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Review Article

Bacteriophages and Their Derivatives as Biotherapeutic Agents in Disease Prevention and Treatment

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The application of bacteriophages for the elimination of pathogenic bacteria has received significantly increased attention worldwide in the past decade. This is borne out by the increasing prevalence of bacteriophage-specific conferences highlighting significant and diverse advances in the exploitation of bacteriophages. While bacteriophage therapy has been associated with the Former Soviet Union historically, since the 1990s, it has been widely and enthusiastically adopted as a research topic in Western countries. This has been justified by the increasing prevalence of antibiotic resistance in many prominent human pathogenic bacteria. Discussion of the therapeutic aspects of bacteriophages in this review will include the uses of whole phages as antibacterials and will also describe studies on the applications of purified phage-derived peptidoglycan hydrolases, which do not have the constraint of limited bacterial host-range often observed with whole phages.

1. Bacteriophage History

Bacteriophages (phages) were first characterised about 100 years ago by [1–3]. Earlier authors, such as Ernest Hankin [4], Nikolay Gamaleya [5], and Frederick Twort [6], are understood to have observed the antibacterial activity of phages without being able to recognise or identify the agents responsible. Nowadays, most recognition for the development of phage therapy goes to Felix d'Herelle who isolated these agents from the stool samples of dysentery patients, named them bacteriophages, and developed the phage assays which remain in use up to the present [7, 8]. He also initiated the first phage therapy experiments in the early 1920s. Research in phage therapy was eclipsed in the West by the advent and increasing widespread successful application of antibiotics in medical practice from the late 1940s. Phage therapy, on the other hand, was declined largely due to variable and unpredictable results, an issue related to the relatively poor understanding of phage biology at the time. Certainly, many of the illnesses that had been treated with phage preparations up to the mid-twentieth century were likely to have not had a bacterial basis. Thus, the results of phage therapy generally tended to be inferior to those observed for antibiotics, since the latter had a broader therapeutic spectrum and, generally, did not require detailed bacteriological knowledge for effective prescribing by practitioners. The use of phages to treat bacterial infections has recently gained attention in Western medicine mainly due to ever-increasing incidence of bacterial resistance to antibiotics and also due to the fact that phage biology, phage-bacteria interaction, and the basis for bacterial infections are vastly better understood than was the case in the mid-twentieth century when phage therapy was eclipsed by antibiotic treatments [7].

2. Sources of Bacteriophages

Phages are found in almost all environments on Earth, ranging from soil, sediments, water (both river and seawater), and in/on living or dead plants/animals. Essentially, phages

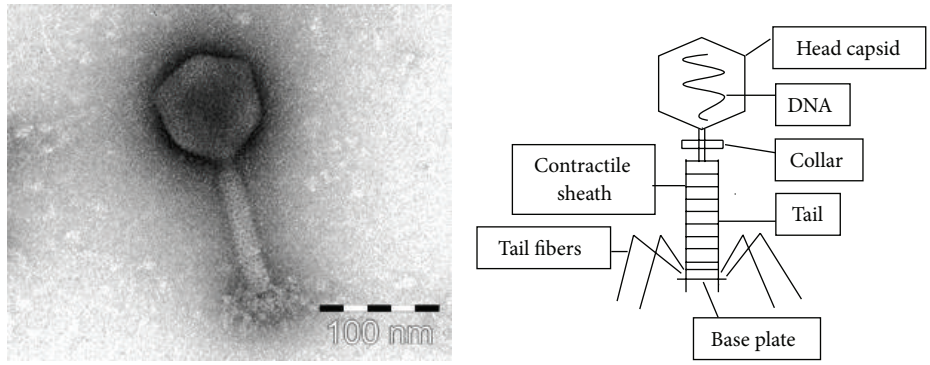

Figure 1: Electron micrograph of a negatively stained *Acinetobacter baumannii* phage (a) and generalised structure of a tailed phage (b).

can be isolated from almost any material that will support bacterial growth. The estimated global phage population size is extraordinarily high. For instance, it is estimated that aquatic environments have a total phage population above 10^{31} [9, 10]. Many terrestrial ecosystems have been shown to harbour 10^{7} phages per gram of soil; and sewage is known to contain in the range of 10^{8}–10^{10} phage per millilitre [11–15].

3. Classification of Bacteriophages

Phages have evolved an array of shapes, sizes, capsid symmetries, and structures. All are composed of nucleic acid (genome) encapsulated by a protein coat (capsid). Phage genomes can be double- or single-stranded DNA or double- or single-stranded RNA. Capsids have been identified in many forms, ranging from small 3D hexagon-like structures to filaments to highly complex structures consisting of a head and a tail (Figure 1). It is estimated that approximately 5,500 bacteriophages have been viewed by electron microscopy since 1959. Of those studied from a morphological perspective, 96.3% had a tailed morphology [16, 17]. Over the years, a sophisticated phage classification system has been drawn up by the International Committee for Taxonomy of Viruses (ICTV) to account for the diversity. Originally the taxonomy of phages was organised according to their morphological characteristics, type of nucleic acid, and presence or absence of envelope or lipid. According to this approach, phages were organised into 14 distinct phage families as shown in Figure 2 [18–20]. More recently, the importance of phage genome sequences comparisons has also been recognised. Some of the phage families have been grouped into orders; for example, the three-tailed phage families (Figure 2) (Myoviridae, Siphoviridae, and Podoviridae) belong to the Caudoviralesorder, and the Archaea-infecting Lipothrixviridae and Rudiviridae phages belong to the Ligamenvirales order.It is noteworthy that many of the other families have not yet been assigned an order. The inoviruses (Inoviridae family) consist of a nonenveloped rod of protein filaments surrounding a circular, ssDNA genome. The microviruses (Microviridae family) possess a linear, ssDNA genome and a nonenveloped,

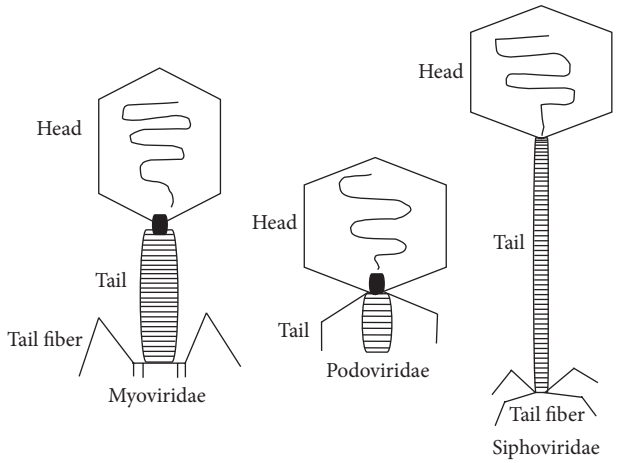

Figure 2: The three-tailed phage families (Myoviridae, Siphoviridae and Podoviridae).

icosahedral capsid. The tectiviruses (Tectiviridae family) and corticoviruses (Corticoviridae family) both possess external icosahedral capsids with a lipid membrane lying directly beneath. These two families differ in terms of capsid and genome organisation. Whereas the corticovirus genome is circular and highly supercoiled, the tectivirus genome is linear with terminal inverted repeats. By contrast, the plasmaviruses (Plasmaviridae family) possess an external lipid envelope, pleomorphic geometry, and a circular genome. They are only known to infect the mycoplasmal genus *Acholeplasma*. The cystoviruses (Cystoviridae family) have a linear, segmented, dsRNA genome. They are charactarised by a double capsid with a surrounding lipid envelope. The leviviruses (Leviviridae family) have a linear, positive-stranded, ssRNA genome and a nonenveloped, spherical capsid (Table 1; Figure 3).

