Author Manuscript Author Manuscript Author Manuscript Author Manuscript

HHS Public Access
Author manuscript
Expert Rev Anti Infect Ther. Author manuscript; available in PMC 2020 December 02.

Published in final edited form as:
Expert Rev Anti Infect Ther. 2019 December ; 17(12): 1011–1041. doi:10.1080/14787210.2019.1694905.

Biological challenges of phage therapy and proposed solutions: a literature review

Katherine M Caflisch[1], **Gina A Suh**[2], **Robin Patel**[*,2,3]

[1]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota, USA

[2]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

[3]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Introduction—In light of the emergence of antibiotic-resistant bacteria, phage (bacteriophage) therapy has been recognized as a potential alternative or addition to antibiotics for use in humans in Western medicine.

Areas covered—This review assessed the scientific literature on phage therapy published between January 1, 2007 and October 21, 2019, with a focus on successes and challenges of this prospective therapeutic.

Expert opinion—Efficacy has been shown in animal models and experimental findings suggest promise for safety of human phagotherapy. Significant challenges remain to be addressed prior to the standardization of phage therapy in the West, including the development of phage resistant bacteria; the pharmacokinetics of phage; and any potential human immune response incited by phagotherapy.

Keywords

Phage; phage resistance; antimicrobial resistance; endolysin; enzybiotics; phage therapy; phagotherapy; antimicrobial therapy

1. Introduction

Less than a century after the discovery of penicillin, the emergence of bacteria no longer susceptible to small-molecule antibiotics is recognized as one of the most significant health threats facing modern medicine. Phages (bacteriophages) are among the strategies being evaluated as a potential replacement or adjunct to antibiotics.

[*]Corresponding author: Robin Patel, Mayo Clinic, 200 First St. SW, Rochester MN 55905, patel.robin@mayo.edu, Phone: 507 284 4272, Fax: 507 538 0579.

Reviewer disclosures
Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Phages are viruses (with single- or double-stranded DNA or RNA genomes) that exclusively infect bacteria. Individual phages are specific for strains of bacterial species. Like other viruses, phages lack a complete replisome and must therefore assume intracellular infection of a host to propagate. Phages undergo receptor-mediated adsorption to the surface of target bacteria prior to injecting their genetic material into the cytoplasm where bacterial replication machinery is subverted to produce new virions [1]. Phages are released following bacterial lysis, thereafter infecting adjacent bacterial hosts. In therapeutic settings, exclusively virulent phages (i.e., those that do not integrate into bacterial genomes) are generally favored over those that can integrate into bacterial genomes due to the predictable time to lysis, and also because host genomic integration and excision of temperate phages risks mobilization or activation of virulence and/or antibiotic resistance genes, and because infection by temperate phages may prevent subsequent phage infection of their bacterial host [2]. A detailed description of the virulent and lysogenic life cycles of phage is found elsewhere [1].

The earliest accounts of phage, circa 1890s, are as storied as they are disputed, particularly, regarding the individual(s) to whom credit for their discovery should be attributed. The majority of sources contend that in 1896, Ernest Hanbury Hankin, a bacteriologist commissioned by the British Commonwealth, observed in the waters of India's Ganges and Yamuna rivers an inhibitory phenomenon which thwarted growth of *Vibrio cholerae* [3], and that in 1898, Russian scientist Nikolay Gamaleya made similar observations in his study of *Bacillus subtilis* [4]. English bacteriologist Frederick Twort is widely remembered as the next noteworthy individual in the phage narrative; in 1915, he observed bacteriophage in bacterial contaminants in experiments with Vaccinia virus, though he ascribed this to a bacterial co-factor negatively impacting cellular viability [2,3]. While this interpretation was ultimately incorrect, Twort's research greatly contributed to the field's understanding of phage biology [2]. Finally, a 1917 publication by Felix d'Herelle at the Pasteur Institute describing an "invisible microbe" [5] leads some sources to ascribe the discovery of phage to him; nevertheless, most sources contend that it was d'Herelle who first developed the notion of using phages therapeutically [6].

Seemingly-successful early human applications of phage in cases of pediatric dysentery [7], cholera [4,8,9], and bubonic plague [6,7] stimulated interest in phage therapy across Europe, the former Soviet Union, and the United States. Failed attempts to reproduce positive findings (possibly secondary to an incomplete biological understanding of phage) however, atrophied scientific interest and even inspired opposition from the Council on Pharmacy and Chemistry of the American Medical Association in 1934 [3,6–9]. The discovery of penicillin, and subsequently other antibiotics, alongside tensions between Eastern and Western political powers, further disincentivized phage use in Western medicine at the time, while they continue to be used in Eastern Europe and the former Soviet Union to this day [6,8]. However, the rise of antibacterial resistance has stimulated renewed Western interest in the therapeutic potential of phage, having already been approved for antibacterial applications in the agriculture and food processing industries [10].

Promising human case reports and case series of Western phagotherapy have been published over the past decade [11–13], including the recovery of a diabetic patient from necrotizing

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

pancreatitis with a multidrug-resistant *Acinetobacter baumannii* infection [14], bacterial eradication from an aortic graft infected with *Pseudomonas aeruginosa* [15], treatment of multidrug-resistant *P. aeruginosa* pneumonia in a cystic fibrosis patient [16], and stabilization of a post-lung transplant *Mycobacterium abscessus* infection in a cystic fibrosis patient [17]. Further, *in vitro* and experimental animal data have also demonstrated activity of phage against drug-susceptible and -resistant bacteria (Figures 2 and 3, Table 1). Nevertheless, therapeutic failure of phage in a handful of human studies, including two clinical trials [18,19], suggests that their clinical utility requires careful definition [19–21]. Biological challenges surrounding phagotherapy, which constitute the focus of this literature review, include i) the risk of selecting for phage-resistant bacteria, ii) pharmacodynamic and pharmacokinetic complexities, and iii) human host interactions. Potential solutions to these challenges are also discussed.

1.1 Review methods

The search strategy consisted of Embase, Cochrane Central Register of Controlled Trials (CCTR), Cochrane Database of Systematic Reviews (CDSR), Ovid MEDLINE, Ovid MEDLINE Daily, Ovid MEDLINE In-Process, Epub Ahead of Print and Other Non-Indexed Citations database surveys for articles published between January 1, 2007 and October 21, 2019 (Supplemental Table).

2. Biological constraints of phage therapy in humans

2.1 Bacterial resistance to phage

Community dynamics underlying bacterial resistance are complex when the antibacterial agent is biologically dynamic, as occurs in phagotherapy. Unlike small molecule antibiotics, phages are shaped *by* environmentally-mediated competitive selection in addition to exerting the same *upon* bacteria. This bidirectional interaction results in co-evolution of bacteria and viruses as each contends for survival. To this end, the former have developed an array of approaches to withstand phage infection that collectively targets every major step of the lytic infection cycle [22] (Figure 1). Phages, in turn, have acquired mechanisms for resisting countermeasures exhibited by bacteria [22,23]. Such an ever-changing environment presents unique challenges in which to assess therapeutic outcomes. It should be noted that the "inevitable" [24] development of phage-resistant bacteria may be biomedically advantageous if the mutation incurred to withstand phage drives a fitness reduction that can be leveraged through secondary antibacterial treatment. For instance, exposure of multidrug-resistant *P. aeruginosa* clinical isolates to virulent phage OMKO1 selected for the loss of its bacterial receptor, MexAB and MexXY efflux pump-associated outer membrane porin, OprM, ultimately renewing the isolates' susceptibility to antibiotics [25]. Ho et al. show that lipotechoic acid (LTA) mutations emerge following NPV phage exposure to *Enterococcus faecalis*, a phenotypic change that can sensitize the organism to daptomycin [26]. Most recently, Sumrall et al. demonstrated *in vitro* that exposure of *Listeria monocytogenes* strain 1042 to virulent phage A511 induced loss of glucose and galactose residues on wall techoic acids, a hallmark of the highly pathogenic serotype 4b, to phenotypically resemble the less invasive serotype 4d [27]. Scanlan, Buckling and Hall suggest that bacteria exposed to phage may be more highly evolved as a consequence of increased selective pressure [28]. Such

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

modifications may lead to concomitant changes in bacterial fitness that further impact response to antimicrobial treatment [29]. One extreme example of this phenomenon was demonstrated by Capparelli et al., who generated phage-resistant *S. aureus* mutants in mice to produce a live-attenuated vaccine [30]. Whereas bacterial fitness attenuation may be associated with phage resistance [31], this is not always the case [32]. Osada et al. found that *S. aureus* SA003 developed resistance to lytic phage φSA012, with transient fitness reduction, ultimately countered by an elevated growth rate [33]. Similarly, Kashiwagi and Yomo reported that an RNA phage Qβ-resistant *Escherichia coli* strain incurred mutations in genomic domains governing cell proliferation, resulting in a compensatory increase in doubling time related to evolution of a new pathway enabling sulfur biogenesis [34].

The emergence of phage-resistant bacteria must be acknowledged as a certain outcome of human phage therapy. The following are currently-known mechanisms by which bacteria can resist phage.

2.1.1 Cell surface and extracellular modifications

2.1.1.1 Receptor adaptations: Bacterial resistance to phage may be achieved by cell surface modification via receptor downregulation, concealment, or conformational change [22,35,36]. Bacteria may possess sensory mechanisms that detect the preliminary, weak molecular interactions of phage binding to garner infection resistance prior to the irreversible phage binding [37]. Point mutations or epigenetic modifications to bacterial phage receptors [38,39], including lipopolysaccharide (LPS), outer membrane proteins, flagella, pili, and capsule-associated proteins, for example, may result in decreased phage adsorption, and ultimately, infectivity. Le et al. described an observation in *P. aeruginosa* following exposure to lytic phage whereby 30% of phage-resistant bacteria exhibited genomic deletion of *galU*, a gene required for biosynthesis of LPS, the particular phage's receptor [35]. Similarly, Fallico et al. reported biochemical modifications to teichoic and lipoteichoic acids on *Lactococcus lactis* IL1403 during early phage exposure to contain D-alanine ester, hypothetically resulting in a conformational change to the phage receptors and limiting phage adsorption and infection [40]. Similar examples include O-antigen acetylation of *N*-acetylmuramic acid in staphylococci [41], and galactosylation of techoic acid in *L. lactis* subsp. *cremoris* SK110 and of cell wall techoic acid in *Staphylococcus pseudintermedius* SP015 and *Staphylococcus aureus* [39,42].

2.1.1.2 Outer Membrane Vesicles: Outer membrane vesicles (OMVs), nonreplicating structures that are part of a functionally-diverse intercellular transport system, and which bud from Gram-negative bacterial cells, may act as a sink during phage invasion. Phages may bind OMVs as a result of similar surface features to that of their parent cell, thereby reducing the likelihood of cellular infection [22].

2.1.1.3 Quorum Sensing: Bacterial cells may also leverage intercellular communication to limit viral infection within the population. Bru et al. recently reported that *in vitro* infection of *P. aeruginosa* UCBPP-PA14 by virulent phage DMS3vir results in decreased swarming motility through a mechanism involving secretion of the quorum sensing molecule PQS thereby spatially segregating uninfected subpopulations; this generalized stress

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

response is also observed when colonies are exposed to aminoglycosides [43]. Induction of PQS also stimulates release of OMVs, potentially compounding the anti-phage effect of quorum sensing [43].

2.1.2 Intracellular Modifications

2.1.2.1 Restriction-Modification (RM) Systems: The most ubiquitous mechanism of defense among bacteria and archaea, RM systems detect and cleave foreign DNA, including phage double-stranded DNA, based on nucleotide methylation of host DNA [44]. (RM systems are considered not to degrade single-stranded DNA or RNA phage.) Harbored by more than 90% of prokaryotes according to some estimates, RM systems play a role in prokaryotic homeostasis analogous to the innate immune system of higher-order organisms [44]. Restriction-modification systems are comprised of two functional subunits – a restriction endonuclease which cleaves (degrades) un-methylated DNA, and a related methyltransferase which methylates host DNA [44]. Such epigenetic modification forms the basis for recognizing and sequestering foreign genomic material [44,45] and is phylogenetically-conserved, with several hypotheses attempting to explain why this might be the case [44]. Korona et al. predict that RM systems stave off global infection of the bacterial community upon phage introduction to allow for the expansion of genetically-diverse bacterial sub-populations[46].

Yet RM systems are notably flawed; inappropriate methylation of foreign DNA may occur with a probability of up to 0.1 per infection allowing certain phages to acquire host-modified nucleic acids, thereby subverting the RM defense barrier [44,47]. If this occurs, all progeny of the methylated phage will maintain this epigenetic mark, enabling viral propagation to continue [44]. Similarly, T-even phages harbor non-canonical base nucleotides that evade recognition and subsequent cleavage by bacterial RM systems [45]. Sneppen et al. used *in silico* analyses of phage-bacterial systems to conclude that such ‘loopholes’ within RM systems generate diversity among phage and bacteria alike, increasing ecological carrying capacity and portending the emergence of RM-resistant “epigenetic phage variants” [44]. These variants, in turn, promote more balanced population dynamics by reducing the net growth rate of the comparatively-fit RM-harboring bacterial population that remains susceptible to the mutant phage [44].

The defense island system associated with restriction modification (DISARM) is a bacterial and archaeal defense system similar to RM that is also governed by methylated self-recognition, with putative differences between the two systems comprising specific gene networks involved in cleavage of foreign DNA; the mechanism and function of DISARM-mediated methylation is incompletely defined [22,48].

2.1.2.2 Abortive Infection: A lack of consensus exists regarding the scope of the diverse systems of defense comprising abortive infection; some maintain that it necessarily requires host cell lysis, whereas others consider the term to merely require that infectivity of internalized virions be quenched [45,47]. Toxin-antitoxin systems represent a manifestation of abortive infection observed in both bacteria and archaea. An unstable antitoxin molecule binds and neutralizes the effect of its toxin dimer in a virally-uninfected cell, resulting in cell

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

survival, whereas phage infection interferes with antitoxin synthesis such that unbound toxin prevents bacterial translation through RNA-mediated means [49]. Other abortive infection processes include attenuation of bacterial cell membrane potential, or altered gene expression or maturation [47]. A consequence that may be associated with abortive infection is lysis of the cell following deactivation of the internalized phage to prevent infection of adjacent bacteria [45,47,49]. Some bacterial possess phage inhibitory chromosomal islands, which are prokaryotic mobile genetic elements that indirectly minimize the burst size of an active viral infection [50]. While abortive infection appears in Gram-positive and -negative bacteria, it is more prevalent in the former [49].

2.1.2.3 Bacteriophage Exclusion (BREX): The most recently-identified innate anti-phage defense system, phage exclusion, is a resistance mechanism found in recombinant *B. subtilis* bearing similarities with RM and abortive infection systems as it enables selective methylation of the host genome to prevent phage propagation; however, it does not subsequently degrade phage [51]. The mechanism underlying phage exclusion is unknown. Such systems may be present in 10% of microbial genomes, according to one estimate [51].

2.1.2.4 Superinfection Exclusion: Superinfection exclusion is a protein-mediated event precluding entry of subsequent, taxonomically-related phage following genomic integration of an earlier temperate phage [32], thereby rendering lysogenized bacteria resistant to secondary infection by certain phages. Superinfection exclusion is a prevalent mechanism in Gram-negative bacteria, occurring less frequently in Gram-positive species.

2.1.2.5 CRISPR/Cas: Clustered regularly interspaced short palindromic repeats (CRISPR), together with CRISPR-associated proteins (Cas), which have revolutionized the field of gene editing, harbor a parallel role in their native bacterial hosts where they are present in 50% of genomes [45,52]. The equivalent of adaptive immunity, CRISPR-Cas systems are comprised of repetitive DNA sequences with exogenously acquired DNA spacers distributed throughout. DNA is inserted into the CRISPR cassette, with Cas1 suspected to play a role [53]. Cells use these spacers as templates to synthesize CRISPR RNAs (crRNA), which may then complex with Cas9 to initiate double strand breaks in the corresponding loci of incoming foreign DNA [53]. Such systems have been shown to maintain viability following recombination: Jakutyte-Giraitiene and Gasiunas recently reported CRISPR3-recombinant *B. subtilis* sourced from *Streptococcus thermophilus*. Anti-phage activity between transformed *B. subtilis* and *S. thermophilus* was comparable, with both showing high recombination efficiency [52]. CRISPR3 enables recognition of replication gene sequences gp6 and gp58 of lytic phage SPP1 and subsequent destruction of the phage in *B. subtilis* [52]. The role of this mechanism in the context of phage resistance is incompletely understood. Prokaryotic Argonaute (pAgo) proteins, implicated in other bacterial defense systems, are thought to be involved in a parallel phage immunity scheme utilizing RNA rather than DNA templates to recognize and degrade foreign nucleic acid [22].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Phage pharmacokinetics

Pharmacokinetics of phagotherapy are more complex than those of fixed composition small-molecule antibiotics [54,55], more closely resembling therapeutic biologics such as stem cells. While adsorption, distribution, metabolism and excretion of phage can theoretically be computed *in silico*, ideal human dosing and route(s) of administration are as yet undefined due to potential elimination by the immune system (itself a changing entity), differential access to sites of infection (and oftentimes varying and unknown bacterial abundance at infected sites), inter-individual (human and bacterial) differences, and inter-phage variables, including diverse capacities of different phages to persist and replicate. Unlike antimicrobial chemotherapeutics which are eliminated by human phase I and phase II enzymes, bacteriophages are eliminated by the reticuloendothelial system in the spleen, but their bioavailability; clearance rate; charge and hydrophobicity; binding affinity to plasma proteins such as human serum albumin, lipoproteins, and glycoproteins remain largely unknown.

