding, exhilarating new biology, molecular mechanisms of RNA-guided DNA targeting and cleavage by the Cas9 enzyme Cas9 in genome engineering effective use of CRISPR-mediated understanding of CRISPR–Cas9 mechanisms genome engineering in clinical applications CRISPR biology exemplified via the transformative discovery of CRISPR–Cas DNA editing structures and phage-encoded anti-CRISPR defences [51]. We're on the verge of entering an exciting new era in phage technology and applications, thanks to advanced molecular methods, devices, and applications in medicines.

6. Conclusions

In this chapter, the lytic bacteriophages' efficacy has been reported. Therapeutic bacteriophages have been shown to prevent the growth and replication of a variety of pathogenic bacteria in humans, resulting in improved recovery, health, and survival of infected individuals. Since no or few side effects have been reported, phage therapy is medically safe and effective against bacterial infections. Personalized treatment is presented for phage-resistant gangrene bacterial strains, burn wounds, chronic ulcers, psoriasis, bacterial diarrhoea, urinary tract infections, pneumonia and tuberculosis. Producing higher-quality phage cocktails against specific bacteria groups and making them readily available in all areas, regardless of geography, economics, or climatic conditions, is, however, advantageous. Phage therapy is one of the most effective methods for controlling microbial infections that occur in a variety of species at different times. Expanding research to other organisms may be one of the most useful techniques for collecting evidence and validating the phage therapy's utility and therapeutic potential. Understanding infection mechanisms, phage tolerance, phage therapy effectiveness on targeted pathogens, and their effects on the normal microbiome can all aid in improving biocontrol strategies. A scientific logical approach is needed to develop long term storage and transport of therapeutic bacteriophages with a common guideline for the use and safety of phage therapy. The production of new medicines, innovative methods, and management practises to mitigate the risk of infectious agents being introduced and to reduce predisposing factors may be needed in the future to control bacterial diseases. The discovery of novel phage-host interaction methods and the understanding of how bacteriophages control their hosts will be aided by future studies on the complexities of phage lifestyles and dynamics, bionomics in natural systems, genome and viriome analysis, proteome analysis, genes coding for their proteins, and DNA polymerase phylogeny. To reduce the risk of infectious agents being introduced and to reduce predisposing factors, future bacterial disease control would depend on the development of new drugs, methods, and management practises. In response to the threat posed by multiresistant "super bugs," the use of phage endolysins, as well as possible applications of these enzymes in medicine, food protection, agriculture and veterinary medicine, biotechnology, and environmental sciences, has increased significantly. The significance and trend of research on bacteriophages and their applications is expected to continue as the quest for new antimicrobials intensifies in the near future.

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Author's contribution

Both the authors (Palaniappan Ramasamy, Govindan Dayanidhi) have made equal contributions in the conception, design, and execution of the described study and in drafting the manuscript writing and discussion.

Conflict of interest

The authors (PR, GD) declare that there is no financial or conflict of interests.

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