4. Bacteriophage Life Cycles

A common characteristic of phages is that, although their genome carries the information required to drive their own

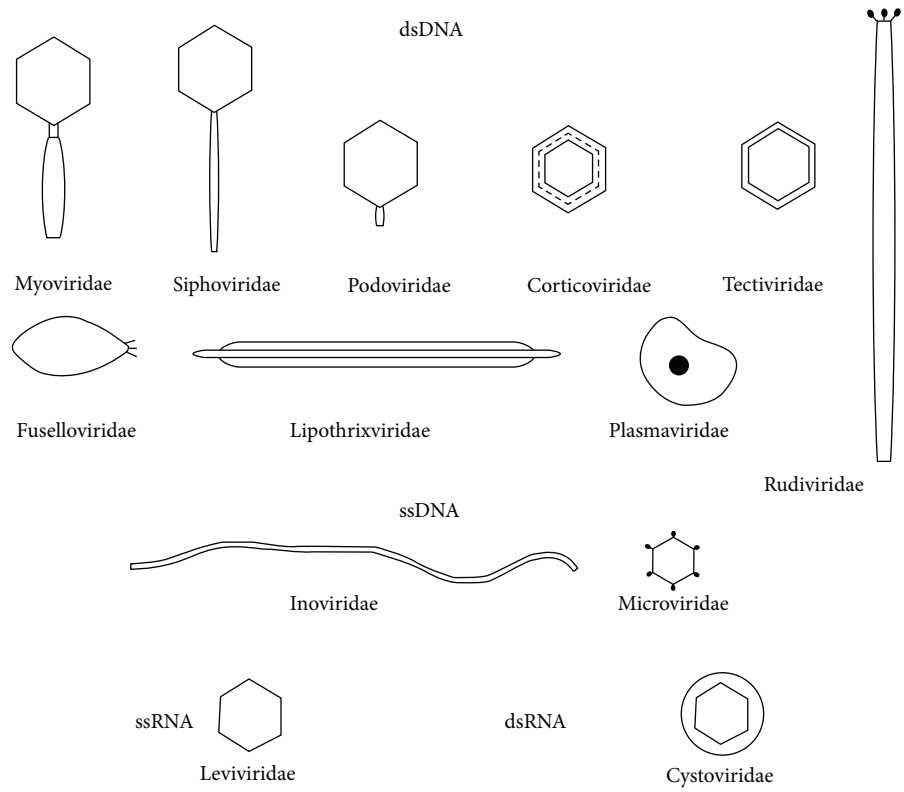

Figure 3: The fourteen phage families based on morphology and genome characteristics. See Table 1 for further details.

multiplication, they completely rely on the energy and protein biosynthetic machinery of their bacterial hosts to complete their lytic cycle, rendering them obligatory intracellular parasites of bacteria [8, 10, 21]. The first contact between a phage and its host happens by random collision, provided that the cell carries specific receptors on its surface. The contact is usually made between the receptor molecules of the host (e.g., teichoic acid in Gram-positives or lipopolysaccharide in Gram-negatives) and specific phage proteins located at the tip of the tail fibre, or at one end of a filamentous phage. Injection of DNA follows immediately after a phage has stably and irreversibly adsorbed to the cell surface [22]. Based on their subsequent propagation cycle, most phages can be broadly divided into two major groups: virulent and temperate (Figure 4). Virulent phages immediately redirect the host metabolism toward the production of new phage virions, which are released upon cell death within several minutes to hours after the initial phage attachment event. This is termed the lytic cycle. Virulent phage infection results in clear plaques on the respective host bacterial lawns. Temperate phages can replicate either by the lytic cycle as described above or by establishing a stable long-term viable relationship with their host bacteria. In this state, the phage DNA is replicated together with the host's chromosome. This is termed the lysogenic cycle, during which viral genes that are detrimental to the host are not expressed [8, 13, 20, 23, 24].

5. Bacterial Resistance to Phages

Bacteria can evolve resistance to phages. These resistance mechanisms are manifested when an interruption occurs during phage development, through specific molecular mechanisms, which have evolved in bacteria throughout their coevolution with phages. Bacteria are able to defend against phage infection almost in every stage of the infection process. By blocking phage receptors, producing an extracellular matrix and competitive inhibitors, bacteria prevent the phage from adsorbing to their surface. This is termed phage adsorption inhibition. Injection of the phage genome can also be inhibited through a process known as injection blocking [25]. Phage inhibition can also occur after phage genome injection into a host as a result of bacterial-encoded endonucleases that recognise and destroy foreign DNA, a phenomenon known as restriction-modification. Bacterial protection of its own DNA is based on modification by methylation at specific points on its DNA sequence, which concomitantly will give protection against restriction endonuclease cleavage. Restriction results in the cleavage of foreign DNA that does not carry the corresponding methylation pattern. Some unmodified phage genomes physically avoid host-mediated restriction (possibly by encountering the methylase enzyme molecule in advance of meeting the endonuclease), and, on being replicated, their genome becomes modified. This enables resulting phage to evade restriction by a particular

Table 1: The fourteen phage families based on morphology and genome characteristics.

Family	Morphology	Nucleic acid
Myoviridae	Nonenveloped, contractile tail	Linear dsDNA
Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA
Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA
Lipothrixviridae	Enveloped, rod-shaped (infect Archea)	Linear dsDNA
Rudiviridae	Nonenveloped, rod-shaped (infect Archea)	Linear dsDNA
Ampullaviridae	Enveloped, bottle-shaped	Linear dsDNA
Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA
Clavaviridae	Nonenveloped, rod-shaped	Circular dsDNA
Corticoviridae	Nonenveloped, isometric	Circular dsDNA
Cystoviridae	Enveloped, spherical	Segmented dsRNA
Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA
Globuloviridae	Enveloped, isometric	Linear dsDNA
Guttavirus	Nonenveloped, ovoid	Circular dsDNA
Inoviridae	Nonenveloped, filamentous	Circular ssDNA
Leviviridae	Nonenveloped, isometric	Linear ssRNA
Microviridae	Nonenveloped, isometric	Circular ssDNA
Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA
Tectiviridae	Nonenveloped, isometric	Linear dsDNA

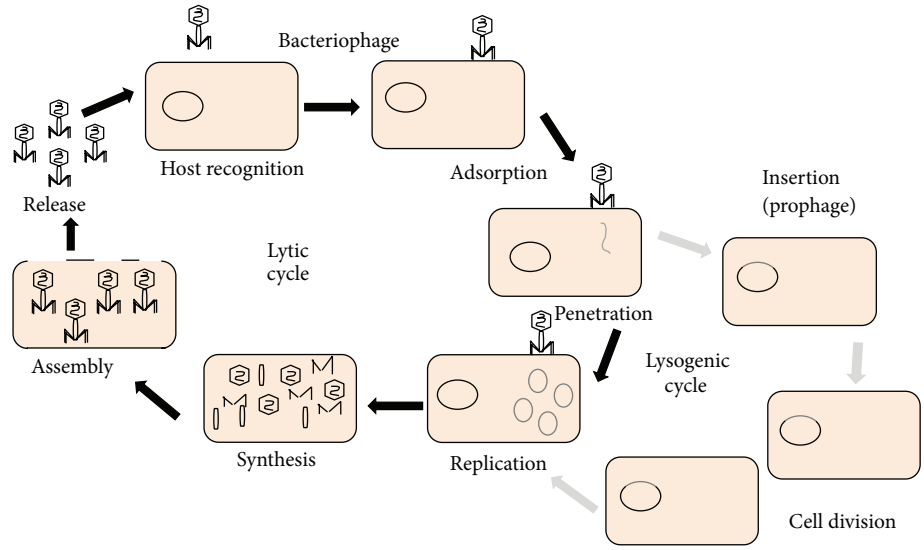

Figure 4: The steps during the bacteriophage lytic and lysogenic life cycles. The well-known bacteriophage Lambda has a choice between both cycles. Some phages are exclusively virulent, never entering the lytic cycle. Others are long-term residents of the bacterial host chromosome and in some cases may have lost the ability to excise and enter the lytic cycle.

host restriction/modification system in subsequent infective cycles [26–28]. Another mechanism of phage resistance termed abortive infection represents a broad range of diverse phage resistance mechanisms whereby the phage-infected cells often die before completing the lytic cycle, thus containing the virus and preventing it from proliferating. Abortive infection mechanisms frequently have a different primary function in bacteria [25]. CRISPRs (clustered regularly interspaced short palindromic repeats) are loci containing multiple, short direct repeats, which are found in the genomes of approximately 40% of sequenced bacteria and 90% of sequenced Archaea [29]. CRISPRs function like a prokaryotic immune system in that they confer a form of acquired immunity to exogenous genetic elements such as plasmids and phages. Short segments of foreign DNA, called spacers, are incorporated into the genome between CRISPR repeats and serve as a "memory" of past exposures. CRISPR spacers are then used to recognise and silence exogenous genetic elements in a manner analogous to RNA in eukaryotic organisms [25, 30, 31]. The mechanism of CRISPR/Cas interference involves three phases (Figure 5).