Pharmacodynamic and pharmacokinetic considerations alike expand in their complexity when phages or lysins are used in combination with other phages or lysins, or other antimicrobial agents altogether. For each, the mechanism of action and removal from the body should be carefully defined prior to determining the dosing regimen in an effort to maximize the antibacterial capacity of such combinations. Some cocktails have shown synergy, while others exhibit additive benefit, and some display antagonism. The order of delivery may impact therapeutic outcome. Kumaran et al. report an experiment in which planktonic and biofilm methicillin-resistant *S. aureus* was treated with phage SB-1 prior to or at the same time as rifampin, daptomycin, fosfomycin, ciprofloxacin, or vancomycin *in vitro* with sequential treatment outperforming simultaneous treatment. Similarly, treatment of *S. aureus* biofilms *in vitro* with phage SATA-8505 following vancomycin, dicloxacillin, cefazolin, tetracycline, or linezolid abrogated the antimicrobial effect [56].

4. Human interactions

The need to identify the impact of “trans-kingdom interactions” in the context of human phagotherapy is paramount to its widespread clinical usage safely and effectively [57]. Within the triad of phage-treated, human-associated bacteria, interactions between phage and bacteria have been well-characterized, as have those between bacteria and humans. Very few studies have, however, considered the possibility and hypothetical nature of any interactions spanning phage and humans. Given the significant reduction in planktonic and adherent *C. difficile* cells and significant increase in phage amplification observed in systems containing bacteria, phage, and HT-29 (human colorectal cancer) cells *in vitro* versus systems lacking HT-29 cells – presumably explained by the close proximity of bacteria and phage that their eukaryotic binding generated – Shan et al. propose that phage-human dynamics be further considered [58].

Perhaps the most obvious eukaryotic niche in which to examine the impact of therapeutic phage is innate and adaptive immune systems. For more than fifty years, phages (particularly, coliphage ΦX174) have upheld a role in clinical practice in the diagnosis of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

primary and secondary immunodeficiencies and continue to be utilized in this way today [59]. As a neoantigen, ΦX174 is processed only via presentation by T to B cells of the humoral immune system, such that inability to develop cell-mediated immunity against the phage indicates immune deficiency.

Despite the diagnostic role of phage in clinical medicine in addition to their myriad therapeutic application in humans, only a handful of studies have assessed phage safety as a primary endpoint [60]. A 2003 phase I trial in Switzerland demonstrated that oral ingestion of T4 phage resulted in neither entry of phage into the bloodstream nor altered liver enzymes [61]. An Egyptian burn wound trial executed in 1990 in which non-purified lytic phages were applied to wounds was not associated with adverse effects [62]. Even in vulnerable populations, including pediatric and immunocompromised patients, no serious adverse effects have been linked to phage therapy [59,63,64]. Some experts maintain that any safety threat to mankind would have been observed long ago, considering the ubiquitous presence of phages in the natural environment, but this supposition is perhaps an oversimplification [65]. Indeed, the safety of individual phage formulations is not just a function of safety of the phage, but is contingent on preparation methods, which are non-standardized [65]. For example, incomplete purification of phage from its bacterial host could result in inadvertent delivery of a bacterial toxin, such as endotoxin and/or an exotoxin.

Related theoretical safety concerns include that systemic application of phage may result in emergent toxicity following rapid bacterial lysis and, in the case of Gram-negative bacteria, release of endotoxin in large quantities, although this has not been observed in practice. (In fact, at least one group has observed diminished endotoxin release by phagotherapy *versus* antibiotic chemotherapy *in vitro* [66]). Dufour et al. reported on this possibility in an *E. coli* model in which two lytic coliphages (536_P1 and LM33_P1) or antibiotics were applied in parallel in *in vitro* systems using *E. coli* 536 and *E. coli* LM33 [67]; they found release of extracellular LPS induced by phage to be similar to that observed with amikacin and lower than that observed with cefoxitin or imipenem [66]. Amplification of anti-inflammatory suppressor of cytokine signaling 3 (SOSC3), IL-1 receptor antagonist, and IL-6, as well as reduction in LPS-induced inflammation by NF-κB p65 phosphorylation inhibition are suggestive of the anti-inflammatory properties of phage in some instances [65,68–70], while evidence for the hyperinflammatory potential of therapeutic phage via toll-like receptor 9 and IFN-γ has been demonstrated in animal models to worsen preexisting conditions [71]. However, in one study, although one of two phages utilized in a murine pneumonia model yielded significant increases in IFN-γ, IL-12, monokine induced by IFN-γ (MIG), monocyte chemoattractant protein-1 (MCP-1), and keratinocyte chemoattractant (KC) in the lungs of uninfected mice, there was no statistically significant increase in these acute-phase inflammatory cytokines in infected, phage- or antibiotic-treated mice over the course of a 20–22 hour infection [72]. In this way, modulation of infection-associated inflammation by phage is variable and likely to be phage species-specific.

As viruses, phages are capable of stimulating innate and adaptive immune systems; given their recent detection in the bloodstream via metagenomic analysis as part of the endogenous human phageome, such an interaction is likely and may influence tolerance and/or efficacy of phagotherapy [65]. It is unclear whether phages' potent bioactivity may be partly

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

attributed to their recruitment of the immune system beyond inherent antibacterial properties [63]. For example, El-Aziz, Elgaml, and Ali observe enhanced complement-mediated antibacterial activity of the innate immune system by virulent phage MMI-*Ps*$_1$ against *P. aeruginosa* in a murine model of acute lung infection [73]. On the other hand, Van Belleghem et al. report functional opsonization of phage by binding the surface of invading bacteria [65]; this may result in hyperinflammation or phage neutralization via secondary adaptive immune responses.

In some applications, there is concern that recognition of circulating phages will result in phage elimination, diminishing phage efficacy. Upon systemic introduction, for instance, phage may be intercepted by tissue proteases or the reticuloendothelial system and delivered to the spleen and liver for degradation [65]. Delivery of specific phage in multiple doses, especially systemically, also begets the possibility of neutralizing antibody production against the phage, possibly stimulating phage destruction. Available reports do not agree on the prevalence of phage-neutralizing antibodies and the degree to which they might impact clinical efficacy of phage therapy [3,74]. The extent of antibody production may vary based on routes of phage administration [3,74]. One study administered *S. aureus*-specific phages 676/Z or A3/R to 122 ill patients twice or thrice daily for 7–91 days and monitored serum anti-phage activity [60]. The investigators found that a cohort of healthy volunteers (n=30) possessed a basal level of anti-phage antibodies similar to that of patients pre-treatment. Serum anti-phage activity increased during phage treatment in a manner dependent upon administration route, with local (which included “gargling, fistula irrigation, irrigation of the abscess cavity, sitz baths, wet compresses, nose drops, ear drops, vaginal irrigation, and inhalations” depending upon infection site [60]) and combined local/oral administration generating greater anti-phage activity than oral or intrarectal administration [75]. Anti-phage activity in patients waned following discontinuation of therapy although it remains a concern whether antibodies demonstrate cross-neutralization of related and unrelated phage species [60].

Finally, a small body of evidence suggests that bacterial lysogeny may directly impact human cells. For instance, one report presented evidence for infection of eukaryotic cells by enterohemorrhagic *E. coli* (EHEC) temperate phage [76]. The temperate phage studied harbored bacterial genes encoding Shiga toxin which were expressed in adjacent eukaryotic cells, likely via secondary translation pathways or using mitochondrial pathways [77]. This report is consistent with a 1971 report describing translation in mammalian fibroblasts of ß-galactosidase with purported origins from temperate phage [78]. Sweere et al. reported that *P. aeruginosa* isolated from wounds lysogenized with temperate phage Pf supported chronic bacterial infection compared with non-lysogenized *P. aeruginosa*, with a proposed mechanism involving phage transcriptome-mediated production of TRIF-dependent type I interferon and reduction of phagocytosis and tumor necrosis factor secretion [79]. The application of a lytic phage treatment in a system containing such lysogenized bacteria might be associated with non-infection via superinfection exclusion or CRISPR, or, as these authors speculate, with exacerbation of bacterial infection by upregulating expression of virulence mechanisms by the bacterium, two clinically-relevant considerations given that temperate phages are present in an estimated 40–50% of bacterial genomes [80].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

5. Concessions to limitations of therapeutic phage

5.1 Phage Modification

5.1.1 Adaptation—In 1961, J.F. Vieu described a phenomenon known as phage adaptation, which is described as the repeated passage of a phage in the presence of its target bacterial host or eukaryotic host in order to increase certain therapeutic parameters, such as selecting for long-circulating variants *in vivo* or evolved receptor binding proteins capable of interacting with evolved bacterial receptors [8,81,82]. Such "directed evolution" has typically been achieved by exposing *one* phage to *one* bacterium, and consecutively testing the resulting phage lysate against resistant colonies until susceptibility is observed [83]. However, the Appelmans Protocol is an alternative adaptation technique commonly utilized in the Republic of Georgia, in which a *cocktail* of phages, rather than a single phage, is tested against a bacterium because the administration of several phages together allows for the possibility of genetic recombination between phages [84].

Though traditionally labor-intensive, the availability of new tools such as multiplex automated genome engineering and phage-assisted continuous evolution has accelerated phage adaptation, subjecting phage to "automatic evolution" and enabling synchrony with adaptation of bacterial hosts [85]. Sybesma et al. found that screening *K. pneumoniae* and *E. coli* phages to identify those with an expanded host range improved treatment efficacy; of 38 strains tested, Pyo-phage cocktail susceptibility increased from 66 to 93% [86,87].

Alternatively, phage adaptation may be unnecessary in place of a screening protocol that detected phages which naturally exhibited extended persistence or broad spectra of activity [88].

5.1.1.1 Engineered Phage: Phage researchers have considered optimizing the therapeutic potential of phages via genome engineering [89–91]. Engineering has been employed to increase the antibacterial capacity of phages intended for therapeutic usage. For example, through modifications to the genome of phage M13mp18, the damage incurred following simultaneous application of modified phage and ofloxacin, gentamicin, or ampicillin resulted in augmented killing efficiency by 5-, 3- and 5.5-fold, respectively, compared with bacteria treated with antibiotics alone, though the effect of phage alone was not reported [92,93]. Phages have also been modified to cross eukaryotic membranes in the case of intracellular infection, with some success [94]. Pouillot, Blois and Iris reported a gene editing technology that enabled pausing of the T4 phage replication cycle for insertion of recombinant genes, followed by re-activation of hybrid phage [95]. This technique might be applied for expansion of host range or to encode a bacterial antigen on a phage capsid to augment immune response for ultimate clearance of bacterial pathogens.

The literature describes a diversity of ways in which natural phages have been modified in an attempt to mitigate eukaryotic immune stimulation, which may be a problematic consequence of systemic administration of wild-type phage [87]. A 2005 study conducted in germ-free mice in which animals were treated with lambda phage bearing E158K capsid resulted in extended circulation when administered by intraperitoneal (IP), intravenous (IV) or oral routes of administration[96]. Paul et al. insertionally inactivated the endolysin gene

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of the *S. aureus*-specific temperate phage P954 to determine whether compromise to the cell membrane exclusively (i.e., due to holin activity) might lead to bacterial cell death without massive release of bacterial antigen [97], a safety concern of phage therapy. *In vitro* studies using eight *S. aureus* strains showed that phages wtP954 and P954 cleared over 90% of bacterial cells, without cell lysis (plaque formation) with the latter. In a systemic infection model in neutropenic mice challenged with 5×10^7 methicillin-resistant *S. aureus* (MRSA) isolate B911 IP, IP P954 phage treatment [200 multiplicity of infection (MOI); defined as the number of virions administered per bacterium] 0- and 2-hours post-infection rescued 100% of mice (n=16) with no adverse events noted, suggesting efficacy of this phage for treatment of bacterial infection in a way that may possibly circumvent lysis-associated safety concerns [97]. (Rescue from lethal bacterial challenge with wtP954 was not reported). Hagens and Bläsi described a similar approach in which modification of phage lambda or phage M13 to exclusively contain a functional holin, resulted in cell death without lysis [98,99], while Bardy et al. developed a holin-deficient T4LyD phage with similar effects [100].

Another widely-recognized exercise in phage modification was described by Lu and Collins in 2009 [92,98]. Following their observations that “directly lethal” antimicrobial approaches resulted in rapid resistance development due to strong selective pressure, the authors overexpressed the nonessential gene *φlexA3* for repression of the SOS response as a means of killing bacterial pathogens without incurring phage resistance [92].

Phage engineering was recently employed for the first time in man for the treatment of extrapulmonary tuberculosis in a young lung transplant recipient with early success [17]. Whereas modifications may prompt unique regulations prior to commercial usage, advantages might outweigh drawbacks of phage engineering if antibacterial activity is augmented and/or selection of resistance blunted. Yet Citorik et al. caution against synthetic phage modifications, expressing concern about unintended consequences, including perhaps, attenuated fitness of phage that may abrogate its therapeutic potential [85]. Although the vast number of phages and the ability to generate novel phages through evolution and adaptation renders pan-resistance unlikely [101], caution should be exercised during experimentation to avoid the emergence of bacteria that are resistant to phage and antibiotics, known as “double-resistant variants” [102]. Any such potential consequences of viral genome modification might theoretically be minimized by incorporating synthetic kill switches [103]. It also stands to reason that engineered phages are as susceptible to continuous, directional evolution as wild-type phages, such that the changes they incur may not be observed in any fixed capacity.

5.1.1.2 Encapsulated Delivery: Nanoparticle delivery systems, such as liposome- or polyethylene glycol (PEG)-based platforms, represent another potential strategy to avoid human immune recognition and facilitate phage-bacterial interactions, although phage encapsulation may counterproductively preclude access to its bacterial receptor, resulting in decreased efficacy [93]. Esteban et al. have developed nano-emulsions that complex with phage to quench their negative charge and reduce electrostatic repulsion by bacteria for increased infectivity [104]. Encapsulation of phage may extend circulation in the body up to eight times that of wild-type phage and evade inactivation by neutralizing antibodies

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[105,106]. In a comparative study, biofilms were preferentially infected by liposome-encapsulated *versus* wild-type phage *in vitro*, especially as biofilm age increased; *in vivo*, liposome-encapsulated phage (KPO1K2) cleared *Klebsiella pneumoniae* B5055 from the lungs of mice in an experimental model when administered up to 72 hours post-infection, whereas un-encapsulated phage (KPO1K2) cleared infection only when administered no more than 6 hours post-infection [105,107]. In a distinct experimental setup, the same group demonstrated that liposome encapsulation of KPO1K2 (MOI=10) killed 95% of intracellular *K. pneumoniae* in *ex vivo* mouse macrophages after 24 hours compared with 21% killed with unencapsulated phage even at increased phage titer [107]. Chadha et al. 2017 similarly considered maintenance in bioactivity of phage when administered intraperitoneally (IP) within liposomes in a mouse *K. pneumoniae* infected burn model [106]. Concealment of phage from the immune system led to a circulation time of encapsulated phages six times that of un-encapsulated phages. Attenuated phage immunogenicity was observed via reduction of cytokine levels compared to baseline; there was 100% 4-day survival of liposome encapsulated phage-treated mice (n=12) when treatment was delayed by 24 hours post-infection compared with 0% survival of unencapsulated phage-treated mice. Lu and Koeris reported antibody neutralization of PEGylated phage in mice pre-vaccinated with phage, indicating suboptimal effects with repeated exposure to the same phage, even when concealed by PEG [98]. This conclusion is supported and extended by findings of Kim et al. in which prior exposure to wild-type or PEGylated A511 *Listeria* phage or Felix-O1 *Salmonella* phage in BALB/c mice resulted in >99% blood clearance of phage within 24 hours following secondary injection, regardless of encapsulation [108].