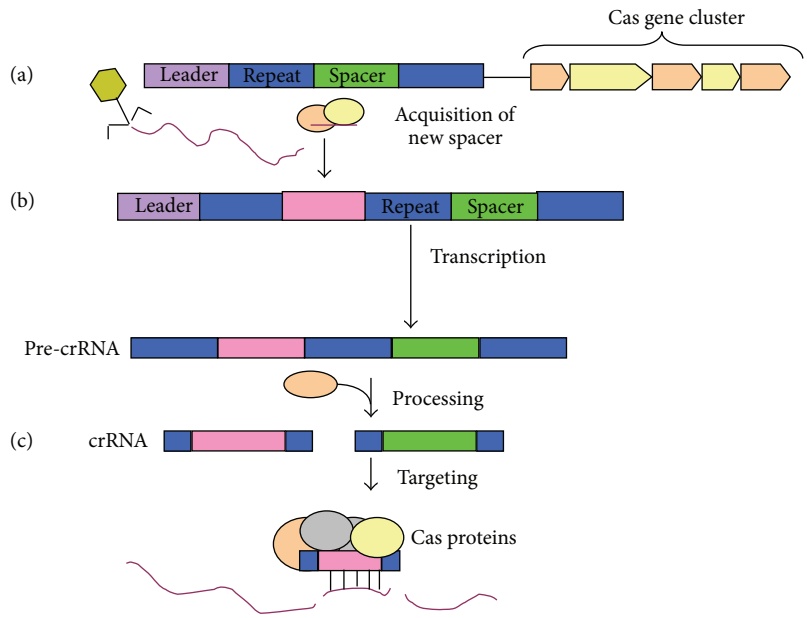

Figure 5: Overview of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) adaptive immunity. (a) Adaptation. The CRISPR arrays are composed of short repeats and intervening sequences derived from foreign invaders. Upon infection with a foreign element (e.g., phages), part of the genome is typically incorporated into the leader end of the CRISPR array and the repeat is duplicated. The CRISPR arrays are located adjacent to a cluster of Cas genes. (b) crRNA generation. The CRISPRs are transcribed into pre-crRNAs that are then processed into mature crRNAs. (c) Interference. The crRNA, in a complex with Cas proteins, binds and degrades the target nucleic acid of the invading element.

Firstly, resistance is acquired via the integration of short sequences from foreign genetic elements (termed spacers) into repetitive genetic elements known as CRISPR arrays. Secondly, CRISPR arrays are then transcribed and processed into small RNAs (crRNAs) by Cas proteins. In the third and final step, targeting of the invading phage or plasmid is mediated by a Cas protein complex that contains crRNAs. During this stage, the crRNA-Cas protein complex then interferes, in a sequence-specific manner, with the foreign nucleic acids [32].

6. Significance of Bacteriophages

6.1. Ecological Importance of Bacteriophages. Phages are more numerous than any other organism in the biosphere, prokaryotes included, and are found in all ecosystems on Earth. There is considerable evidence of the significant role that phages play in prokaryotic evolution in terms of gene transfer by transduction and also in terms of controlling bacterial populations in specific niches. The concentration of phages in natural waters indicates that phage infection may be an important factor in recycling of nutrients as a result of prokaryotic cell lysis and thus influence levels of planktonic microorganisms [33]. For example Hankin in 1896 investigated the difference between the cholera outbreaks along the Ganges and Jumna Rivers in India; Hankin found an unknown source of antibacterial activity against *V. cholerae* in the river and then suggested that this unidentified substance, which passed through fine porcelain filters and was heat labile, was responsible for limiting the spread of cholera epidemics [4]. At the same time, an interesting relationship between *V. cholerae* and vibriophages in the environment was also reported and suggested that the cessation of cholera epidemic was due to the spread of bacteriophages from convalescent cases [34]. It is also noted that significant quantities of phages are also found in soil and in the gut of animals where they are likely to have similar roles [35].

6.2. Economic Importance of Bacteriophages. In the dairy industries, many different lactic acid bacteria are used as starter cultures in the production of products such as cheese and yoghurt. Infections of starter cultures by lytic phages can lead to the slowing or arrest of fermentations and subsequent loss of production. Thus, phages of lactic acid bacteria are a real threat to the milk fermentation industry because of (a) its global magnitude and (b) the fact that in a typical factory, there is multiple filling of vats providing ample opportunities for phages to propagate to high numbers in a single day's production, which may typically see many vats of several thousand litres filled repeatedly with milk each day [36, 37]. The fact that the substrate is a nonsterile liquid facilitates easier dissemination of phage particles. Thus, a typical observation in a cheese factory might be to see the starter culture "phaging out" after 15 vats of the planned 40, resulting in reduced product output by the industry. Such a detrimental economic impact has resulted in major investment in phage-resistant starter culture research and the genetic improvement of industrial starter strains of lactic acid bacteria [25]. In the area of food safety, phages also are becoming very important. Since 2006, phages have been

approved for the elimination of *Listeria monocytogenes* in food products [38]. A study using phage for biocontrol of *Salmonella* on both cooked and raw beef found significant reductions when samples were incubated at both refrigeration and room temperature conditions [39], and this application is likely to expand in the future.

6.3. Medical and Therapeutic Application of Bacteriophages. The first use of phages as therapeutic agents in humans started in 1919, shortly after their discovery (Figure 6), when they were successfully used to treat severe cases of bacterial dysentery in four children in Paris, France. All of the treated children recovered from what otherwise could have been a fatal infection. The study was conducted in close collaboration with Felix d'Herelle, one of the discoverers of phages. Shortly afterwards, in 1921, Richard Bruynoghe and Joseph Maisin used phages to treat a staphylococcal skin disease [40]. Despite this promising beginning in the preantibiotic era, the success of early phage therapy was short-lived [41, 42]. This was due to a variety of factors that included (1) a lack of understanding of phage biology, (2) poor experimental techniques, (3) poor quality of phage preparations, (4) a lack of understanding of the underlying causes of ailment being treated, and (5) ultimately the discovery and ease of use of antibiotics. The first evidence of the nature of phage only became available in 1936 when Schlesinger reported the composition of phage particles being 50% protein and 50% nucleic acid and later with the first electron microscopic observation of phages [43, 44]. Thus, the application of phages as therapeutic agents was heavily compromised. As a result, phage therapy and its associated research were abandoned in Western countries, but they continued in Poland and in countries within the Former Soviet Union (FSU). For example, the Bacteriophage Institute in Tbilisi (now the George Eliava Institute of Bacteriophage, Microbiology and Virology) is still researching phage therapy applications and supplies phage for the treatment of various bacterial infections [8, 10, 42, 45].