Hybrid nanoparticle systems have also been employed to optimize phage delivery, as in the case of Chhibber et al.'s use of a transferosome, a synthetic liposome containing phosphatidylcholine, to enhance permeability. Intramuscular (IM) administration of their phage cocktail unbound or transferosome-bound (MOI 10) 30 minutes post-infection with 10^7 cfu/mL of *S. aureus* rescued 100% of rats, whereas IM administration 12 hours post-infection rescued 100% of rats treated with transferosome-bound phage and 0% of rats treated with free phage at the study endpoint two weeks later [109].

The effect of encapsulation on phage immunogenicity is more nuanced. In one study, for instance, mice to which *K. pneumoniae* was intranasally administered were treated with liposome-encapsulated KPO1K2 phage; they exhibited a decrease in proinflammatory IL-1β and TNFα, but an increase in anti-inflammatory IL-10 compared to infected, unencapsulated phage-treated and infected, untreated controls [105]. Other groups utilizing PEG nanoparticles to deliver phage [104,108] collectively report a decreased T_h-1 response and decreased levels of inflammatory cytokines IFN-γ and IL-6, in addition to extended circulatory duration [93,100,108].

5.1.1.3 Phage Component-Based Therapies: While whole phages have been the historical focus of phagotherapy, the use of phage component-based molecules as antibacterial agents has been recently investigated. Such approach features simplicity of regulatory execution, and perhaps more limited bacterial resistance [110–113], prevention of genomic integration, rapid antibacterial effect even at low concentrations, and ease of quality control and storage measures relative to conventional phage therapy [114–119]. The dosing

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

regimen of phage components resembles that of small-molecule antibiotics and they also do not replicate in their bacterial hosts as whole phages do, enabling simplified administration [110]. Certain phage components may even directly enhance bacterial sensitivity to conventional antibiotics, such as PA-PP, a serine protease that degrades outer membrane porin protein in *P. aeruginosa* PAR50 [120]. A potential drawback of employing phage enzymes over conventional phage therapy is the technical difficulty of their acquisition [121].

Over the course of endogenous phage infection of a bacterium, expression of lysins during intracellular biosynthesis provides the means for dissemination of new virions as lysins bind peptidoglycan components from the interior face, perforating the cell wall leading to osmotic rupture. Vázquez and García describe the enzybiotic approach as lysins "repurposed," due to their extracellular, rather than intracellular, administration to effect the same lethal outcome [115]. Virulent phage enzymes were recently named the most promising alternatives to antibiotics by a pipeline portfolio review in *Lancet Infectious Disease* [113]. In fact, enzymes secreted over the course of the phage's life cycle have in recent decades been considered for use as antibacterial agents in place of conventional phage therapy. Two clinically-relevant classes of phage enzymes include depolymerases and endolysins (also known as lysins or peptidoglycan hydrolases [PGHs]). To a lesser extent, the prospect of utilizing "small chemical molecules" that mediate initial phage binding has been envisaged owing to their bacteriostatic effects [113]. Waseh et al. isolated the tailspike protein, P22sTsp, of lytic phage P22, which plays a role in docking P22 at its bacterial receptor. Three doses of P22sTsp were administered to chicks beginning 1 or 18 hours after initial oral challenge with *Salmonella enterica* serovar Typhimurium and every 24 hours thereafter [122]. P22sTsp caused aggregation of *S. enterica* serovar Typhimurium concomitant with significant reductions in bacterial density of the liver, spleen and cecum following the final, third dose of P22sTsp among animals initially treated 1 hour post-infection *versus* sham-treated controls, potentially suggesting an immune-enhancing mechanism underlying phage activity resembling antibody-mediated agglutination. While the precise mechanism of action is unknown, it is speculated that allosteric modulation of O antigen following phage-bacterial complexing impedes the organism's motility [122].

Endolysins are enzymes produced by double-stranded DNA phages that hydrolyze the cell wall of bacteria. Those targeting Gram-positive bacteria are generally composed of two domains, an enzymatically active domain (EAD) and a cell wall binding domain (CBD) [123]. In contrast, most endolysins targeting Gram-negative bacteria lack a CBD [119], which reflects the presence of the intervening outer membrane; this structure generally limits the efficacy of lysins against these organisms, with exceptional lysins exhibiting amphipathic components capable of traversing the outer membrane [116]. In the same vein, Gram-negative lysins may be synthetically optimized by fusion with outer membrane permeabilizers, as in the case of Artilysin® [113,124,125]. Some lysins harbor two distinct EADs, which may minimize resistance development. As agents that may induce the phenomenon known as "lysis from without" in high concentrations, bacterial entry is not required [126]; their exclusive interaction at the bacterial surface may mitigate resistance development [111]. Their enzymatic activity may promote removal of bacterial structures such as extracellular polymeric substance that may otherwise be unaffected by conventional

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

antimicrobial agents [113]. Lysins are immunogenic due to their protein composition; the dose administered must account for their rapid removal from the circulation [110]. They may also display synergy when combined with antibiotics or one another, or enhance complement-mediated activity of the innate immune system [112,123,127,128] (Table 2).

Recombinant lysin systems have also been developed, typically capitalizing on protein engineering technology to modify the lysin gene product with the purpose of increasing its spectrum of activity (spanning genera, in some instances [125]), or increasing its circulation time in order to reach the infected site [113,115]. To better address *Streptococcus pneumoniae* infections, Blazquez et al. synthesized a chimeric lysin comprised of natural phage lysins Pal and LytA, both of which are *N*-acetylmuramoyl-L-alanine-amidases with bactericidal activity against *S. pneumoniae*, with the latter representing the major pneumococcal autolysin [129]. The recombination of the Pal catalytic domain with the consolidated CBD of LytA formed the PL3 chimeric endolysin, resulting in a reduction of all 10 tested choline-containing Gram-positive bacterial strains including *S. pneumoniae* and other streptococci *in vitro*; Pal and LytA were not tested alone, though other studies have demonstrated comparatively reduced potency and efficacy. Application of PL3 in a zebrafish (*Danio rerio*) embryo *S. pneumoniae* D39 infection model reduced mortality to 0%, from 40% in non-treated, infected embryos [129]. Daniel et al. developed a chimeric lysin, ClyS, in which the N-terminal domain of the Twort phage was fused with the C-terminal cell wall targeting domain of φNM3, which lacks Sh3b domain binding peptidoglycan peptide bridges, to which bacteria may easily become resistant [112]. Others have considered cleaving the C-terminal to increase lysis, as the binding component precludes the catalytic subunit from functioning [130]. In one of the largest comparative studies of recombinant lysins, Schmelcher et al. reported that six of the nine PGH compounds administered IP 30 minutes after IP challenge of BALB/c mice with MRSA NRS382 exhibited similar activity to vancomycin [131]. The experimental success of recombinant lysin technology has piqued the interest of the pharmaceutical industry. Zhang et al. demonstrated activity of phage IME-EF1 endolysin against enterococci *in vitro*; this phage was active against four of 20 strains tested, whereas treatment with its endolysin killed 11 strains [132]. Subcutaneous implantation of a catheter colonized with *A. baumannii* and subsequently treated with PlyF307 endolysin resulted in a population reduction of 2 logs by 28 hours later, with the same endolysin able to clear bacterial EPS *in vitro* [124]. Lastly, Hathaway and colleagues loaded either a combination of endolysin $CHAP_K$ and lysostaphin into poly(*N*-isopropylacrylamide) (PNIPAM)-based vesicles creating a thermoresponsive polymer-based approach which allowed for the ejection of antimicrobial cargo following phase transition induced by inflammation-associated heat release for topical administration to staphylococcal infections [133,134]. *In vitro* testing demonstrated a 0.5 fold reduction in bacterial density at 32°C in PNIPAM-encapsulated *versus* unencapsulated cocktail, but a fourfold reduction in cell density at 37°C in PNIPAM-encapsulated *versus* unencapsulated cocktail [133].

Table 2 summarizes experimental animal and moth larval studies involving lysins. Examples of endolysin products under development include Tonabacase (Intron Biotechnology, Inc.), a *S. aureus*-specific endolysin currently in a phase II clinical trial for IV administration in bacteremia [135], and Exebacase (previously CF-301, ContraFect) which in 2018 completed a phase II clinical trial of IV administration for treatment of *S. aureus* bacteremia and

endocarditis [136,137]. Results of the trial demonstrated that combination therapy of Exebacase and standard of care antibiotics generated a significant increase in response rate *versus* antibiotics alone in patients with MRSA bacteremia or endocarditis [137].

5.2 Pharmacologic Optimization

5.2.1 Phage Cocktails—The application of phage 'cocktails' comprised of phages bearing divergent mechanisms of action may increase the formulation's spectrum of activity and decrease the potential for development of resistance [138]. Experts have advised that cocktails be comprised of between two and 10 phages [24,139]. The *in vitro* study of a cocktail of phage DRA88 and phage K containing distinct host spectra showed activity against 74% of 95 *S. aureus* isolates tested in the planktonic state, with individual applications being active against only 60 and 64%, respectively, as measured by spot plating, a modification of the double overlay plaque assay [140]. *In vitro* population reduction of phage cocktail-treated *S. aureus* was observed for all three isolates tested (15981, MRSA 252, H325), with a range of MOIs achieving similar effects, although lower MOIs required an extended time period to do so [140]. Nonetheless, phage formulation via cocktails is not a foolproof antibacterial strategy; if each phage in a cocktail is not maintained at a sufficient dose, or if constituent phages do not target the same bacteria, resistance may occur as readily as it would with single phages [141]. The majority of clinical trials evaluating phage therapy past and present have been administered as cocktails rather than individual viruses (Table 3).

5.2.2 Combination Therapy—Phages have also been combined with other antimicrobial agents, including antibiotics, with considerable success. One objective of combination therapy is to reduce the mutant selection window, or the range in drug concentration spanning the minimum inhibitory concentration and the mutant prevention concentration, to minimize resistance-selecting population expansion in the presence of the therapeutic [142]. Combination therapy using phage may be pursued with natural substances and/or antibiotics. First described in 2007, phage antibiotic synergy (PAS) is a phenomenon wherein the antibacterial effect observed following the combination of phage with sub-inhibitory concentrations of antibiotic enhances phage activity [143,144]. Johnson and Garcia evaluated hydrogel-embedded MR-5 phage, linezolid, or a combination thereof for their abilities to inhibit MRSA biofilm formation *in vitro*. Combination therapy resulted in significant reductions in bacterial colonization *versus* no treatment or phage or linezolid alone, and was associated with lower mutation rates compared with those observed in the monotherapy groups [145]. The binding of phage to surface receptors that contribute to bacterial pathogenicity can diminish bacterial virulence and/or lead to selection against receptor expression among progeny, resulting in comparatively treatable infection, as discussed above [143]. Combination therapy was shown to limit the acquisition of φSan23 phage resistance to ~33% of a *S. enterica* subsp. *enterica* serovar Enteritidis population, while treatment with phage alone resulted in phage resistance in ~90% of the population [36]. Higher drug concentrations combined with phages do not always support greater antibacterial effects since phages require a certain bacterial density (replication threshold) before phage replication can occur [143]. Recent work from Kim et al. revealed the highly-anticipated mechanism underlying some forms of PAS to be cellular expansion mediated by DNA damage response pathways in the presence of antibiotic or other environmental

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

stressors, including, but not limited to, reactive oxygen species [146]. Phages in combination with antibiotics may also, however, elicit additive, antagonistic, or facilitative effects (the last being an effect in which combination therapy generates greater activity than either agent alone but less activity than the sum of the effects of the individual agents) beside synergistic effects [143].

PAS is observed in biofilms as well as planktonic cultures, as demonstrated by Akturk et al. in the treatment of mono- and dual-species biofilms with *in vitro* phage, antibiotics, or combination therapy administered simultaneously or staggered [147]. They report that treatment of 48 hour monospecies biofilms with phage for 6 hours, MIC or 8 x MIC concentrations of gentamicin, ciprofloxacin, and meropenem for 24 hours, combination phage and MIC or 8 x MIC concentrations of gentamicin, ciprofloxacin, or meropenem for 24 hours, or combination phage (for 6 hours) and MIC or 8 x MIC concentrations of gentamicin, ciprofloxacin, or meropenem for 18 hours thereafter led to statistical reductions in *P. aeruginosa* biofilm-associated bacteria, while significant reductions were only seen in *S. aureus* biofilms treated with phage and ciprofloxacin or gentamicin at MIC and 8 x MIC concentrations. Overall, combination treatment of phage for six hours followed by 18 hours gentamicin (MIC or 8 x MIC) or ciprofloxacin (8 x MIC) supported the greatest reduction in *P. aeruginosa* monospecies biofilm-associated bacterial density.

In 48 hour dual-species biofilms, simultaneous combination therapy did not yield synergistic activity. Sequential combination therapy of EPA1 followed 6 hours later by gentamicin (MIC) yielded anti-biofilm synergy against *P. aeruginosa* constituents while *S. aureus* density was not significantly altered.

Although phage antibiotic synergy classically implicates lytic phages, recent data proposes that a similar phenomenon may occur by temperate phages. Population modelling suggests that possession of a prophage may render its *P. aeruginosa* host more sensitive to antibiotics, even if the host is antibiotic-resistant, as antibiotic exposure may place stress on the bacterium driving induction of temperate phage and resulting in cell lysis [148].

Overall, combination therapies offer a potentially effective, though complex, solution to phage resistance.

5.2.3 Selective Administration—Adjusting therapeutic phage formulations has been shown to increase biological stability of phage as well as increase the dose that reaches the site(s) of infection. In a cohort of pediatric patients with pulmonary infection, Leung et al. evaluated the impact on phage activity in spray-dried *versus* spray freeze-dried formulations. Superior results were found with the latter, which is able to accommodate larger viral loads, although some damage was observed to phage through the delivery process, dependent to an extent on the phage utilized [149]. The addition of L-leucine and trehalose excipients increased stability and bioactivity of phage solutions targeting *P. aeruginosa*, increasing the mouth-throat fraction to 58%, and preserving properties exhibited by phage prior to drying [150]. Results were corroborated in a similar study subjecting *P. aeruginosa* and *Burkholdheria cenocepacia* phages to spray-drying [151]. In a similar manner, antacids may be used to neutralize gastric acidity, allowing phage transit following oral administration

[152]. Likewise, dosing schedules may be designed to deliver higher phage titers at fewer time points to minimize development of antibodies and/or avoid consequences of antigen-antibody binding [102].

Phage therapy may not be ideal for every infection. Administration into various body fluids may functionally reduce viral titers via biochemical interactions. For instance, serum, but not albumin or fibrinogen [153], inhibits phage-bacterial relations, and, as mentioned above, phages administered orally may be inactivated by the acidity of the gastric compartment [154]. Further, not all bacteria are equally susceptible to infection by lytic phage, at least those discovered by extant detection methods, suggesting the need for improved "phage hunting" methods [155]. Indeed, for some microorganisms, such as *C. difficile*, only a small number of lytic phages have been identified. Nevertheless, efforts to identify additional phages or lysing targeting *C. difficile* are underway [50,156–159].

6. Conclusion

While experimental findings suggest promise for the safety and, in some cases, efficacy of phage therapy, many questions remain [160]. Improved *in vivo* models and human studies are needed to better understand selection of phage resistance, and to inform pharmacokinetics and pharmacodynamics of phage and its interactions with human tissues, in the context of human phage therapy [161]. As with other aspects of this highly individualized treatment regimen, the precise effects of phage administration upon the prokaryotic and eukaryotic milieu to which it is introduced is likely to be phage species-dependent [88,162,163].

7. Expert opinion

Although multiple factors led to the discontinuation of therapeutic phage use in Western medicine until recently, the impending antimicrobial resistance crisis justifies its reconsideration. Moreover, the authors contend that the role of phage therapy may not be limited to cases of dire resistance, but that phages may be considered much earlier in a patient's therapeutic course, perhaps even as first-line therapy, allowing for the preservation of conventional antibiotics. This would represent a major paradigm-shift in the treatment of infections with the potential for a significant impact on reducing antibiotic usage. Phage therapy therefore should be seen as not only a reaction to antibiotic resistance, but more significantly, a way to address a root cause of development of resistance.

The authors encourage well-documented, well-controlled clinical trials of phage (and/or phage products) to more fully establish the disease(s) against which they may be beneficial, how best to select appropriate phages (and/or phage products), how best to dose and administer them, their potential interaction with the immune system and its impact on therapeutic outcome, and the selection of phage (and/or phage product) resistance.

As with other newly emerging individualized therapies, phage therapy also has the potential to challenge the current pathways by which drugs are regulated. Individualized gene and cell therapies call for a re-evaluation of current regulatory processes, and bring to light new ethical and safety questions.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

7.1 Novel technologies beget novel potential

Although the current 're-discovery' of therapeutic phages exemplifies an unusual narrative in scientific discovery, the present is an ideal time for the revitalization of phage research [63] given recent technological advancements that afford new experimental possibilities. For instance, the past decade has witnessed the refinement of animal models through which to investigate preclinical utility, including use of the greater wax moth (*Galleria mellonella*) as a small, cost-effective, IACUC-exempt organism that is easy to maintain and possesses an immune system analogous to that of humans [164]. The sequencing revolution, the advent of -omics technologies, the emergence of the multidisciplinary field of systems biology, as well as the breadth of molecular [165–167] and visualization techniques [168–170] all poise the field to address unanswered questions asked a century ago which, in many ways, precluded the possibility of evidence-based clinical application [98,165]. For example, genome sequencing of candidate phages can now provide information regarding their ability to lysogenize bacteria and therefore to mobilize or enhance production of virulence factors [171]. Genome engineering technologies have afforded the expression of phage recombinases in bacteria that indicate prior phage exposure under laboratory conditions by way of altered gene expression [85]. Computational prediction models using inter-species genomics data through the use of machine learning is a promising method by which phage-bacteria pairs can be identified rapidly and on a large scale [172].

Systems biology has also lent contributions which have included the development of tools that accelerate phage adaptation to maintain relevance as a bacterial predator. Insights to be gleaned from this approach include determination of phage predation rates amd phage behavior in diverse, multicellular communities [85]. While laboratory experiments are needed to confirm the veracity of *in silico* predictions, modeling represents a powerful tool for exploring ecological parameters governing phage-bacterial interactions in a low-risk environment prior to *in vitro* and *in vivo* experimentation [36,142].

7.2 The need for more, better preclinical and clinical studies

We recognize that the greatest impediment to standardized phagotherapy is well-controlled clinical trials. The lack of randomized controlled trial data and the complexity of regulatory restrictions required has driven much of the recent human data generation to be from compassionate use cases. However, case reports and series often include the concomitant use of other antimicrobial agents given the primary clinical objective of positive patient outcomes [11]. This kind of approach can make it difficult to draw conclusions as to the effects of phage therapy alone. Additional insight is critical for the determination of how best to select appropriate phages or phage enzymes, as well as how to dose and administer them; pharmacokinetic and pharmacodynamic properties must also be better defined. Perhaps, randomized controlled clinical trials performed in well-defined infectious diseases (even if not caused by drug-resistant bacteria) should be considered. As an unconventional approach, bioethicists Anomaly and Savulescu suggest recruiting healthy individuals to a representative population sample for infectious diseases studies in which participants are challenged with a pathogen and subsequently administered an experimental treatment regimen, and compensating them fairly for the potentially high risks of involvement [173].

If shown to be efficacious, a goal of present and future research vis-à-vis phage therapy will be to develop a pipeline through which phage(s) targeting individual patients' bacterial isolates can be identified and administered as primary or adjunctive therapy, or to identify appropriate phage cocktails. Some countries have begun this process already: The Queen Astrid Military Hospital in Brussels, Belgium has published standardized guidelines for phage treatment in the setting of osteomyelitis, while Germany's Phage 4 Cure initiative aims to develop Good Manufacturing Practice (GMP)-grade processes for the assimilation of phage therapy into the regulatory confines of Western medicine expected to meet "international quality standards" [12]. Nevertheless, several challenges remain, including the creation of clinical laboratory standards for phage testing and appropriate regulation for such a potentially individualized anti-infective approach [174].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Larry J. Prokop, MLS, for his careful search and compilation of source material from several medical databases and support throughout the writing and revision process, and to Kerryl Greenwood-Quaintance, MS, proofreading the manuscript.

Funding

R Patel is supported by UM1 AI104681, R01 AR056647, R01 AI091594, R01 AI134770 and R21 AI125870. K M Caflisch is supported by T32 AR056950.

Declaration of interest

R Patel has research grant support from ContraFect, a company which has financial interest in the subject matter discussed in this manuscript. In addition, she reports other relationships not directly relevant to the subject matter discussed in this manuscript, including research grant support from CD Diagnostics, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, ContraFect, TenNor Therapeutics Limited and Shionogi, serving a consultant to Curetis, Specific Technologies, Next Gen Diagnostics, PathoQuest, Selux Diagnostics and Qvella (all monies are paid to Mayo Clinic), and receiving travel reimbursement from ASM and IDSA, an editor's stipend from IDSA, and honoraria from the NBME, Up-to-Date and the Infectious Diseases Board Review Course. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. Guttman B, Raya R, Kutter E. Basic phage biology In: Kutter E, Sulakvelidze A, editors. Bacteriophages: Biology and applications: CRC Press; 2004.
2. Summers W History of phage research and phage therapy In: Waldor M, DI F,SL A, editors. Phages: their role in bacterial pathogenesis and biotechnology: ASM Press; 2005 p. 3–17.
3. Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens [Review]. Virulence. 2014;5(1): 209–218.
4. Chanishvili N Bacteriophages as therapeutic and prophylactic means: summary of the Soviet and post Soviet experiences [Review]. Curr Drug Deliv. 2016 01 5;13(3):309–323. [PubMed: 27090515]
5. On an invisible microbe antagonistic to dysentery bacilli. Note by d'Herelle MF, presented by Roux M Comptes Rendus Academie des Sciences 1917; 165:373–5. Bacteriophage. 2011 2011/01/01;1(1):3–5.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

6. Fruciano E, Bourne S. Phage as an antimicrobial agent: d'Herelle's heretical theories and their role in the decline of phage prophylaxis in the West [Conference Paper]. Can J Infect Dis Med. 2007 Jan-Feb;18(1):19–26.
7. Summers WC. Bacteriophage research: early history In: Kutter E, Sulakvelidze A, editors. Bacteriophages: biology and applications. 1 ed: CRC Press; 2004 p. 5–27.
8. Abedon ST, Kuhl SJ, Blasdel BG, et al. Phage treatment of human infections [Review]. Bacteriophage. 2011 Mar-Apr;1(2):66–85. [PubMed: 22334863]
9. Summers WC. History of phage research and phage therapy In: Waldor MK, Friedman DI, Adhya SL, editors. Phages: their role in bacterial pathogenesis and biotechnology. Washington, D.C.: ASM Press; 2005 p. 3–17.
10. Vivas R, Barbosa AAT, Dolabela SS, et al. Multidrug-resistant bacteria and alternative methods to control them: an overview. Microb Drug Resist. 2019 Jul-Aug;25(6):890–908. [PubMed: 30811275]
11. Tkhilaishvili T, Winkler T, Muller M, et al. Bacteriophages as adjuvant to antibiotics for the treatment of periprosthetic joint infection caused by multidrug-resistant Pseudomonas aeruginosa [Journal Article]. Antimicrob Agents Chemother. 2019 9 16;16:16.
12. Onsea J, Soentjens P, Djebara S, et al. Bacteriophage application for difficult-to-treat musculoskeletal infections: development of a standardized multidisciplinary treatment protocol. Viruses. 2019;11(10).
13. Maddocks S, Petrovic Fabijan A, Ho J, et al. Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by Pseudomonas aeruginosa. Am J Respir Crit Care Med. 2019;200(9):1179–1181. [PubMed: 31437402]
14. Schooley RT, Biswas B, Gill JJ, et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. Antimicrob Agents Chemother. 2017;61(10).
15. Chan BK, Turner PE, Narayan D, et al. Phage treatment of an aortic graft infected with Pseudomonas aeruginosa. Evol Med Public Health. 2018;2018(1):60–66. [PubMed: 29588855]
16. Law N, Logan C, Yung G, et al. Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant Pseudomonas aeruginosa infection in a cystic fibrosis patient. Infection. 2019 5 17.
17. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med. 2019 05;25(5):730–733. [PubMed: 31068712]
18. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. Lancet Infect Dis. 2019;19(1):35–45. [PubMed: 30292481]
19. Sarker SA, Sultana S, Reuteler G, et al. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. EBioMedicine. 2016 01 2;4:124–137. [PubMed: 26981577]
20. Jennes S, Merabishvili M, Soentjens P, et al. Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas aeruginosa septicaemia in a patient with acute kidney injury-a case report. Crit Care. 2017;21(1):129–129. [PubMed: 28583189]
21. Rhoads DD, Wolcott RD, Kuskowski MA, et al. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care. 2009 6;18(6):237–8, 240–3. [PubMed: 19661847]
22. Azam AH, Tanji Y. Bacteriophage-host arm race: an update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy. Appl Microbiol Biotechnol. 2019 3;103(5):2121–2131. [PubMed: 30680434]
23. Taylor VL, Fitzpatrick AD, Islam Z, et al. The diverse impacts of phage morons on bacterial fitness and virulence. Adv Virus Res. 2019;103:1–31. [PubMed: 30635074]
24. Yuan Y, Wang L, Li X, et al. Efficacy of a phage cocktail in controlling phage resistance development in multidrug resistant Acinetobacter baumannii. Virus Res. 2019 15 10;272(197734).
25. Chan BK, Sistrom M, Wertz JE, et al. Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. Sci Rep. 2016 05/26/online;6:26717.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

26. Ho K, Huo W, Pas S, et al. Loss-of-function mutations in epaR confer resistance to ϕNPV1 infection in Enterococcus faecalis OG1RF. Antimicrob Agents Chemother. 2018;62(10):e00758–18. [PubMed: 30104266]
27. Sumrall ET, Shen Y, Keller AP, et al. Phage resistance at the cost of virulence: Listeria monocytogenes serovar 4b requires galactosylated teichoic acids for InlB-mediated invasion. PLoS Pathog 2019 01 10;15(10):e1008032. [PubMed: 31589660]
28. Scanlan PD, Buckling A, Hall AR. Experimental evolution and bacterial resistance: (co)evolutionary costs and trade-offs as opportunities in phage therapy research. Bacteriophage. 2015;5(2).
29. Chatterjee A, Johnson CN, Luong P, et al. Bacteriophage resistance alters antibiotic-mediated intestinal expansion of enterococci. Infect Immun. 2019;87 (6)(e00085–19). [PubMed: 30936157]
30. Capparelli R, Nocerino N, Lanzetta R, et al. Bacteriophage-resistant Staphylococcus aureus mutant confers broad immunity against staphylococcal infection in mice. PLoS One. 2010;5(e11720). [PubMed: 20661301]
31. Kvachadze L, Balarjishvili N, Meskhi T, et al. Evaluation of lytic activity of staphylococcal bacteriophage Sb-1 against freshly isolated clinical pathogens. Microb Biotechnol. 2011 9;4(5): 643–650. [PubMed: 21481199]
32. Obeng N, Pratama AA, Elsas JDV. The significance of mutualistic phages for bacterial ecology and evolution [Review]. Trends Microbiol. 2016 01 6;24(6):440–449. [PubMed: 26826796]
33. Osada K, Takeuchi I, Miyanaga K, et al. Coevolution between Staphylococcus aureus isolated from mastitic milk and its lytic bacteriophage ΦSA012 in batch co-culture with serial transfer. Biochem Eng J. 2017;126:16–23.
34. Kashiwagi A, Yomo T. Ongoing phenotypic and genomic changes in experimental coevolution of RNA bacteriophage Qβ and Escherichia coli. PLoS Genet. 2011;7(8):e1002188–e1002188. [PubMed: 21829387]
35. Le S, Yao X, Lu S, et al. Chromosomal DNA deletion confers phage resistance to Pseudomonas aeruginosa. Sci Rep-UK. 2014;4:4738.
36. Holguin AV, Cardenas P, Prada-Penaranda C, et al. Host resistance, genomics and population dynamics in a Salmonella Enteritidis and phage system. Viruses. 2019 2 22;11(2).
37. Debarbieux L Bacterial sensing of bacteriophages in communities: the search for the Rosetta stone [Review]. Curr Opin Microbiol. 2014 8;20:125–130. [PubMed: 24952283]
38. Chadha P, Katare OP, Chhibber S. In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice [Journal Article]. Microb Pathog. 2016 10;99:68–77. [PubMed: 27498362]
39. Azam AH, Kadoi K, Miyanaga K, et al. Analysis host-recognition mechanism of staphylococcal kayvirus SA039 reveals a novel strategy that protects Staphylococcus aureus against infection by Staphylococcus pseudintermedius Siphoviridae phages. Appl Microbiol Biotechnol. 2019 16 8;103(16):6809–6823. [PubMed: 31236618]
40. Fallico V, Ross RP, Fitzgerald GF, et al. Genetic response to bacteriophage infection in Lactococcus lactis reveals a four-strand approach involving induction of membrane stress proteins, D-alanylation of the cell wall, maintenance of proton motive force, and energy conservation. J Virol. 2011 11;85(22):12032–12042. [PubMed: 21880765]
41. Bera A, Biswas R, Herbert S, et al. The presence of peptidoglycan O-acetyltransferase in various staphylococcal species correlates with lysozyme resistance and pathogenicity. Infect Immun. 2006 8;74(8):4598–604. [PubMed: 16861647]
42. Sijtsma L, Wouters JT, Hellingwerf KJ. Isolation and characterization of lipoteichoic acid, a cell envelope component involved in preventing phage adsorption, from Lactococcus lactis subsp. cremoris SK110. J Bacteriol. 1990 12;172(12):7126–30. [PubMed: 2123864]
43. Bru JL, Rawson B, Trinh C, et al. PQS produced by the Pseudomonas aeruginosa stress response repels swarms away from bacteriophage and antibiotics. J Bacteriol. 2019;26.
44. Sneppen K, Semsey S, Seshasayee ASN, et al. Restriction modification systems as engines of diversity. Front Microbiol. 2015;6 6(528).
45. Stern A, Sorek R. The phage-host arms race: shaping the evolution of microbes [Review]. BioEssays. 2011 1;33(1):43–51. [PubMed: 20979102]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

46. Korona R, Levin BR. Phage-mediated selection and the evolution and maintenance of restriction-modification. Evolution. 1993 4;47(2):556–575. [PubMed: 28568719]
47. Seed KD. Battling phages: how bacteria defend against viral attack. PLoS Pathog. 2015 6;11(6):e1004847. [PubMed: 26066799]
48. Ofir G, Melamed S, Sberro H, et al. DISARM is a widespread bacterial defence system with broad anti-phage activities. Nat Microbiol. 2018 1;3(1):90–98. [PubMed: 29085076]
49. Fineran PC, Blower TR, Foulds IJ, et al. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. P Natl Acad Sci USA. 2009 20 1;106(3):894–899.
50. Dalmasso M, Hill C, Ross RP. Exploiting gut bacteriophages for human health [Review]. Trends Microbiol. 2014 7;22(7):399–405. [PubMed: 24656964]
51. Goldfarb T, Sberro H, Weinstock E, et al. BREX is a novel phage resistance system widespread in microbial genomes. EMBO J. 2015;34(2):169–183. [PubMed: 25452498]
52. Jakutyte-Giraitiene L, Gasiunas G. Design of a CRISPR-Cas system to increase resistance of Bacillus subtilis to bacteriophage SPP1. J Ind Microbiol Biot. 2016 01 8;43(8):1183–1188.
53. Makarova KS, Wolf YI, van der Oost J, et al. Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements. Biol Direct. 2009 8 25;4:29. [PubMed: 19706170]
54. Chan BK, Abedon ST. Phage therapy pharmacology: phage cocktails. Adv Appl Microbiol. 2012;78:1–23. [PubMed: 22305091]
55. Abedon ST, Thomas-Abedon C. Phage therapy pharmacology [Review]. Curr Pharm Biotechnol. 2010 1;11(1):28–47. [PubMed: 20214606]
56. Kumaran D, Taha M, Yi Q, et al. Does treatment order matter? Investigating the ability of bacteriophage to augment antibiotic activity against Staphylococcus aureus biofilms. Front Microbiol. 2018;9:127. [PubMed: 29459853]
57. Santiago-Rodriguez TM, Hollister EB. Human virome and disease: high-throughput sequencing for virus discovery, identification of phage-bacteria dysbiosis and development of therapeutic approaches with emphasis on the human gut. Viruses. 2019 7;11 (7)(656).
58. Shan J, Ramachandran A, Thanki AM, et al. Bacteriophages are more virulent to bacteria with human cells than they are in bacterial culture; insights from HT-29 cells. Sci Rep. 2018 23 3;8(1): 5091. [PubMed: 29572482]
59. Smith LL, Buckley R, Lugar P. Diagnostic immunization with bacteriophage ΦX 174 in patients with common variable immunodeficiency/hypogammaglobulinemia. Front Immunol. 2014;5:410–410. [PubMed: 25221555]
60. Lusiak-Szelachowska M, Zaczek M, Weber-Dabrowska B, et al. Phage neutralization by sera of patients receiving phage therapy. Viral Immunol. 2014;27(6):295–304. [PubMed: 24893003]
61. Vandenheuvel D, Lavigne R, Brussow H. Bacteriophage therapy: advances in formulation strategies and human clinical trials [Review]. Ann Rev Virol. 2015 09 11;2:599–618. [PubMed: 26958930]
62. Abul-Hassan HS E-TkMB, Gomaa R. Bacteriophage therapy of Pseudomonas burn wound sepsis. Ann Burns Fire Disasters. 1990;3(4).
63. Borysowski J, Gorski A. Is phage therapy acceptable in the immunocompromised host? [Short Survey]. Int J Infect Dis. 2008 9;12(5):466–471. [PubMed: 18400541]
64. Fortuna W, Miedzybrodzki R, Weber-Dabrowska B, et al. Bacteriophage therapy in children: facts and prospects [Review]. Med Sci Monit. 2008 8;14(8):RA126–RA132. [PubMed: 18668009]
65. Van Belleghem JD, Dabrowska K, Vaneechoutte M, et al. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses. 2018 12 25;11(1).
66. Dufour N, Delattre R, Ricard JD, et al. The lysis of pathogenic Escherichia coli by bacteriophages releases less endotoxin than by β-lactams. Clin Infect Dis. 2017 6 1;64(11):1582–1588. [PubMed: 28329379]
67. Dufour N, Clermont O, La Combe B, et al. Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b: H4 Escherichia coli clonal complex. J Antimicrob Chemother. 2016 01 11;71(11):3072–3080. [PubMed: 27387322]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