The increasing prevalence of antibiotic resistance and multidrug-resistance in bacterial pathogens led the Western scientific community to reassess the potential applications of phages and phage products in the treatment of certain infectious diseases [44, 46–48], such as *Pseudomonas* spp. [49], vancomycin-resistant *Enterococci* [50–52], antibiotic-resistant *Staphylococci* [53, 54], multidrug-resistant *Klebsiella pneumonia* [55], imipenem-resistant [56, 57] and multidrug-resistant *Pseudomonas aeruginosa* [58, 59], antibiotic-resistant strains of *Escherichia coli* [60], and methicillin-resistant *Staphylococcus aureus* [61]. Today, there are several different antibacterial strategies derived from phages including enzybiotics (cloned host-specific, phage-encoded lytic enzymes introduced to combat bacteria without the whole phage) and whole-phage therapy (introducing whole, viable phage to attack the infecting bacteria) [8, 62–64]. Typically, whole-phage preparations may contain one or a small number of phage strains, each with a broad range of activity within a bacterial genus; or, alternatively, phage may be applied as a mixture of several phages, which as a mixture has activity against a broad range of strains/species [2]. This approach with whole phages is largely based on the phage preparations used throughout the Former Soviet Union (FSU). Available data suggest that the use of phages as antibacterial agents is rather simple and has many advantages over antibiotics [8, 19, 53, 54, 65]. These advantages may be summarised as follows: (1) phages are specific and, therefore, cannot eliminate ecologically important bacteria (e.g., gut microflora); (2) phages cease to function soon after all their specific target host bacterial cells are destroyed and, hence, will disperse harmlessly; (3) human patients who are allergic to antibiotics can be treated with phages with no side effects; (4) phages are safe to use because they have no effect on mammalian cells; (5) phages can be administered in various routes—for example, topically, intravenous, or orally; (6) phages reproduce exponentially; hence, a single dose can be sufficient to treat an infection; (7) when resistant bacterial strains arise in the host, the phage has capabilities to overcome this resistance by mutating in step with the evolving bacteria; (8) production of phages is simple and inexpensive; (9) phages are ubiquitous and, thus, regarded as safe.

There are, however, still some disadvantages that must be considered when using phage therapy: (1) phage specificity implies that the causative bacterial pathogens have to be identified by the medical practitioner prior to phage administration, and, also, the lytic spectrum of the phage may be limited to only one subtype of bacterial pathogen; (2) low or no efficacy has been reported in certain cases, but this may be attributed to either incorrect diagnosis of the disease or insufficient phage dose, together with an ineffective phage delivery approach; (3) phage administration requires a neutralised environment, which, for example, is generally not found in the digestive system of animals due to the presence of gastric secretions [12, 21, 66].

In comparing the advantages and disadvantages, phages do certainly have several characteristics that confer on them strong potential as therapeutic agents. In addition, the large and ever-increasing number of publications shows that the scientific and technical understanding of phage therapy is constantly improving, and given the increasing need for nonantibiotic therapies, it is likely that phage therapy will become a reality in the near future.

6.4. Recent Research in the Use of Phages as Antimicrobial Agents. The last decade has brought several substantial developments in phage therapy, which is currently leading to greater interest in Western medicine as an alternative to antibiotics in the treatment of infections caused by multidrug-resistant bacteria [67]. The recent increase in interest began to a large extent with a Polish study first reported in 1985 in which phages were applied in 114 cases of suppurative bacterial infections in children followed by scientific analysis. Positive therapeutic results were obtained in 109 (95.6%) cases; patients had a wide range of bacterial infections caused by the pathogenic *Staphylococci, Klebsiella, Escherichia, Proteus,* and *Pseudomonas* bacteria [68]. In a follow-up to this study, phage preparations were administered to patients in various age groups with a wide range of antibiotic-resistant infections caused by the aforementioned

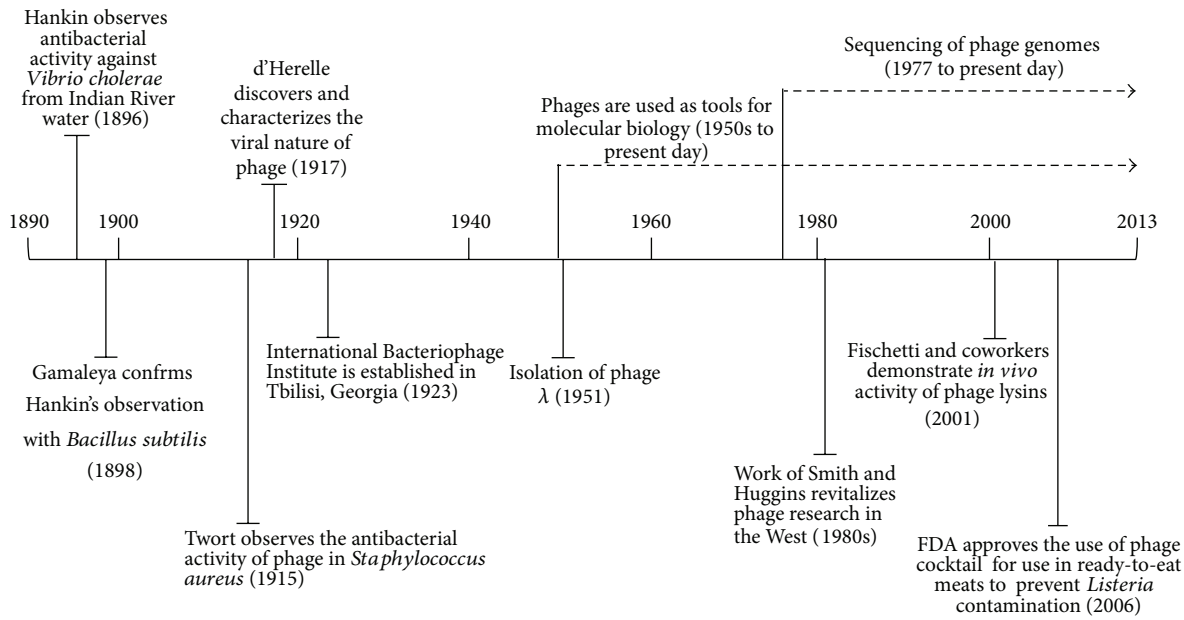

Figure 6: Timeline of major milestones in phage history.

pathogens. The patient ages ranged from one week to 86 years of age. The phages were administered orally three times per day, locally by direct application on wounds, or by dropping a phage suspension into the eye, ear, or nose. In most cases, bacterial sensitivity to the phage was monitored and different phages were applied in situations where phage resistance had occurred. In one report from this study, the results from 550 cases were reported from 1981 to 1986. These results showed that over 92% of patients were cured, and about 6.9% of patients showed an improvement in condition in contrast to only 0.7% of the cases of patients where the phage therapy proved to be ineffective [23, 69]. Another suppurative chronic skin infection study by the same group used phage to treat the infections in 31 patients ranging in age from 12 to 86 years old, whose infections were caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus,* and *E. coli*. Of the 31 cases, 77% showed improvements in condition [70]. Later, the same group reported a broader study with a larger number of patients and obtained similar results [71]. These infections included suppurative wound infections, gastroenteritis, sepsis, osteomyelitis, dermatitis, empyemas, and pneumonia: and again, pathogens included *Staphylococcus, Streptococcus, Klebsiella, Escherichia, Proteus, Pseudomonas, Shigella,* and *Salmonella* spp. More recently in the same group, antibiotic-resistant septicaemia was treated with phage therapy in 94 patients. In 71 of these cases, antibiotic treatment was continued in conjunction with phage therapy and in the remaining 23 cases phage alone was administered. Of the 94 cases, complete recovery was achieved in 85.1% of cases, whereas in 14.9% of cases phage therapy was ineffective [47]. The Polish scientists reported a success rate of 80–95% [72, 73], which applied to the older studies in which phage preparations were produced by the institute and distributed to local hospitals and individual patients. It is noteworthy that most patients, some of them with acute rather than chronic infections, were not directly monitored by the institute staff. Therefore, not all data reported could be directly verified. In 2005, the phage therapy centre was established at the institute, which is responsible for direct patient care, supervision, and monitoring according to the current standards of the EU and FDA under the Declaration of Helsinki. Only patients with antibiotic-resistant infections have been accepted and the published results suggest notable success rates of approximately 40% for this group of patients in whom all available therapy had failed [74]. The results have been supported by a more recent but similar British study, which also demonstrated significant efficacy of phages against *Escherichia coli, Acinetobacter* spp., *Pseudomonas* spp., and *Staphylococcus aureus* [75]. In this case, phage therapies in a group of 1,307 patients ranging in age from 4 weeks to 86 years from 1987 to 1999 were investigated. Full recovery occurred in 85.9% of cases, and an improvement in condition occured in 10.9% of cases, while no improvement was observed in 3.8% of cases. As with the earlier study, patients had a wide range of bacterial infections caused by the pathogens *Staphylococcus, Klebsiella, Escherichia, Enterobacter, Proteus,* and *Pseudomonas*. Similar important work with similar findings was performed at the Eliava Institute for Bacteriophage, Microbiology and Virology in Tbilisi, Georgia, and the details have been reviewed by Sulakvelidze and Kutter [44]. A Swiss group led by Brussow performed a safety test on phage administration in human volunteers. In this study, 15 healthy adult volunteers received T4 coliphage in their drinking water, at a concentration up to 10^5 PFU/mL. No adverse effects identified in volunteers receiving phage T4 [76]. Also Sarker et al. gave a 9-phage cocktail to 15 healthy adult volunteers. The phages were detected in 64% of the stool samples when subjects were treated with higher titer phage, compared to 30% and 28% when treated with lower-titer phage. No *Escherichia coli* was present in initial