68. Van Belleghem JD, Clement F, Merabishvili M, et al. Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by Staphylococcus aureus and Pseudomonas aeruginosa phages. Sci Rep. 2017 8 14;7(1):8004. [PubMed: 28808331]
69. Zhang L, Hou X, Sun L, et al. Corrigendum: Staphylococcus aureus bacteriophage suppresses LPS-induced inflammation in MAC-T bovine mammary epithelial cells. Front Microbiol. 2018;9:2511–2511. [PubMed: 30450085]
70. Zhang L, Hou X, Sun L, et al. Staphylococcus aureus bacteriophage suppresses LPS-induced inflammation in MAC-T bovine mammary epithelial cells. Front Microbiol. 2018;9:1614–1614. [PubMed: 30083140]
71. Gogokhia L, Buhrke K, Bell R, et al. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. Cell Host Microbe. 2019 2 13;25(2):285–299.e8. [PubMed: 30763538]
72. Dufour N, Delattre R, Chevallereau A, et al. Phage therapy of pneumonia is not associated with an overstimulation of the inflammatory response compared to antibiotic treatment in mice. Antimicrob Agents Chemother. 2019;63 (8)(e00379–19). [PubMed: 31182526]
73. Abd El-Aziz AM, Elgaml A, Ali YM. Bacteriophage therapy increases complement-mediated lysis of bacteria and enhances bacterial clearance after acute lung infection with multidrug-resistant Pseudomonas aeruginosa. J Infect Dis. 2019;219(9):1439–1447. [PubMed: 30476337]
74. Qadir MI. Phage therapy: a modern tool to control bacterial infections [Review]. Pak J Pharm Sci. 2015 01 1;28(1):265–270. [PubMed: 25553704]
75. Lusiak-Szelachowska M, Zaczek M, Weber-Dabrowska B, et al. Antiphage activity of sera during phage therapy in relation to its outcome. Future Microbiol. 2017 2;12(2):109–117. [PubMed: 27643529]
76. Bentancor LV, Bilen MF, Mejias MP, et al. Functional capacity of Shiga-toxin promoter sequences in eukaryotic cells. PLoS One. 2013;8(2):e57128. [PubMed: 23451160]
77. Bentancor LV, Mejias MP, Pinto A, et al. Promoter sequence of Shiga toxin 2 (Stx2) is recognized in vivo, leading to production of biologically active Stx2. MBio. 2013 10 1;4(5):e00501–13. [PubMed: 24085779]
78. Lengeling A, Mahajan A, Gally DL. Bacteriophages as pathogens and immune modulators? mBio. 2013;4 (6)(e00868–13). [PubMed: 24222490]
79. Sweere JM, Van Belleghem JD, Ishak H, et al. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. Science. 2019;363(6434):eaat9691. [PubMed: 30923196]
80. Howard-Varona C, Hargreaves KR, Abedon ST, et al. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. ISME J. 2017;11(7):1511–1520. [PubMed: 28291233]
81. Merril CR, Biswas B, Carlton R, et al. Long-circulating bacteriophage as antibacterial agents. Proc Natl Acad Sci USA. 1996;93(8):3188–3192. [PubMed: 8622911]
82. Vieu J Interet des bacteriophages dans le traitement de staphylococcies. Vie Med. 1961;42:823–829. [PubMed: 13781256]
83. Botka T, Pantucek R, Maslanova I, et al. Lytic and genomic properties of spontaneous host-range Kayvirus mutants prove their suitability for upgrading phage therapeutics against staphylococci. Sci. 2019 02 4;9(1):5475.
84. Burrowes BH, Molineux IJ, Fralick JA. Directed in vitro evolution of therapeutic bacteriophages: the Appelmans Protocol. Viruses. 2019;11(3).
85. Citorik RJ, Mimee M, Lu TK. Bacteriophage-based synthetic biology for the study of infectious diseases [Review]. Curr Opin Microbiol. 2014 6;19(1):59–69. [PubMed: 24997401]
86. Sybesma W, Zbinden R, Chanishvili N, et al. Bacteriophages as potential treatment for urinary tract infections. Front Microbiol. 2016 11 4;7 Apr(465).
87. Goodridge LD. Designing phage therapeutics [Review]. Curr Pharm Biotechnol. 2010 1;11(1):15–27. [PubMed: 20214605]
88. Serwer P, Wright ET, Lee JC. High murine blood persistence of phage T3 and suggested strategy for phage therapy. BMC Res Notes. 2019;12(1):560–560. [PubMed: 31488211]
89. Diez-Martinez R, De Paz HD, Garcia-Fernandez E, et al. A novel chimeric phage lysin with high in vitro and in vivo bactericidal activity against Streptococcus pneumoniae. J Antimicrob Chemother. 2014 12 11;70(6):1763–1773.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

90. Weber W, Fussenegger M. Emerging biomedical applications of synthetic biology [Review]. Nat Rev Genet. 2012 1;13(1):21–35.
91. Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections [Review]. Front Pharmacol. 2019;10 (5)(513).
92. Lu TK, Collins JJ. Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. P Natl Acad Sci USA. 2009 24 3;106(12):4629–4634.
93. Viertel TM, Ritter K, Horz HP. Viruses versus bacteria-novel approaches to phage therapy as a tool against multidrug-resistant pathogens. J Antimicrob Chemother. 2014 9;69(9):2326–2336. [PubMed: 24872344]
94. Puiu M, Julius C. Bacteriophage gene products as potential antimicrobials against tuberculosis [Review]. Biochem Soc Trans. 2019;47(3):847–860. [PubMed: 31085613]
95. Pouillot F, Blois H, Iris F. Genetically engineered virulent phage banks in the detection and control of emergent pathogenic bacteria. Biosecur Bioterror. 2010 01 6;8(2):155–169. [PubMed: 20569057]
96. Vitiello CL, Merril CR, Adhya S. An amino acid substitution in a capsid protein enhances phage survival in mouse circulatory system more than a 1000-fold. Virus Res. 2005 12;114(1–2):101–3. [PubMed: 16055223]
97. Paul V, Sundarrajan S, Rajagopalan S, et al. Lysis-deficient phages as novel therapeutic agents for controlling bacterial infection. BMC Microbiol. 2011;11(195).
98. Lu TK, Koeris MS. The next generation of bacteriophage therapy [Review]. Curr Opin Microbiol. 2011 10;14(5):524–531. [PubMed: 21868281]
99. Hagens S, Blasi U. Genetically modified filamentous phage as bactericidal agents: a pilot study. Lett Appl Microbiol. 2003;37(4):318–23. [PubMed: 12969496]
100. Bardy P, Pantucek R, Benesik M, et al. Genetically modified bacteriophages in applied microbiology [Review]. J Appl Microbiol. 2016 01 9;121(3):618–633. [PubMed: 27321680]
101. Donlan RM. Preventing biofilms of clinically relevant organisms using bacteriophage. Trends Microbiol. 2009 2;17(2):66–72. [PubMed: 19162482]
102. Gorski A, Miedzybrodzki R, Weber-Dabrowska B, et al. Phage therapy: combating infections with potential for evolving from merely a treatment for complications to targeting diseases [Review]. Front Microbiol. 2016 26 9;7(1515).
103. Planson AG, Carbonell P, Grigoras I, et al. A retrosynthetic biology approach to therapeutics: from conception to delivery [Review]. Curr Opin Biotechnol. 2012 12;23(6):948–956. [PubMed: 22475981]
104. Esteban PP, Jenkins ATA, Arnot TC. Elucidation of the mechanisms of action of bacteriophage K/nano-emulsion formulations against S. aureus via measurement of particle size and zeta potential. Colloids Surf B Biointerfaces. 2016 3 01;139:87–94. [PubMed: 26700237]
105. Singla S, Harjai K, Katare OP, et al. Bacteriophage-loaded nanostructured lipid carrier: improved pharmacokinetics mediates effective resolution of Klebsiella pneumoniae-induced lobar pneumonia. J Infect Dis. 2015 15 7;212(2):325–334. [PubMed: 25605867]
106. Chadha P, Katare OP, Chhibber S. Liposome loaded phage cocktail: enhanced therapeutic potential in resolving Klebsiella pneumoniae mediated burn wound infections [Journal Article]. Burns. 2017 11;43(7):1532–1543. [PubMed: 28502784]
107. Singla S, Harjai K, Katare OP, et al. Encapsulation of bacteriophage in liposome accentuates its entry in to macrophage and shields it from neutralizing antibodies. PLoS One. 2016 4;11(e0153777). [PubMed: 27115154]
108. Kim KP, Cha JD, Jang EH, et al. PEGylation of bacteriophages increases blood circulation time and reduces T-helper type 1 immune response. Microb Biotechnol. 2008 5;1(3):247–57. [PubMed: 21261844]
109. Chhibber S, Shukla A, Kaur S. Transfersomal phage cocktail is an effective treatment against methicillin-resistant Staphylococcus aureus-mediated skin and soft tissue infections. Antimicrob Agents Chemother. 2017;61(10).
110. Pastagia M, Euler C, Chahales P, et al. A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive Staphylococcus aureus strains. Antimicrob Agents Chemother. 2011 2;55(2):738–744. [PubMed: 21098252]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

111. Ji W, Huang Q, Sun L, et al. A novel endolysin disrupts Streptococcus suis with high efficiency. FEMS Microbiol Lett. 2015 31 10;362(fnv205).

112. Daniel A, Euler C, Collin M, et al. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2010 4;54(4):1603–1612. [PubMed: 20086153]

113. Abdelkader K, Gerstmans H, Saafan A, et al. The preclinical and clinical progress of bacteriophages and their lytic enzymes: the parts are easier than the whole. Viruses. 2019 1 24;11(2).

114. Benesik M, Novacek J, Janda L, et al. Role of SH3b binding domain in a natural deletion mutant of Kayvirus endolysin LysF1 with a broad range of lytic activity [In Press]. Virus Genes. 2017 29 8:1–10. [PubMed: 29151222]

115. Vazquez R, Garcia P. Synergy between two chimeric lysins to kill Streptococcus pneumoniae. Front Microbiol. 2019;10 (6)(1251). [PubMed: 31231338]

116. Wu M, Hu K, Xie Y, et al. A novel phage PD-6A3, and its endolysin Ply6A3, with extended lytic activity against Acinetobacter baumannii. Front Microbiol. 2019;10 (1)(3302).

117. Schuch R, Khan BK, Raz A, et al. Bacteriophage lysin CF-301, a potent antistaphylococcal biofilm agent. Antimicrob Agents Chemother. 2017 7;61(e02666–16). [PubMed: 28461319]

118. Zhou Y, Zhang H, Bao H, et al. The lytic activity of recombinant phage lysin LysK amidase against staphylococcal strains associated with bovine and human infections in the Jiangsu province of China. Res Vet Sci. 2017 01 4;111:113–119. [PubMed: 28235706]

119. Schmelcher M, Donovan DM, Loessner MJ. Bacteriophage endolysins as novel antimicrobials. Future Microbiol. 2012;7(10):1147–1171. [PubMed: 23030422]

120. Al-Wrafy F, Brzozowska E, Gorska S, et al. Identification and characterization of phage protein and its activity against two strains of multidrug-resistant Pseudomonas aeruginosa. Sci Rep. 2019 17 9;9(1):13487. [PubMed: 31530875]

121. Chen W, Hwang YY, Gleaton JW, et al. Optimization of a peptide extraction and LC-MS protocol for quantitative analysis of antimicrobial peptides. Future Sci OA. 2019;5 (1)(FSO348). [PubMed: 30652017]

122. Waseh S, Hanifi-Moghaddam P, Coleman R, et al. Orally administered P22 phage tailspike protein reduces Salmonella colonization in chickens: prospects of a novel therapy against bacterial infections. PLoS One. 2010;5(e13904). [PubMed: 21124920]

123. Latka A, Maciejewska B, Majkowska-Skrobek G, et al. Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process [Review]. Appl Microbiol Biotechnol. 2017 01 4;101(8):3103–3119. [PubMed: 28337580]

124. Lood R, Winer BY, Pelzek AJ, et al. Novel phage lysin capable of killing the multidrug-resistant Gram-negative bacterium Acinetobacter baumannii in a mouse bacteremia model. Antimicrob Agents Chemother. 2015 01 4;59(4):1983–1991. [PubMed: 25605353]

125. Raz A, Serrano A, Hernandez A, et al. Isolation of phage lysins that effectively kill Pseudomonas aeruginosa in mouse models of lung and skin infection. Antimicrob Agents Chemother. 2019;63 (7)(e00024–19). [PubMed: 31010858]

126. Abedon ST. Lysis from without. Bacteriophage. 2011 Jan-Feb;1(1):46–49. [PubMed: 21687534]

127. Karau MJ, Schmidt-Malan SM, Yan Q, et al. Exebacase in addition to daptomycin is more active than daptomycin or Exebacase alone in methicillin-resistant Staphylococcus aureus osteomyelitis in rats. Antimicrob Agents Chemother. 2019:AAC.01235–19.

128. Schuch R, Lee HM, Schneider BC, et al. Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant Staphylococcus aureus-induced murine bacteremia. J Infect Dis. 2014;209(9):1469–1478. [PubMed: 24286983]

129. Blazquez B, Fresco-Taboada A, Iglesias-Bexiga M, et al. PL3 amidase, a tailor-made lysin constructed by domain shuffling with potent killing activity against pneumococci and related species. Front Microbiol. 2016 28 7;7 Jul(1156).