stool samples, and no amplification of phage was observed. One percent of the administered oral phage was recovered from the feces. No adverse events were observed by self-report, clinical examination, or from laboratory tests for liver, kidney, and hematology function. In addition, no impact was seen on the fecal microbiota composition with respect to bacterial 16S rRNA from stool [77].

7. Phages Therapy in Animal Models of Human Infection

Notwithstanding the above promising human studies, several animal models have also been conducted in keeping with the normal process in new drug or anti-infective development studies. These models have allowed for the evaluation and comparison of the different possible administration routes, timings, and dose-titres of phage. The use of animals as models for microbiological infections has been a fundamental part of infectious disease research, and many research groups have also applied this to phage and their studies are summarised in Table 2. The study of Smith and Huggins 1982 mentioned that a single intramuscular dose of one anti-K1 phage was more effective than multiple intramuscular doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim plus sulphafurazole in curing mice of a potentially lethal intramuscularly or intracerebrally induced infection of *Escherichia coli* strain; it was at least as effective as multiple intramuscular doses of streptomycin. A notable study by Biswas et al. [50] used the virulent phage ENB6 from raw sewage, and its activity was tested against a wide range of clinical isolates of vancomycin-resistant *Enterococcus faecium* (VRE). The study reports that the induction of a bacteremia in mice with a high dose of VRE (10^9 CFU) and an incubation time of 45 h after injection can be fully cured by a single intraperitoneal (i.p.) injection dose of 3×10^8 PFU of phage ENB6. Another recent interesting use of phage therapy showed the efficacy of the lytic phage 9882 isolated from hospital sewage in overcoming virulent beta-lactamase- (ESBL-) producing *E. coli* strains. Notably, this study examined the timing of administration of the therapeutic phage and showed that it is of major importance for the total elimination of infectious bacteria. For example, the efficacy of the activity of the phage varied up to 100% if the phage was introduced 40 min after the bacterial infection to 60% if the phage was introduced 60 min after infection. This study also showed that therapeutic efficacy was directly linked to the functional capability of the phage and not to any other immunological reaction of the host that may be thought to have been triggered by the physical introduction of the phage [56]. These researchers used the same strategy to examine the effectiveness of phages in the treatment of imipenem-resistant *Pseudomonas aeruginosa* (IMPR-Pa). The phage (isolated from hospital sewage and designated phage A392) was shown to have *in vitro* lytic activity against a wide range of clinical isolates of IMPR-Pa. The phage was shown to overcome a high-dose, systemic bacterial infection as well as wound infection in mice. Thus, the study demonstrated that the therapeutic efficacy of the phages was independent of the administration routes, but overall, the timing of administration is very important [57]. Another study focused on the details of multiplicity of infection (MOI) of phage against multidrug-resistant uropathogenic *E. coli* (UPEC). Phage T4 and a newly isolated phage (KEP10) were injected at an MOI of 60 in mice that were administrated with a UPEC strain. After seven days, 100 and 90% of mice treated with T4 and KEP10, respectively, had survived. In the control group, where no phages were administered, all the mice died within three days [78]. In addition, lower multiplicities of infection (0.03 and 0.003) resulted in a reduced rescue of animals (60 and 40%) [79].

An intraperitoneal phage administration study involving phage SS specific for *K. pneumoniae* was conducted to treat mice that had been challenged by intranasal inoculation with *K. pneumoniae* (10^8 CFU/mL). A single intraperitoneal injection of 10^{10} CFU/mL phage SS administered immediately after intranasal inoculation challenge was sufficient to rescue 100% of animals from the *K. pneumoniae*-mediated respiratory infections [80]. In addition, in a rabbit model of wound infection, abscess formation in rabbits was prevented when 2×10^9 PFU of phage LS2a was injected simultaneously with 8×10^7 CFU of *S. aureus* into the same subcutaneous site [81]. The studies reported above were performed under different conditions but demonstrate that the experimental conditions for phage therapy are dependent on the specific infection being treated. Furthermore, these studies illustrate the importance of timing of phage administration, MOI, and route of phage administration in some cases. In addition, phages can have enhanced therapeutic efficacy when they are (i) virulent for the corresponding bacterial host, (ii) essentially free of contaminating bacterial toxin, and (iii) capable of evading the reticuloendothelial system (RES). A notable study by Merril et al. [65] identified phages that were selected for their ability to survive longer than control phage in the blood of mice. The development of such phage may provide important tools for the treatment of bacterial diseases. Therefore, before phages are administered to humans and animals for treatment, (especially for new phages) animal models are an important initial step to help determine potential experimental conditions down the line, such as route and timing of administration and MOI.

8. Use of Phage to Eliminate Biofilms

Biofilm formation is an important bacterial survival strategy, particularly on surfaces. Biofilms are microbial structures consisting of microbial cells surrounded by an exopolymeric matrix. In humans, biofilms are responsible for many pathologies, including those associated with the use of medical devices [82]. Since the first phage/biofilm study was reported in 1995 [83], there has been an increased interest in using phage to eliminate biofilms (Table 3). This is due to the ability of phages in general to replicate at the site of an infection and in some cases to produce enzymes that degrade the extracellular polymeric substance matrix of a biofilm [84]. Significantly, the development of extracellular polysaccharide-based matrices by biofilm bacteria does not

Table 2: Use of bacteriophages to control pathogenic bacteria in animal models of human infection.

Infection hosts	Bacteria	Phage	Main outcome	Reference
Mice	*E. coli*	Anti-K1	Better mice survival rates with phage	Smith and Huggins, 1982 [119]
BALB/c mice	*Klebsiella*	*Klebsiella pneumoniae* bacteriophage	Rescue of generalized *Klebsiella* infection	Bogovazova et al., 1991 [120]
Guinea pigs	*P. aeruginosa*	BS24	Skin graft protection from bacteria by phage	Soothill, 1994 [75]
Mice	*E. coli, S. typhimurium*	λ and P22	Identification, isolation, and subsequent use of long circulating phage	Merril et al., 1996 [65]
Chickens and calves	*E. coli H247 (O18 : K1 : H7)*	ΦR	Protection against morbidity and mortality	Barrow et al., 1998 [121]
Hamsters	*C. difficile*	CD140	5/6 hamster survived in the phage-treated group compared with none in the control	Rdamesh, 1999 [122]
BALB/c mice	*Helicobacter pylori*	M13	Reduction of stomach colonization by *Helicobacter*	Cao et al., 2000 [123]
Mice	*E. faecium*	ENB6	100% survival 45 min after phage administration	Biswas et al., 2002 [50]
Mice	*V. vulnificus*	CK-2, 153A-5, and 153A-7	Different results of mice protection depending on the phage used. CK-2 and 153A-5 protected mice, whereas 153A-7 did not	Cerveny et al., 2002 [124]
Mice	*E. coli*	LW and LH	Mortality rates in mice varied depending on the phage used	Bull et al., 2002 [125]
Mice	*S. aureus*	MR11	Better mouse survival rates with phage administration (MOI40.1) straight after bacterial administration	Matsuzaki et al., 2003 [126]
Chicken skin	*Salmonella enterica* and *Campylobacter jejuni*	P125589 and P22	Reduction by 2 log units in bacterial abundance over 48 hours	Goode et al., 2003 [127]
BALB/c mice	*Pseudomonas aeruginosa*	Phage Pf3R	Higher survival rate and reduced inflammatory response after 12–24 hours	Hagens et al., 2004 [128]
Rabbits	*S. aureus*	LS2a	Reduction in abscess size in phage-treated animals and no difference when phage administration was delayed	Wills et al., 2005 [81]
Mice	*E. coli* O157 : H7	SP15, SP21, and SP22	Successive daily phage administration was required to reduce cell numbers from the gastrointestinal tract	Tanji et al., 2005 [129]
Chicken	*Salmonella enteritidis*	CNPSA 1, CNPSA 3, and CNPSA 4	Reduction of *Salmonella enteritidis* counts in treated chicken cuts	Fiorentin et al., 2005 [130]
Chicken	*Salmonella typhimurium*	*Salmonella*-specific phages	Reduction in *Salmonella* counts in cecum and ileum treated chickens	Toro et al., 2005 [131]
Mice	*P. aeruginosa*	A392	100% survival rate 60 min after phage administration. Reduced survival rates when phages were administrated at 180 and 360 min	Wang et al., 2006b [57]
Mice	*E. coli*	9882	100% survival at 24–168 h after phage administration (40 min after bacterial administration)	Wang et al., 2006a [56]
Shrimp	*Vibrio harveyi*	Siphoviridae	Improved larval survival	Vinod et al., 2006 [132]
Mice	*P. aeruginosa*	Pa1, Pa2, and Pa11	87% protection against bacterial infection in mouse burn model compared with 6% in the untreated group after intraperitoneal injection	McVay et al., 2007 [133]

Table 2: Continued.

Infection hosts	Bacteria	Phage	Main outcome	Reference
Mice	*P. aeruginosa*	KPP10	Survival rates of 66.7% for the phage-treated group versus 0% for the saline-treated control group	Watanabe et al., 2007 [134]
Mice	*E. coli*	T4 and KEP10	100% survival rate with T4. 90% survival rate with KEP10	Nishikawa et al., 2008 [78]
Mice	*E. faecalis*	EF24C	100% survival rate with a phage MOI of 0.1	Uchiyama et al., 2008 [51]
Mice	*P. aeruginosa*	CSV-31	100% protection observed when phage was administrated 45 min after bacterial challenge	Vinodkumar et al., 2008 [58]
Mice	*K. pneumonia*	SS	Immediate administration of phage resulted in 100% protection; this decreased after 3 h and no protection at 6 h was observed after bacterial challenge	Chhibber et al., 2008 [80]
Mice	*K. pneumoniae*	Kpn5	Phage was able to rescue mice from infection caused by *K. pneumoniae*	Kumari et al., 2010 [135]
Mice	*P. aeruginosa*	PA1	No viable bacteria were found in organ samples after 48 h of the phage treatment	Tiwari et al., 2011 [136]
Mice	*P. aeruginosa*	NH-4 and MR299-2	Killing the pathogen in the lungs of infected mice after phage mixture administration	Alemayehu et al., 2012 [137]
Mice	*E. coli*	EC200(PP)	100% survival mice 1 and 7 h after phage administration	Pouillot et al., 2012 [138]

protect cells from lysis by bacteriophage [85]. Phages evidently penetrate the extracellular biofilm matrix that binds macromolecules and cells to eliminate their target bacterial cells [86].

9. Phage Endolysins as Therapeutics

Many phages encode specific peptidoglycan-degrading enzymes, also known as murein hydrolases or endolysins (lysins), which are responsible for lysis of the host bacterial cell at the end of the lytic cycle. To achieve access to the cell wall, endolysins require a second lysis factor—a small membrane protein designated a holin, which permits penetration of the plasma membrane [87–91]. Both endolysin and holin proteins are produced in the late stage of the lytic cycle, when they accumulate in the cytosol of the host cell. At a genetically specific time, holin protein forms pores in the plasma membrane, thus, providing access for the endolysin to reach its target in the peptidoglycan where it will cause rapid cell lysis with the concomitant release of mature phage progeny (Figure 7) [92, 93]. Many endolysins studies to date display a two- or three-domain modular structure [94, 95] with an N-terminal catalytic domain(s) and a C-terminal cell wall-binding domain [96–99] (Figure 7(b)). Lysins are classified into five different groups depending on their cleavage site within the peptidoglycan. These are (1) N-acetyl-β-D-muramidase (lysozymes); (2) lytic transglycosylase; (3) N-acetyl-β-D-glucosaminidases (glycosidases), which hydrolyse the β-1-4 glycosidic bond in the sugar moiety of the cell wall; (4) N-acetylmuramoyl-L-alanine amidases, which cleave the amide bond connecting the sugar and peptide moieties of the bacterial cell wall, and (5) L-alanoyl-D-glutamate endopeptidases and interpeptide bridge-specific endopeptidases, which attack the peptide moiety of the cell wall peptidoglycan (Figure 8) [96, 100–102]. If purified and applied exogenously, endolysins are only effective against Gram-positive bacteria; the outer membrane of Gram-negatives prevents access of exogenous Gram-negative endolysins [103].

Lysins have recently received considerable attention in the context of exploitation as novel antibacterials, and their potential has been extensively reviewed [24, 100, 104].

A variety of *in vitro* tests and animal models using different purified preparations of lysins, either alone, in combination, or together with classical antibiotics, have demonstrated the potential of many phage lysins as therapeutics or biocontrol agents, and those that have been well researched are summarised in Tables 4(a) and 4(b).

The pioneer of endolysin antibacterial research is undoubtedly Vincent Fischetti, whose group showed that the streptococcal lysin encoded by phage C1 is specific for groups A, C, and E Streptococci [105, 106]. The addition of 1,000 U of purified lysin *in vitro* within five seconds resulted in 100% inhibition of 10^7 CFU/mL of group A Streptococci. Furthermore, in a mouse model of infection, protection of mice from group A Streptococci colonisation was evident. In this case, a single dose of lysin (250 U) was added to

Table 3: Phages and bacterial hosts used in biofilm eradication studies.