130. Shang Nelson D. Contributions of net charge on the PlyC endolysin CHAP domain. Antibiotics. 2019 05/28;8:70.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

131. Schmelcher M, Shen Y, Nelson DC, et al. Evolutionarily distinct bacteriophage endolysins featuring conserved peptidoglycan cleavage sites protect mice from MRSA infection. J Antimicrob Chemother. 2014 20 10;70(5):1453–1465.
132. Zhang W, Mi Z, Yin X, et al. Characterization of Enterococcus faecalis phage IME-EF1 and its endolysin. PLoS One. 2013 13 11;8(e80435).
133. Hathaway H, Ajuebor J, Stephens L, et al. Thermally triggered release of the bacteriophage endolysin CHAPK and the bacteriocin lysostaphin for the control of methicillin resistant Staphylococcus aureus (MRSA) [Journal Article]. J Control Release. 2017 1 10;245:108–115. [PubMed: 27908758]
134. Hathaway H, Alves DR, Bean J, et al. Poly(N-isopropylacrylamide-co-allylamine) (PNIPAM-co-ALA) nanospheres for the thermally triggered release of bacteriophage K. Eur J Pharm Biopharm. 2015 10;96:437–41. [PubMed: 26423908]
135. ClinicalTrials.gov. Phase IIa clinical study of N-Rephasin® SAL200 2017 [updated 8 Aug 2018]. Available from: https://clinicaltrials.gov/ct2/show/NCT03089697?term=SAL-200&rank=3
136. ClinicalTrials.gov. Safety, efficacy and pharmacokinetics of CF-301 vs. placebo in addition to antibacterial therapy for treatment of S. aureus bacteremia 2018 Available from: https://clinicaltrials.gov/ct2/show/NCT03163446?term=CF-301&rank=1
137. Fowler J, Vance G., Das A, Lipka J, et al. Exebacase (lysin CF-301) improved clinical responder rates in methicillin resistant Staphylococcus aureus (MRSA) bacteremia including endocarditis compared to standard of care antibiotics (SOC) alone in a first-in-patient phase II study [30 4 2019]. Available from: https://d1io3yog0oux5.cloudfront.net/_53ec10a3e327f1e291fe5c77b7504a7a/contrafect/db/226/1306/pdf/ECCMID+ORAL+Ph2_FINAL.pdf
138. Worthington RJ, Melander C. Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol. 2013;31(3):177–184. [PubMed: 23333434]
139. Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy [Review]. Future Microbiol. 2013 6;8(6):769–783. [PubMed: 23701332]
140. Alves DR, Gaudion A, Bean JE, et al. Combined use of bacteriophage K and a novel bacteriophage to reduce Staphylococcus aureus biofilm formation. Appl Environ Microb. 2014 11;80(21):6694–703.
141. Nilsson AS. Phage therapy--constraints and possibilities. Ups J Med Sci. 2014;119(2):192–198. [PubMed: 24678769]
142. Hill JA, Cowen LE. Using combination therapy to thwart drug resistance. Future Microbiol. 2015;10(11):1719–26. [PubMed: 26597425]
143. Tagliaferri TL, Jansen M, Horz H-P. Fighting pathogenic bacteria on two fronts: phages and antibiotics as combined strategy. Front Cell Infect Microbiol. 2019;9:22–22. [PubMed: 30834237]
144. Comeau AM, Tetart F, Trojet SN, et al. Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. PLoS One. 2007 8 29;2(8):e799. [PubMed: 17726529]
145. Kaur S, Harjai K, Chhibber S. Bacteriophage mediated killing of Staphylococcus aureus in vitro on orthopaedic K wires in presence of linezolid prevents implant colonization. PLoS ONE. 2014;9(3):e90411–e90411. [PubMed: 24594764]
146. Kim M, Jo Y, Hwang YJ, et al. Phage-antibiotic synergy via delayed lysis. Appl Environ Microbiol. 2018 11 15;84(22).
147. Akturk E, Oliveira H, Santos SB, et al. Synergistic action of phage and antibiotics: parameters to enhance the killing efficacy against mono and dual-species biofilms. Antibiotics (Basel). 2019 7 25;8(3):25.
148. Clifton SM, Kim T, Chandrashekhar JH, et al. Lying in wait: modeling the control of bacterial infections via antibiotic-induced proviruses. mSystems. 2019;4 (5)(e00221–19). [PubMed: 31575664]
149. Leung SSY, Parumasivam T, Gao FG, et al. Production of inhalation phage powders using spray freeze drying and spray drying techniques for treatment of respiratory infections. Pharm Res. 2016 01 6;33(6):1486–1496. [PubMed: 26928668]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

150. Chang RY, Wong J, Mathai A, et al. Production of highly stable spray dried phage formulations for treatment of Pseudomonas aeruginosa lung infection. Eur J Pharm Biopharm. 2017 12;121:1–13. [PubMed: 28890220]
151. Matinkhoo S, Lynch KH, Dennis JJ, et al. Spray-dried respirable powders containing bacteriophages for the treatment of pulmonary infections. J Pharm Sci. 2011 12;100(12):5197–5205. [PubMed: 22020816]
152. Huh H, Wong S, St Jean J, et al. Bacteriophage interactions with mammalian tissue: therapeutic applications. Adv Drug Deliv Rev. 2019 1 17.
153. Dixon DV, Hosseinidoust Z, Tufenkji N. Effects of environmental and clinical interferents on the host capture efficiency of immobilized bacteriophages. Langmuir. 2014 3 25;30(11):3184–90. [PubMed: 24617341]
154. Miedzybrodzki R, Klak M, Jonczyk-Matysiak E, et al. Means to facilitate the overcoming of gastric juice barrier by a therapeutic staphylococcal bacteriophage A5/80. Front Microbiol. 2017;8:467. [PubMed: 28386250]
155. Garretto A, Miller-Ensminger T, Wolfe AJ, et al. Bacteriophages of the lower urinary tract [Review]. Nat Rev Urol. 2019 01 7;16(7):422–432. [PubMed: 31073244]
156. Abedon ST. Ecology of anti-biofilm agents II: bacteriophage exploitation and biocontrol of biofilm bacteria [Review]. Pharmaceuticals. 2015 09 9;8(3):559–589. [PubMed: 26371011]
157. Hargreaves KR, Clokie MRJ. Clostridium difficile phages: still difficult? Front Microbiol. 2014;5 4(184).
158. Rashid SJ, Barylski J, Hargreaves KR, et al. Two novel myoviruses from the north of Iraq reveal insights into Clostridium difficile phage diversity and biology. Viruses. 2016 16 11;8(310).
159. Yang J, Yang H. Non-antibiotic therapy for Clostridioides difficile infection: a review [Review]. Crit Rev Clin Lab Sci. 2019.
160. Bassetti M, Poulakou G, Ruppe E, et al. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach [Review]. Intens Care Med. 2017 01 10;43(10):1464–1475.
161. Bull JJ, Gill JJ. The habits of highly effective phages: population dynamics as a framework for identifying therapeutic phages. Front Microbiol. 2014;5(618).
162. Akanda ZZ, Taha M, Abdelbary H. Current review-The rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2018 4;36(4):1051–1060. [PubMed: 28971508]
163. Naghizadeh M, Karimi Torshizi MA, Rahimi S, et al. Effect of serum anti-phage activity on colibacillosis control by repeated phage therapy in broilers. Vet Microbiol. 2019 7;234:61–71. [PubMed: 31213273]
164. Abbasifar R, Kropinski AM, Sabour PM, et al. Efficiency of bacteriophage therapy against Cronobacter sakazakii in Galleria mellonella (greater wax moth) larvae. Arch Virol. 2014 9;159(9):2253–2261. [PubMed: 24705602]
165. Eyer L, Pantucek R, Ruzickova V, et al. New perspectives of the phage therapy [Review]. [Nove perspektivy fagove terapie.]. Klin Mikrobiol Infekc Lek. 2007;13(6):231–235. [PubMed: 18320502]
166. Eyer L, Pantucek R, Zdrahal Z, et al. Structural protein analysis of the polyvalent staphylococcal bacteriophage 812. Proteomics. 2007 1;7(1):64–72.
167. Maszewska A, Wojcik E, Ciurzynska A, et al. Differentiation of polyvalent bacteriophages specific to uropathogenic Proteus mirabilis strains based on the host range pattern and RFLP. Acta Biochim Pol. 2016;63(2):303–310. [PubMed: 26730411]
168. Zhang X, Guo H, Jin L, et al. A new topology of the HK97-like fold revealed in Bordetella bacteriophage by cryoEM at 3.5 A resolution. eLife. 2013 17 12;2013(e01299). [PubMed: 24347545]
169. Schofield DA, Molineux IJ, Westwater C. 'Bioluminescent' reporter phage for the detection of Category A bacterial pathogens. JoVE. 2011 (53):e2740. [PubMed: 21775956]
170. Dubrovin EV, Popova AV, Kraevskiy SV, et al. Atomic force microscopy analysis of the Acinetobacter baumannii bacteriophage AP22 lytic cycle. PLoS One. 2012 11 10;7(e47348).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

171. McCallin S, Alam Sarker S, Barretto C, et al. Safety analysis of a Russian phage cocktail: from metagenomic analysis to oral application in healthy human subjects. Virology. 2013 01 9;443(2): 187–196. [PubMed: 23755967]

172. Leite DMC, Brochet X, Resch G, et al. Computational prediction of inter-species relationships through omics data analysis and machine learning. BMC Bioinformatics. 2018 11 20;19(Suppl 14):420. [PubMed: 30453987]

173. Anomaly J, Savulescu J. Compensation for cures: Why we should pay a premium for participation in 'challenge studies'. Bioethics. 2019 01 9;33(7):792–797. [PubMed: 31135070]

174. Caflisch KM, Patel R. Implications of bacteriophage- and bacteriophage component-based therapies for the clinical microbiology laboratory. J Clin Microbiol. 2019:JCM.00229–19.

175. Regeimbal JM, Jacobs AC, Corey BW, et al. Personalized therapeutic cocktail of wild environmental phages rescues mice from Acinetobacter baumannii wound infections. Antimicrob Agents Chemother. 2016 10;60(10):5806–16. [PubMed: 27431214]

176. Leshkasheli L, Kutateladze M, Balarjishvili N, et al. Efficacy of newly isolated and highly potent macteriophages in a mouse model of XDRAB bacteremia. J Glob Antimicrob Resist. 2019;14.

177. Jeon J, Ryu CM, Lee JY, et al. In vivo application of bacteriophage as a potential therapeutic agent to control OXA-66-like carbapenemase-producing Acinetobacter baumannii strains belonging to sequence type 357. Appl Environ Microbiol. 2016 7 15;82(14):4200–4208. [PubMed: 27208124]

178. Guang-Han O, Leang-Chung C, Vellasamy KM, et al. Experimental phage therapy for Burkholderia pseudomallei infection. PLoS One. 2016 7;11(e0158213). [PubMed: 27387381]

179. Carmody LA, Gill JJ, Summer EJ, et al. Efficacy of bacteriophage therapy in a model of Burkholderia cenocepacia pulmonary infection. J Infect Dis. 2010 15 1;201(2):264–271. [PubMed: 20001604]

180. Nale JY, Spencer J, Hargreaves KR, et al. Bacteriophage combinations significantly reduce Clostridium difficile growth in vitro and proliferation in vivo. Antimicrob Agents Chemother. 2016 2;60(2):968–981. [PubMed: 26643348]

181. Tothova L, Celec P, Babickova J, et al. Phage therapy of Cronobacter-induced urinary tract infection in mice. Med Sci Monit. 2011;17(7):BR173–BR178. [PubMed: 21709627]

182. Nishikawa H, Yasuda M, Uchiyama J, et al. T-even-related bacteriophages as candidates for treatment of Escherichia coli urinary tract infections. Arch Virol. 2008 3;153(3):507–515. [PubMed: 18188500]

183. Vahedi A, Soltan Dallal MM, Douraghi M, et al. Isolation and identification of specific bacteriophage against enteropathogenic Escherichia coli (EPEC) and in vitro and in vivo characterization of bacteriophage. FEMS Microbiol Lett. 2018;365(16).

184. Rastogi V, Yadav P, Verma A, et al. Ex vivo and in vivo evaluation of microemulsion based transdermal delivery of E. coli specific T4 bacteriophage: a rationale approach to treat bacterial infection. Eur J Pharm Sci. 2017 30 9;107:168–182. [PubMed: 28711712]

185. Dufour N, Debarbieux L, Fromentin M, et al. Treatment of highly virulent extraintestinal pathogenic Escherichia coli pneumonia with bacteriophages. Crit Care Med. 2015 20 6;43(6):e190–e198. [PubMed: 25803649]

186. Bull JJ, Otto G, Molineux IJ. In vivo growth rates are poorly correlated with phage therapy success in a mouse infection model. Antimicrob Agents Chemother. 2012 2;56(2):949–954. [PubMed: 22106213]

187. Aleshkin AV, Rubalskii EO, Volozhantsev NV, et al. A small-scale experiment of using phage-based probiotic dietary supplement for prevention of E. coli traveler's diarrhea. Bacteriophage. 2015;5(3).

188. Maura D, Galtier M, Le Bouguenec C, et al. Virulent bacteriophages can target O104:H4 enteroaggregative Escherichia coli in the mouse intestine. Antimicrob Agents Chemother. 2012 12;56(12):6235–6242. [PubMed: 23006754]

189. Pouillot F, Chomton M, Blois H, et al. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b: H4-ST131 Escherichia coli strain producing CTX-M-15. Antimicrob Agents Chemother. 2012 7;56(7):3568–3575. [PubMed: 22491690]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

190. Uchiyama J, Rashel M, Takemura I, et al. In silico and in vivo evaluation of bacteriophage phiEF24C, a candidate for treatment of Enterococcus faecalis infections. Appl Environ Microbiol. 2008 7;74(13):4149–4163. [PubMed: 18456848]

191. Al-Zubidi M, Widziolek M, Court EK, et al. Identification of novel bacteriophages with therapeutic potential targeting Enterococcus faecalis. Infect Immun. 2019;26.

192. Cheng M, Liang J, Zhang Y, et al. The bacteriophage EF-P29 efficiently protects against lethal vancomycin-resistant Enterococcus faecalis and alleviates gut microbiota imbalance in a murine bacteremia model. Front Microbiol. 2017 09 5;8:837. [PubMed: 28536572]

193. Malik R, Chhibber S. Protection with bacteriophage KO1 against fatal Klebsiella pneumoniae-induced burn wound infection in mice [Journal Article]. J Microbiol Immunol Infect. 2009 4;42(2):134–40. [PubMed: 19597645]

194. Kumari S, Harjai K, Chhibber S. Topical treatment of Klebsiella pneumoniae B5055 induced burn wound infection in mice using natural products. J Infect Dev Ctries. 2010 6 30;4(6):367–77. [PubMed: 20601788]

195. Thiry D, Passet V, Danis-Wlodarczyk K, et al. New bacteriophages against emerging lineages ST23 and ST258 of Klebsiella pneumoniae and efficacy assessment in Galleria mellonella larvae. Viruses. 2019 5;11 (5)(411).

196. Kumari S, Harjai K, Chhibber S. Efficacy of bacteriophage treatment in murine burn wound infection induced by Klebsiella pneumoniae [Journal Article]. J Microbiol Biotechnol. 2009 6;19(6):622–8. [PubMed: 19597322]

197. Hung C-H, Kuo C-F, Wang C-H, et al. Experimental phage therapy in treating Klebsiella pneumoniae-mediated liver abscesses and bacteremia in mice. Antimicrob Agents Chemother. 2011 4;55(4):1358–65. [PubMed: 21245450]

198. Chhibber S, Kaur S, Kumari S. Therapeutic potential of bacteriophage in treating Klebsiella pneumoniae B5055-mediated lobar pneumonia in mice. J Med Microbiol. 2008 12;57(12):1508–1513. [PubMed: 19018021]

199. Kumari S, Harjai K, Chhibber S. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by Klebsiella pneumoniae B5055. J Med Microbiol. 2011 2;60(Pt 2):205–10. [PubMed: 20965914]

200. Lin TL, Hsieh PF, Huang YT, et al. Isolation of a bacteriophage and its depolymerase specific for K1 capsule of Klebsiella pneumoniae: implication in typing and treatment. J Infect Dis. 2014;210(11):1734–1744. [PubMed: 25001459]

201. Carrigy NB, Larsen SE, Reese V, et al. Prophylaxis of Mycobacterium tuberculosis H37Rv infection in a preclinical mouse model via inhalation of nebulized bacteriophage D29. Antimicrob Agents Chemother. 2019;16.

202. Trigo G, Martins TG, Fraga AG, et al. Phage therapy is effective against infection by Mycobacterium ulcerans in a murine footpad model. PLoS Negl Trop Dis. 2013 4;7 (4)(e2183). [PubMed: 23638204]

203. Yilmaz C, Colak M, Yilmaz BC, et al. Bacteriophage therapy in implant-related infections: an experimental study [Journal Article]. J Bone Joint Surg Am. 2013 1 16;95(2):117–25. [PubMed: 23324958]

204. Henry M, Lavigne R, Debarbieux L. Predicting in vivo efficacy of therapeutic bacteriophages used to treat pulmonary infections. Antimicrob Agents Chemother. 2013 12;57(12):5961–5968. [PubMed: 24041900]

205. Fong SA, Drilling AJ, Ooi ML, et al. Safety and efficacy of a bacteriophage cocktail in an in vivo model of Pseudomonas aeruginosa sinusitis. Transl Res. 2019 4;206:41–56. [PubMed: 30615845]

206. Cafora M, Deflorian G, Forti F, et al. Phage therapy against Pseudomonas aeruginosa infections in a cystic fibrosis zebrafish model. Sci Rep. 2019 2019/02/06;9(1):1527.

207. Watanabe R, Matsumoto T, Sano G, et al. Efficacy of bacteriophage therapy against gut-derived sepsis caused by Pseudomonas aeruginosa in mice. Antimicrob Agents Chemother. 2007 2;51(2): 446–452. [PubMed: 17116686]

208. Roach DR, Leung CY, Henry M, et al. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. Cell Host Microbe. 2017 12 7;22(1):38–47.e4. [PubMed: 28704651]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

209. Heo YJ, Lee YR, Jung HH, et al. Antibacterial efficacy of phages against Pseudomonas aeruginosa infections in mice and Drosophila melanogaster. Antimicrob Agents Chemother. 2009 6;53(6):2469–2474. [PubMed: 19364866]

210. Hall AR, De Vos D, Friman VP, et al. Effects of sequential and simultaneous applications of bacteriophages on populations of Pseudomonas aeruginosa in vitro and in wax moth larvae. Appl Environ Microbiol. 2012 8;78(16):5646–5652. [PubMed: 22660719]

211. Debarbieux L, Leduc D, Maura D, et al. Bacteriophages can treat and prevent Pseudomonas aeruginosa lung infections. J Infect Dis. 2010 01 4;201(7):1096–1104. [PubMed: 20196657]

212. Vinodkumar C, Kalsurmath S, Neelagund Y. Utility of lytic bacteriophage in the treatment of multidrug-resistant Pseudomonas aeruginosa septicemia in mice. Indian J Pathol Micr. 2008 01 7;51(3):360–366.

213. Beeton ML, Alves DR, Enright MC, et al. Assessing phage therapy against Pseudomonas aeruginosa using a Galleria mellonella infection model. Int J Antimicrob Agents. 2015 20 7;46(2):196–200. [PubMed: 26100212]

214. Shivshetty N, Hosamani R, Ahmed L, et al. Experimental protection of diabetic mice against lethal P. aeruginosa infection by bacteriophage. Biomed Res Int. 2014;2014(793242). [PubMed: 24999476]

215. Pabary R, Singh C, Morales S, et al. Antipseudomonal bacteriophage reduces infective burden and inflammatory response in murine lung. Antimicrob Agents Chemother. 2016 2;60(2):744–751. [PubMed: 26574007]

216. McVay CS, Velasquez M, Fralick JA. Phage therapy of Pseudomonas aeruginosa infection in a mouse burn wound model. Antimicrob Agents Chemother. 2007 6;51(6):1934–8. [PubMed: 17387151]

217. Basu S, Agarwal M, Kumar Bhartiya S, et al. An in vivo wound model utilizing bacteriophage therapy of Pseudomonas aeruginosa biofilms [Journal Article]. Ostomy Wound Manage. 2015 8;61(8):16–23.

218. Tiwari BR, Kim S, Rahman M, et al. Antibacterial efficacy of lytic Pseudomonas bacteriophage in normal and neutropenic mice models. J Microbiol. 2011 12;49(6):994–999. [PubMed: 22203564]

219. Oechslin F, Piccardi P, Mancini S, et al. Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. J Infect Dis. 2017;215(5):703–712. [PubMed: 28007922]

220. Nikkhahi F, Dallal MMS, Alimohammadi M, et al. Phage therapy: assessment of the efficacy of a bacteriophage isolated in the treatment of salmonellosis induced by Salmonella enteritidis in mice. Gastroenterol Hepatol Bed Bench. 2017;10(2):125–130. [PubMed: 28702136]

221. Chhibber S, Kaur T, Sandeep K. Co-therapy using lytic bacteriophage and linezolid: effective treatment in eliminating methicillin resistant Staphylococcus aureus (MRSA) from diabetic foot infections [Journal Article]. PLoS One. 2013;8(2):e56022. [PubMed: 23418497]

222. Prazak J, Iten M, Cameron DR, et al. Bacteriophages improve outcome in experimental Staphylococcus aureus ventilator associated pneumonia. Am J Respir Crit Care Med. 2019;01.

223. Gupta R, Prasad Y. Efficacy of polyvalent bacteriophage P-27/HP to control multidrug resistant Staphylococcus aureus associated with human infections. Curr Microbiol. 2011 1;62(1):255–260. [PubMed: 20607539]

224. Rasool MH, Yousaf R, Siddique AB, et al. Isolation, characterization, and antibacterial activity of bacteriophages against methicillin-resistant Staphylococcus aureus in Pakistan. Jundishapur J Microbiol. 2016 10;9(e36135).

225. Lehman SM, Mearns G, Rankin D, et al. Design and preclinical development of a phage product for the treatment of antibiotic-resistant Staphylococcus aureus infections. Viruses. 2019 1 21;11(1).

226. Hsieh SE, Lo HH, Chen ST, et al. Wide host range and strong lytic activity of Staphylococcus aureus lytic phage Stau2. Appl Environ Microb. 2011 2;77(3):756–761.

227. Pincus NB, Reckhow JD, Saleem D, et al. Strain specific phage treatment for Staphylococcus aureus infection is influenced by host immunity and site of infection. PLoS One. 2015 24 4;10(e0124280).

228. Capparelli R, Parlato M, Borriello G, et al. Experimental phage therapy against Staphylococcus aureus in mice. Antimicrob Agents Chemother. 2007 8;51(8):2765–2773. [PubMed: 17517843]
229. Takemura-Uchiyama I, Uchiyama J, Osanai M, et al. Experimental phage therapy against lethal lung-derived septicemia caused by Staphylococcus aureus in mice. Microbes Infect. 2014 6;16(6):512–517. [PubMed: 24631574]
230. Zimecki M, Artym J, Kocidba M, et al. Prophylactic effect of bacteriophages on mice subjected to chemotherapy-induced immunosuppression and bone marrow transplant upon infection with Staphylococcus aureus. Med Microbiol Immunol. 2010 5;199(2):71–79. [PubMed: 19953264]
231. Kishor C, Mishra RR, Saraf SK, et al. Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. Indian J Med Res. 2016 1;143:87–94. [PubMed: 26997019]
232. Seth AK, Geringer MR, Nguyen KT, et al. Bacteriophage therapy for Staphylococcus aureus biofilm-infected wounds: a new approach to chronic wound care. Plast Reconstr Surg. 2013 2;131(2):225–34. [PubMed: 23357984]
233. Yen M, Cairns LS, Camilli A. A cocktail of three virulent bacteriophages prevents Vibrio cholerae infection in animal models. Nat Commun. 2017 01 2;8(14187).
234. Jaiswal A, Koley H, Ghosh A, et al. Efficacy of cocktail phage therapy in treating Vibrio cholerae infection in rabbit model. Microbes Infect. 2013 2;15(2):152–156. [PubMed: 23159467]
235. Jaiswal A, Koley H, Mitra S, et al. Comparative analysis of different oral approaches to treat Vibrio cholerae infection in adult mice. Int J Med Microbiol. 2014 5;304(3–4):422–430. [PubMed: 24656386]
236. Shamim Hasan Zahid M, Nashir Udden SM, Faruque ASG, et al. Effect of phage on the infectivity of Vibrio cholerae and emergence of genetic variants. Infect Immun. 2008 11;76(11): 5266–5273. [PubMed: 18794293]
237. Bhowmick TS, Koley H, Das M, et al. Pathogenic potential of vibriophages against an experimental infection with Vibrio cholerae O1 in the RITARD model. Int J Antimicrob Ag. 2009 6;33(6):569–573.
238. Bhandare S, Colom J, Baig A, et al. Reviving phage therapy for the treatment of cholera. J Infect Dis. 2019 2 15;219(5):786–794. [PubMed: 30395214]
239. Jun JW, Shin TH, Kim JH, et al. Bacteriophage therapy of a Vibrio parahaemolyticus infection caused by a multiple-antibiotic-resistant O3:K6 pandemic clinical strain. J Infect Dis. 2014 01 7;210(1):72–78. [PubMed: 24558119]
240. Oliveira H, Mendes A, Fraga AG, et al. K2 capsule depolymerase is highly stable, is refractory to resistance, and protects larvae and mice from Acinetobacter baumannii sepsis. Appl Environ Microbiol. 2019;85(17).
241. Lin H, Paff ML, Molineux IJ, et al. Therapeutic application of phage capsule depolymerases against K1, K5, and K30 capsulated E. coli in mice. Front Microbiol. 2017 16 11;8(2257).
242. Singh PK, Donovan DM, Kumar A. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from Staphylococcus aureus endophthalmitis. Antimicrob Agents Chemother. 2014 8;58(8):4621–4629. [PubMed: 24890598]
243. Gupta R, Prasad Y. P-27/HP endolysin as antibacterial agent for antibiotic resistant Staphylococcus aureus of human infections. Curr Microbiol. 2011 7;63(1):39–45. [PubMed: 21519800]
244. Chopra S, Harjai K, Chhibber S. Potential of combination therapy of endolysin MR-10 and minocycline in treating MRSA induced systemic and localized burn wound infections in mice. Int J Med Microbiol. 2016 01 12;306(8):707–716. [PubMed: 27616281]
245. Imanishi I, Uchiyama J, Tsukui T, et al. Therapeutic potential of an endolysin derived from kayvirus S25–3 for staphylococcal impetigo. Viruses. 2019 9;11 (9)(769).
246. Yang H, Gong Y, Zhang H, et al. ClyJ Is a novel pneumococcal chimeric lysin with a cysteine- and histidine-dependent amidohydrolase/peptidase catalytic domain. Antimicrob Agents Chemother. 2019 4;63(4).
247. Wright A, Hawkins CH, Anggard EE, et al. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; A preliminary report of efficacy. Clin Otolaryngol. 2009 8;34(4):349–357. [PubMed: 19673983]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Article highlights

- Growing antibiotic resistance, in addition to promising historical and current phage studies, has renewed interest in human phagotherapy
- To expedite consideration of phages as antibacterial agents, certain biological challenges associated with their use should be addressed, including acquired bacterial resistance to phage infection; the pharmacologic complexity of phage relative to small-molecule antibiotics; and interactions between the human immune system and phage
- Challenges may be allayed through the use of phage cocktails, phage modifications, encapsulation, and/or deployment of phage products

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

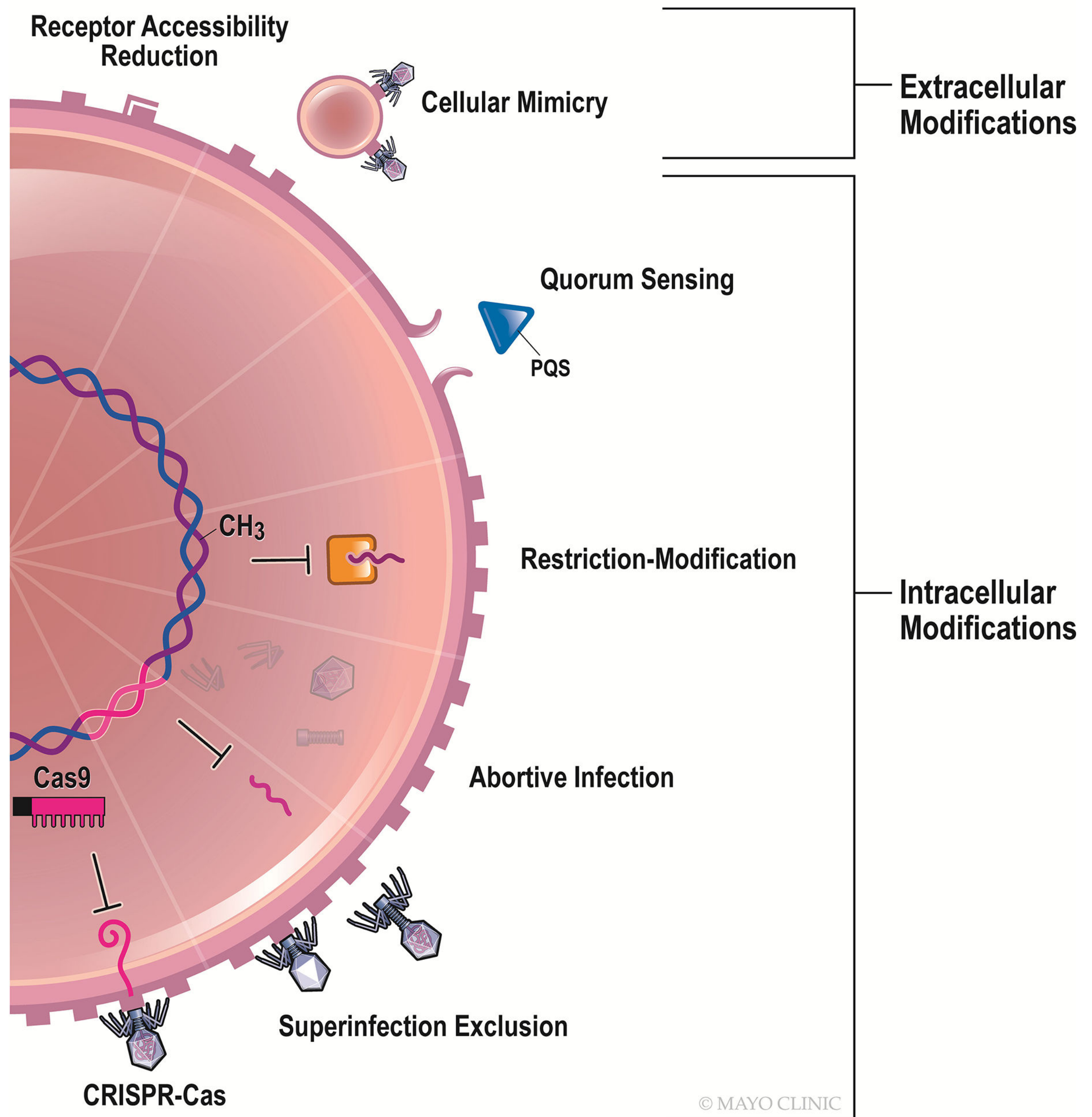

Figure 1.
Compensatory bacterial resistance mechanisms to phage.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

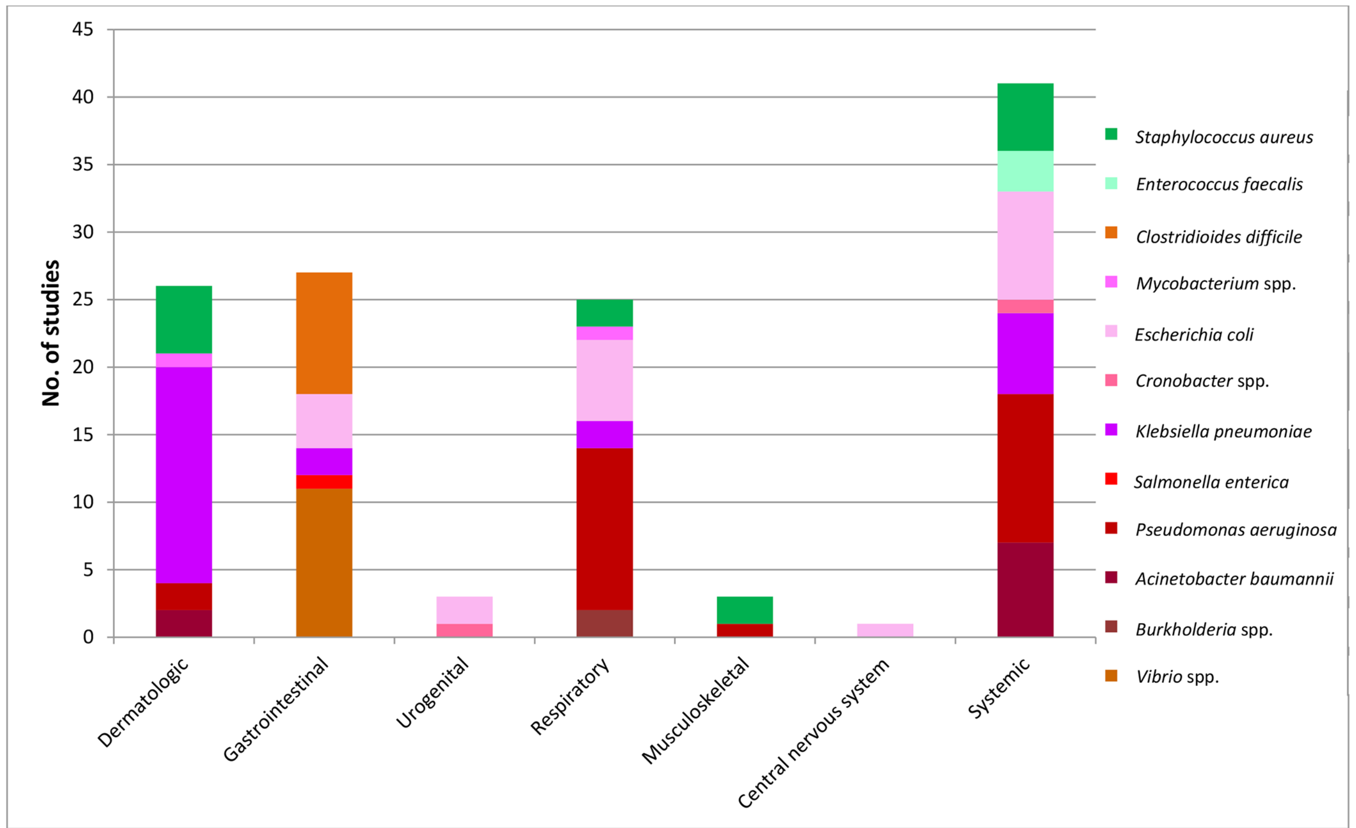

Figure 2.