Year	Bacteria	Phage	Reference
1995	*E. coli*	T4	Doolittle et al., 1995 [83]
1996	*E. coli, P. aeruginosa*	T4, E79	Doolittle et al., 1996 [139]
1998	*E. agglomerans*	53b SF153b	Hughes et al., 1998 [140]
2001	*E. coli*	K-12 T4	Corbin et al., 2001 [141]
2004	*P. fluorescens*	φS1	Sillankorva et al., 2004 [142]
2005	*E. coli*	O157 : H7 KH1	Sharma et al., 2005 [143]
2008	*P. fluorescens*	φS1	Sillankorva et al., 2008 [144]
2010	*K. pneumoniae*	KPO1K2	Verma et al., 2010 [145]
2010	*L. monocytogenes*	P100	Soni and Nannapaneni, 2010 [146]
2011	*S. aureus*	SAP-26	Rahman et al., 2011 [147]
2011	*P. aeruginosa*	PAO1 and ATCC 10145	Pires et al., 2011 [148]
2012	*P. aeruginosa*	φMR299-2 and φNH-4	Alemayehu et al., 2012 [137]
2012	*E. coli*	vB_EcoP_ACG-C91, vB_EcoM_ACG-C40, vB_EcoS_ACG-M12	Chibeu et al., 2012 [149]
2012	*S. aureus*	K	Kelly et al., 2012 [85]
2012	*A. baumannii*	AB7-IBB1	Yele et al., 2012 [150]
2012	*A. baumannii*	AB7-IBB2	Thawal et al., 2012 [151]

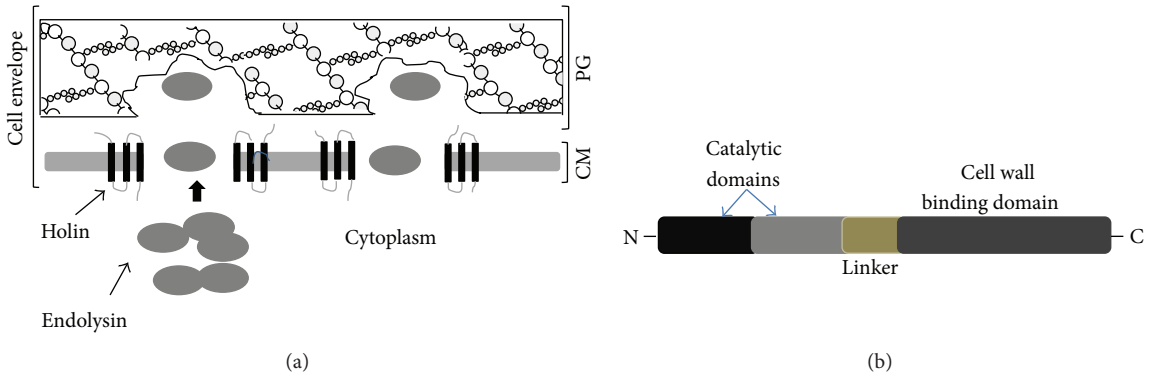

Figure 7: Schematic representation of the modular structure (a) and mode of action (b) of phage-encoded endolysins. Most endolysins are characterised by one or two catalytic domains and one cell wall-binding domain involved in substrate recognition. Access of the endolysin to the peptidoglycan (PG) layer is often aided by insertion of the holin into the cytoplasmic membrane (CM).

the oral cavity of mice before the addition of 10^7 CFU/mL of group A Streptococci. Indeed, in an additional experiment following administration of lysin (500 U) to mice that were heavily colonised with group A Streptococci, no Streptococci were detected 2 h after treatment [106]. The same research group also studied the *Streptococcus pneumoniae* phage lysin enzyme (Pal) and demonstrated that it was able to eradicate 15 common serotypes of *Pneumococci* [105]. It has clearly been shown that the use of purified Cpl-1 and/or Pal 1 lysins was very efficient in curing heavy infections caused by *S. pneumoniae* strain 6B and the acute otitis caused by *S. pneumoniae* [107, 108]. Fischetti's group also focused on a *Bacillus anthracis* phage lysin and showed that it could be exploited for the detection and elimination of this pathogen, which has associations with bioterrorism. In this case, the lysin was identified from phage γ of *B. anthracis* and was found to be effective against vegetative cells as well as germinating spores. The lysins tested were PlG and PlyPH. The latter is especially resistant to a wide pH range [109, 110]. Other lysins were also shown to have a great deal of lytic activity against streptococci; however, it is noteworthy that LySMP manifested a prominent lytic activity that is greater than that seen with the whole phage [111–113].

Staphylococcal phage MR11 was originally reported to be active against *Staphylococcus* infections in mice. Its lysin was subsequently cloned and designated MV-L lysin and used to eliminate MRSA in the nasal cavities of mice. Complete elimination of bacteria was observed in one of nine mice treated with MV-L lysin. The remaining mice had much lower CFU/nasal cavity numbers than the untreated controls. In an additional experiment with a model of systemic MRSA disease after 60 days, all mice treated with MV-L lysin directly

Table 4: Phage lysins targeting pathogenic bacteria.

Bacteria	Phage	Lysins	Activity	References
S. pneumoniae	Cp1	Cpl-1	Muramidase	Loeffler et al., 2001 [105]; Jado et al., 2003 [107]; McCullers et al., 2007 [108]
S. pneumoniae	Dp-1	Pal	Amidase	Jado et al., 2003 [107]
S. pyogenes	C1	C1	Amidase	Nelson et al., 2001 [106]
B. anthracis	γ	PlyG	Amidase	Schuch et al., 2002 [109]
B. anthracis	Ames prophage	PlyPH	Amidase	Yoong et al., 2006 [110]
E. faecalis and *E. faecium*	Phi1	PlyV12	Amidase	Yoong et al., 2004 [114]
S. aureus	MR11	MV-L	Endopeptidase and amidase	Rashel et al., 2007 [99]
S. pyogenes	C1	PlyC	Amidase	Hoopes et al., 2009 [152]
S. agalactiae	B30	GBS lysin	Muramidase and endopeptidase	Pritchard et al., 2004 [153]
S. aureus	P68	Lys16	Endopeptidase	Takáč et al., 2005 [154]
S. aureus	K	LysK	Amidase and endopeptidase	O'Flaherty et al., 2005 [53]
S. aureus	MR11	MV-L	Amidase and endopeptidase	Rashel et al., 2007 [99]
L. monocytogenes	A118	Ply118	Amidase	Gaeng et al., 2000 [155]
L. monocytogenes	A511	Ply511	Amidase	Gaeng et al., 2000 [155]
L. monocytogenes	A500	Ply500	Endopeptidase	Loessner et al., 2002 [91]
S. pneumoniae	ΦDp-1	Pal, S	Amidase and endopeptidase	Loeffler et al., 2001 [105]
S. agalactiae	LambdaSa1 prophage	LambdaSa1 prophage lysin	Glycosidase	Pritchard et al., 2007 [156]
S. agalactiae	LambdaSa2 prophage	LambdaSa2 prophage lysin	Glycosidase and endopeptidase	Pritchard et al., 2007 [156]
S. uberis	(ATCC700407) prophage	Ply700	Amidase	Celia et al., 2008 [111]
S. suis	SMP	LySMP	Glycosidase and endopeptidase	Wang et al., 2009 [113]
B. anthracis	Bcp1	PlyB,	Muramidase	Porter et al., 2007 [157]
S. aureus	Phi11 and Phi12	Phi11 lysin	Amidase and endopeptidase	Sass and Bierbaum, 2007 [158]
S. aureus	ΦMR11	MV-L	Amidase and endopeptidase	Rashel et al., 2007 [99]
S. aureus	ΦH5	LysH5	Amidase and endopeptidase	Obeso et al., 2008 [112]
S. warneri	ΦWMY	LysWMY	Amidase and endopeptidase	Yokoi et al., 2005 [159]
Streptococci (GBS)	ΦNCTC 11261	PlyGBS	Muramidase and endopeptidase	Cheng et al., 2005 [160]
C. perfringens	Φ3626	Ply3626	Amidase	Zimmer et al., 2002 [115]
C. difficile	ΦCD27	CD27 lysin	Amidase	Mayer et al., 2008 [161]
E. faecalis	Φ1	PlyV12	Amidase	Yoong et al., 2004 [114]
A. naeslundii	ΦAv-1-	Av-1 lysin	Putative amidase/ muramidase	Delisle et al., 2006 [162]
L. gasseri	ΦgaY	LysgaY	Muramidase	Sugahara et al., 2007 [163]
S. aureus	ΦSA4	LysSA4	Amidase and endopeptidase	Mishra et al., 2013 [164]
S. haemolyticus	ΦSH2	SH2	Amidase and endopeptidase	Schmelcher et al., 2012 [165]
B. thuringiensis	ΦBtCS33	PlyBt33	Amidase	Yuan et al., 2012 [166]
L. monocytogenes	ΦP40	PlyP40	Amidase	Eugster and Loessner, 2012 [167]
L. monocytogenes	ΦFWLLm3	LysZ5	Amidase	Zhang et al., 2012 [168]
B. cereus	ΦBPS13	LysBPS13	Amidase	Park et al., 2012 [169]
S. aureus	ΦGH15	LysGH15	Amidase and endopeptidase	Gu et al., 2011 [170]
S. aureus	ΦvB_SauS-PLA88	HydH5	Endopeptidase and glycosidase	Rodríguez et al., 2011 [171]
E. faecalis	ΦF168/08	Lys168	Endopeptidase	Proença et al., 2012 [172]
E. faecalis	ΦF170/08	Lys170	Amidase	Proença et al., 2012 [172]

Table 4: Continued.

Bacteria	Phage	Lysins	Activity	References
S. aureus	ΦP-27/HP	P-27/HP	Nonspecified	Gupta and Prasad, 2011 [173]
C. perfringens	ΦSM101	Psm	Muramidase	Nariya et al., 2011 [174]
C. sporogenes	Φ8074-B1	CS74L	Amidase	Mayer et al., 2012 [175]
S. typhimurium	ΦSPN1S	Lysin SPN1S	Glycosidase	Lim et al., 2012 [176]
C. michiganensis	ΦCMP1	CMP1	Peptidase	Wittmann et al., 2010 [177]
C. michiganensis	ΦCN77	CN77	Peptidase	Wittmann et al., 2010 [177]
A. baumannii	ΦAB2	LysAB2	Glycosidase	Lai et al., 2011 [178]
B. cereus	ΦB4	LysB4	Endopeptidase	Son et al., 2012 [179]
P. aeruginosa	ΦKMV	KMV45	Nonspecified	Briers et al., 2011 [117]
C. tyrobutyricum	ΦCTP1	Ctp1l	Glycosidase	Mayer et al., 2010 [180]
P. aeruginosa	ΦEL	EL188	Transglycosylase	Briers et al., 2007 [181]
P. aeruginosa	ΦKZ	KZ144	Transglycosylase	Briers et al., 2007 [181]
S. aureus		Ply187	Nonspecified	Mao et al., 2013 [182]
P. fluorescens	ΦOBP	OBPgp279	Glycosidase	Walmagh et al., 2012 [116]
L. monocytogenes	ΦP35	PlyP35	Amidase	Eugster et al., 2011 [183]
L. fermentum	ΦPYB5	Lyb5	Muramidase	Hu et al., 2010 [184]
S. pneumoniae	ΦCP-7	Cpl-7	Muramidase	Bustamante et al., 2010 [185]
P. chlororaphis201	Φ2-1	201φ2-1gp229	Glycosidase	Walmagh et al., 2012 [116]
S. enterica	ΦPVP-SE1)	PVP-SE1gp146	Glycosidase	Walmagh et al., 2012 [116]
Corynebacterium	ΦBFK20	BFK20	Amidase	Gerova et al., 2011 [186]
E. faecalis	ΦEFAP-1	EFAL-1	Amidase	Son et al., 2010 [187]
Lactobacilli	lamdaSA2	LysA, LysA2, and LysgaY	Nonspecified	Roach et al., 2013 [188]
S. aureus		SAL-1	Nonspecified	Jun et al., 2013 [189]

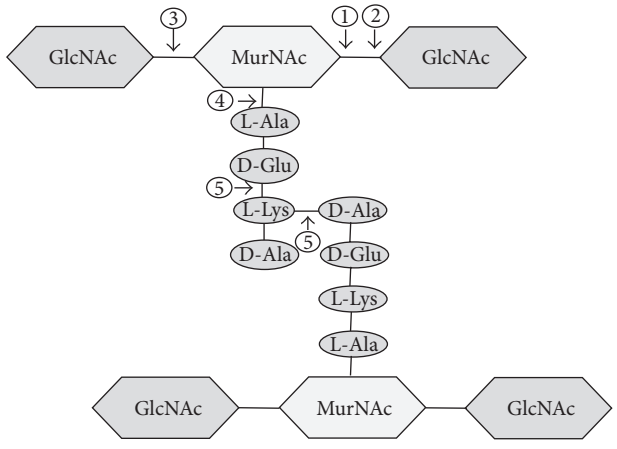

Figure 8: Typical peptidoglycan structure of Gram-positive bacteria, showing lysin cleavage sites. The cleavage sites are indicated: (1) N-acetyl-β-D-muramidase (lysozymes), (2) lytic transglycosylase, (3) N-acetyl-β-D-glucosaminidase, (4) N-acetylmuramoyl-L-alanine amidases, and (5) endopeptidase. Abbreviations: GlcNAc (N-acetyl glucosamine), MurNAc (N-acetyl muramic acid).

or 30 min after bacterial administration survived compared with 60% survival 60 min after bacterial administration [99]. In the genus *Enterococcus,* the lysin PlyV12 was found to have activity not only against its host *E. faecalis* but also against other Gram-positive pathogens, such as Staphylococci and Streptococci. In this case, the authors suggested that this might be due to a common surface structure between these pathogens [114].

The use of lysins is not limited to animal models of infection and the control of infection caused by virulent bacteria. Their use was recently extended to the area of food safety. This is illustrated by the use of the lysin Ply3626 that is active against *C. perfringens* [115], really third most common cause of food-borne illness. Investigations in the area of Gram-negative endolysins as antibacterial applications have also been undertaken, for example, in the case of *Pseudomonas aeruginosa*. While such endolysins (unlike those of Gram-positives) generally have a broad target range among Gram-negative genera, their application as antibacterials is compromised by the presence of the outer membrane. Thus, antibacterial activity has only been shown to be possible after treatment of the outer membrane of Gram-negative cells with EDTA [116] or by the fusion of hydrophobic amino acids to the endolysin, which enables the movement of the endolysin across the outer membrane. The latter approach has been recently developed in endolysins by the group of Lavigne in Belgium [117] and was based on earlier observations where the action of lysozyme against *E. coli* was enhanced by the fusion of a hydrophobic pentapeptide onto

the C-terminus [118]. The advantage of the use of lysins over other antibacterials (or antibiotics) is their specificity for one bacterial pathogen without disturbing other nonpathogenic bacterial flora. There is also a very low chance of bacterial resistance to endolysins, due to the fact that resistance would necessitate an alteration in fundamental peptidoglycan structure. To date no lysin-resistant bacteria have been identified. Thus, lysins could be effective antibacterials in an age of increasing antibiotic resistance. It is worthy of mention that one potential concern in the use of lysins is the development of lysin-neutralising antibodies. Unlike antibiotics, which are small molecules that are generally not immunogenic, endolysins are proteins that stimulate an immune response when delivered both mucosally and systemically. Despite the limited studies on endolysin immunogenicity, it has been reported that highly immune serum slows, but does not block the killing of bacteria by lysins [87, 88, 114].

10. Conclusions

The continued, world-wide antibiotic resistance problem requires the exploitation of inexpensive, natural, available, safe, and efficient therapeutic agents. Consequently, investigations of phage confirmed that they can be specific and highly effective in lysing targeted pathogenic bacteria. The safety of such therapies has been demonstrated by their wide clinical use in Eastern Europe and the Former Soviet Union. Phages are stable and easy to purify at a relatively low cost. They are naturally widespread in many environments on Earth and play an important role in bacterial ecology and evolution. Therefore, investigations of the use of phage for the elimination of pathogenic bacteria are well justified and from the cases discussed here it is clear that use of phages or their lytic enzymes has a considerable array of applications as therapeutics in the modern medical and veterinary fields.

Conflict of Interests

The authors declare no conflict of interests.

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