In vivo phage efficacy studies published between January 1, 2007 and October 21, 2019, by infection and basscterial type.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

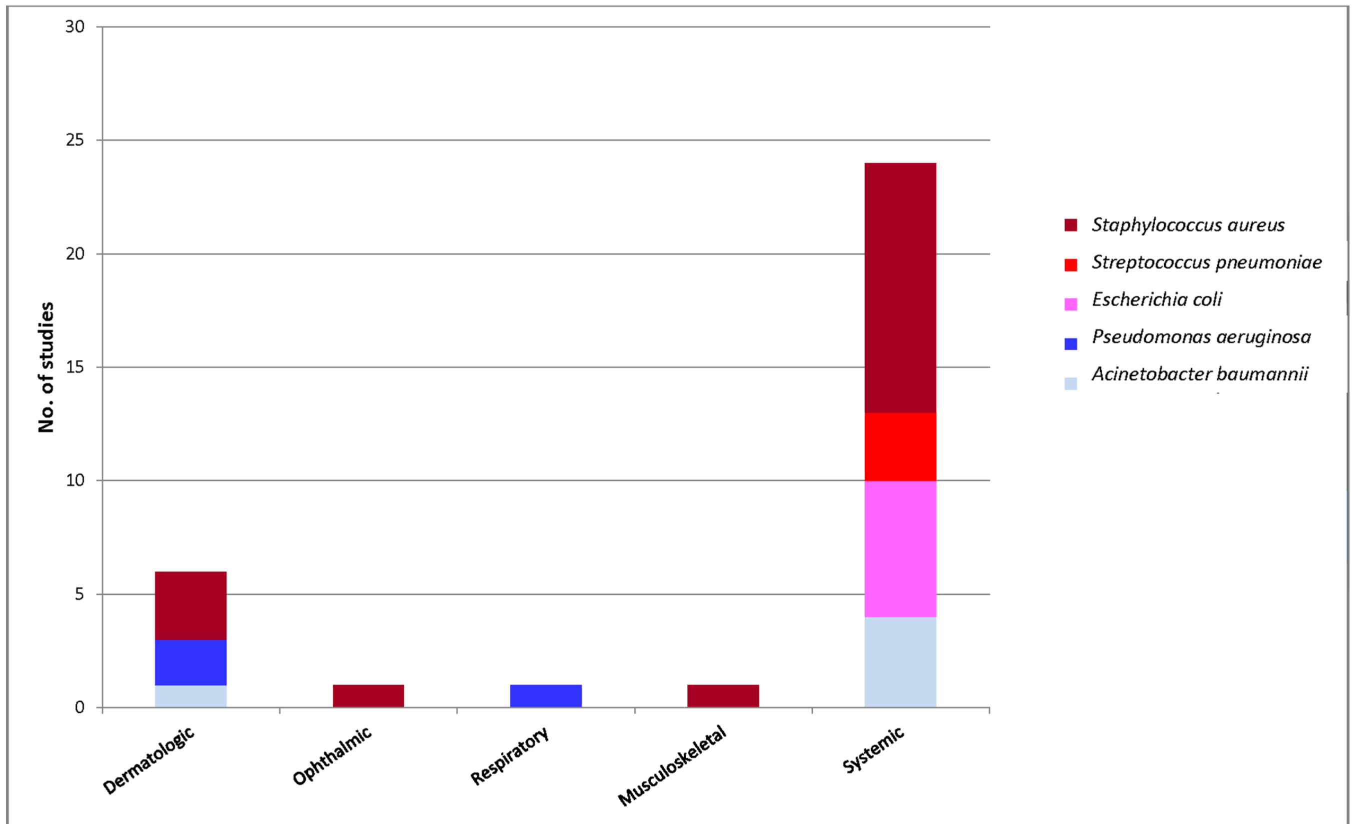

Figure 3.

In vivo lysin efficacy studies published between January 1, 2007 and October 21, 2019, by infection and bacterial type.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1.

Experimental animal and moth larvae model phage efficacy studies published between January 1, 2007 and October 21, 2019.

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
Acinetobacter baumannii	Cocktail: AB-Army1, AB-Navy1, AB-Navy2, AB-Navy3, AB-Navy 4	Mouse, wound infection	5×10^4 cfu AB5075 to wound; 4×10^9 pfu phage intraperitoneal (IP) and topically (5×10^9 pfu) 4, 24, and 48 hours post-infection	Infected, cocktail treated wounds exhibited decreased bacterial abundance at day 5 and wound size at days 9 and 13 compared with AB-Army1-treated and untreated animals	[175]
	Cocktail: AB-Army1, AB-Navy1, AB-Navy2, AB-Navy3, AB-Navy 4	*Galleria mellonella*, systemic infection	Proleg injection (10^7 cfu AB5075/mL); Proleg injection (10^7 cfu AB-Army1-primed AB5075/mL)	95% survival (5 day survival analysis) *G. mellonella* infected with AB-Army1 primed AB5075 *versus* 5% survival in wild-type AB5075-infected *G. mellonella*	[175]
	vB_AbaS_D0, vB_AbaP_D2	Mouse, systemic infection	IP administration ($10\times LD_{100}$, 2×10^7 cfu AB9/mouse); 100 μL vB_AbaS_D0 (10^9 pfu/mL), vB_AbaP_D2 (10^9 pfu/mL), or cocktail (10^9 pfu/mL) 2 hours post-infection	0% survival of infected, untreated mice; 50% survival of vB_AbaS_D0-treated mice; 90% survival of vB_AbaP_D2-treated mice; 100% survival of cocktail-treated mice; phage-resistant mutant bacteria sampled from blood 48 hours post-infection demonstrated significant incidence in vB_AbaP_D2-treated mice *versus* other treatment groups	[24]
	vB_AbaM_3090, vB_AbaM_3054	Mouse, systemic infection	IP administration (6×10^7 cfu FER /100 μL); IP administration of 50mg/kg imipenem, vB_AbaM_3090 and vB_AbaM_3054 alone and in combination (6×10^9 pfu/200 μL) 1 hour post-infection	At 7 days post-infection, 0% survival of infected, untreated mice, 17% survival of antibiotic-treated mice, and 80–100% survival of monophage and dual phage treated mice, between which differences in survival insignificant	[176]
		G. mellonella, systemic infection	Proleg injection (5×10^5 cfu/5μL) [FER]; Proleg injection vB_AbaM_3090 or vB_AbaM_3054 alone or in combination (5×10^7 pfu/10 μL) or imipenem (5 mg/kg) 30 minutes post-infection	Increased survival among phage-treated and antibiotic-treated groups (83–100% survival at 80 hours post-infection) *versus* 0% survival of infected, untreated controls	
	BΦ-C62	Mouse (immunocompromised C57BL/6), systemic infection	1×10^9 cfu/mL [carbapenem-resistant clinical isolates, n=45] intranasally (IN); Phage IP (MOI 0.1, 1 or 10) 30 minutes post-infection	0% survival among infected, non-treated mice 3 days post-infection; dose-dependent effects on survival in phage treated mice at 3 days post-infection: 100% at MOI=10, 50% at MOI=1, 16% at MOI=0.1; reduced amounts of bacteria in lungs of phage-treated mice between 6- and 9-fold relative to	[177]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
				infected, untreated mice at day 1 post-infection	
Burkholderia pseudomallei	C34	Mouse, melioidosis	100 cfu [clinical isolates, n=43] IN; 2×10^8 pfu 24 hours before or 2 hours after infection	Phage improved survival to 33% from 0% in controls, with no differences between pre- and post-treated groups, and extended median survival to 13 days in pre-treated and 11 days in post-treated mice compared to 8 days in controls	[178]
Burkholderia cenocepacia	BcepIL02	Mouse, lung infection	10^7–10^8 AU0728 or K56–2 cfu intratracheally; Phage (MOI=100) IN or IP 24 hours post-infection	Phage treatment (IP) decreased AU0728 bacterial loads in lung but not K-562 bacterial loads under same treatment conditions	[179]
Clostridioides difficile	ΦCDHM1	Hamster, gastroenteritis	10^4 cfu/mL spores [CD105HE1] per os (PO) every 8 hours for up to 36 hours; 1×10^8 pfu/mL individual or mixed phage PO	Reduction in *C. difficile* in treated *versus* control animals with extended survival in treated animals; 2- and 4-phage combination-treated animals exhibited comparable bacterial reductions of 2 and 4 $\log_{10}$ in gut epithelium and lumen, respectively; 4-phage combination prolonged time to death *versus* infected, untreated animals	[180]
	ΦCDHM2				
	ΦCDHM3				
	ΦCDHM4				
	ΦCDHM5				
	ΦCDHS1				
	ΦCDHS1-ΦCDHS12				
	ΦCDHS5-ΦCDHS6				
	Cocktail: ΦCDHM1, ΦCDHM2, ΦCDHM3 ΦCDHM4 ΦCDHM5, ΦCDHS1				
Cronobacter sakazakii	vB_CsaM_GAP161	*G. mellonella*, systemic infection	10×10^5 cfu HPB 3253 ($5\times$ LD_{50}) injection; Phage injection, site unspecified (MOI 8) at 1 and 0.5 hours pre-infection, and 0, 1, 2, 4 hours post-infection	Pre-treatment or simultaneously-treated larvae increased survival *versus* infected, untreated controls; post-treatment group comparable survival to controls	[164]
Cronobacter turicensis	Cocktail: P2, D2	Mouse, urinary tract infection	Bacterial-seeded (1×10^{11} cfu 290708/07/mL) transurethral catheter; Immediate IP phage treatment (10^{11} pfu/mL)	Renal but not bladder bacterial colonization reduced in phage-treated animals	[181]
Escherichia coli	T4	Mouse, urinary tract infection	5×10^9 cfu uropathogenic *E. coli* (ECU5) transurethrally; Phage IP at various MOI concurrent with bacterial inoculation	Bacterial inoculum 100% lethal in untreated mice; phage (MOI 60) rescued 100% mice	[182]
	Unspecified	Mouse, gastroenteritis	200 μL of 2×10^8 cfu/mL PO; PO administration of ciprofloxacin (160 μL of 0.5 g/mL), phage (200 μL/10 g of 2×10^9 pfu/mL), or a combination	No weight or behavioral changes, or bacterial detection in mice treated with phage 24 hours before or after infection; weight loss and behavioral changes noted in antibiotic or	[183]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
			24 hours post-infection, or phage 24 hours pre-infection	combination-treated animals secondary to dysbiosis, though no bacterial detection observed in these groups	
	KEP10	Mouse, urinary tract infection	5×10^9 cfu [uropathogenic *E. coli*, ECU5] transurethrally; Phage IP at various MOI concurrent with bacterial inoculation	Bacterial inoculum 100% lethal in infected, untreated mice; phage (MOI 60) rescued 90% mice	[182]
	T4	Rat, gastroenteritis	10^5–10^7 cfu ATCC 11303 PO; 3.6×10^7 pfu/mL transdermal application	83% phage-treated *versus* 0% untreated rats survived	[184]
	536_P1	Mouse, lung infection	1×10^7 or 4×10^7 cfu 536-lux IN; Phage (MOI 0.3 or 3) IN 2 hours post-infection	Phage rescued 100% animals from death, compared with 25% survival in infected, non-treated controls; mortality reduction from 80% to 25% with the use of an adapted phage	[185]
	536_P7	Mouse, lung infection	1.5×10^7 cfu PDP302 IN; Phage (MOI 10) IN 2 hours post-infection	Phage rescued 20% animals from death compared with 12% survival in infected, non-treated controls; phage adaptation increased survival to 75% from 20%	[185]
	K1-ind1	Mouse, systemic infection	2–3×10^8 cfu CAB1 or CAB281 IM; 10^2–10^8 phage intramuscularly (IM) administered concurrently	K1 capsule-dependent phages yielded 6 $\log_{10}$ reduction (specimen unspecified) following minimum treatment dose *versus* K1 capsule-independent phages	[186]
	K1-ind2				
	K1-ind3				
	K1H				
	K1G				
	K1E				
	K1–5				
	Cocktail: EcD7, V18, SE40, SI3, CH1, Lm1, ST11	Mouse, gastroenteritis	5×10^7 cfu K12 C600/mL daily for 3 days (route unspecified); 10^6 pfu/mL PO concomitantly and up to 24 hours post-infection	Bacteria in stool of untreated mice at 10^4 cfu/g stool, with no bacteria in phage-treated mice	[187]
	Cocktail: CLB_P1, CLB_P2, CLB_P3	Mouse, gastroenteritis	55989Str PO (dose unspecified); 3×10^8–10^{10} pfu/mL PO for 24 hours days 3 to 4 post-infection	Bacterial colonization in ileum treated mice reduced by 88% *versus* controls, although rebound occurred such that median bacterial density was similar across control and treated groups by day 7 post-infection	[188]
	EC200^{PP}	Rat, systemic infection	10^3–10^6 cfu S242 (ciprofloxacin-resistant clinical isolate)/mL IP; 10^8 pfu subcutaneously (SQ) 7 or 24 hours post-infection	100% rescue and bacterial elimination in blood with 7 hour post-infection treatment; 50% rescue with 24 hour post-infection treatment	[189]
	EC200^{PP}	Rat, meningitis	10^6–10^8 cfu S242 (ciprofloxacin-resistant clinical isolate)/mL intrathecally;	100% untreated meningitis-induced rats died by 36 hours post-infection, treatment with 10^8 pfu 1 or 7	[189]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
			10^8 pfu IP 1 or 7 hours post-infection	hours post-infection rescued 100%	
Enterococcus faecalis	ΦEF24C	Mouse, systemic infection	10^9–10^{10} cfu EF14 or VRE2/mL IP (LD_{100}); Range of MOI (100, 10, 1, 0.1, 0.01, 0.001, 0.0001) administered once, IP beginning 20 minutes post-infection	Phage rescued 100% at MOI 10, 1, 0.1, and 0.01 compared to 0% survival of controls; MOI 0.001 and 0.0001 did not impact survival	[190]
	SHEF2	Zebrafish, systemic infection	3 $\times 10^4$ OS16 cfu via embryonic microinjection 2 nL (MOI 20) SHEF2 via embryonic microinjection 2 hours post-infection	84% survival of phage-treated individuals *versus* 27% survival of infected, non-treated controls at 72 hours post-infection	[191]
	EF-P29	Mouse, systemic infection	2 $\times 10^9$ cfu VREF 002 IP (2× LD_{100}); 4×10^3, 4×10^4, 4×10^5, 4×10^6, 4×10^7pfu IP 1 hour post-infection	4×10^5 and 4×10^6 pfu phage rescued 100% mice	[192]
Klebsiella pneumonaie	K01	Mouse, wound infection	10×10^6 cfu B5055/mL SQ; Phage SQ or IP (dose unspecified) 30 minutes and 6 hours post-infection	Bacterial colonization in blood, lung, peritoneum reduced 3+ hours post-infection with IP or SQ phage	[193]
	Kpn5	Mouse, burn wound infection	10^8 cfu B5055topically (LD_{100}); MOI 1 or 200 topically 4, 12 and 24 hours post-infection	Survival of low-and high-dose phage-treated animals 0 and 66%, respectively	[194]
	vB_KpnP_K1-ULIP33	*G. mellonella*, systemic infection	Proleg injection (10^4 cfu SA12/10 μL); Phage administered via proleg injection (MOI 10) 1 hour pre- or post-infection	0–30% infected, untreated larvae; 0–30% uninfected, treated larvae; 90% larvae administered phage prophylactically; and 100% larvae treated after bacterial inoculation survived 4 days post-infection	[195]
	vB_KpnP_KL106-ULIP47		Proleg injection (10^3 cfu 2198/10 μL); Phage administered (vB_KpnP_KL106-ULIP47 and vB_KpnP_KL106-ULIP54 alone and combined) via proleg injection (MOI 10) 1 hour pre- or post-infection	0–10% infected, untreated larvae; 0–30% uninfected, treated larvae; and 80–100% treated larvae survived 4 days post-infection; no significant difference between survival rates of monophage- *versus* polyphage-treated larvae	
	vB_KpnP_KL106-ULIP54				
	Cocktail: vB_KpnP_KL06-ULIP47, vB_KpnP_KL1o6-ULIP54				
	Kpn5	Mouse, burn wound infection	SQ B5055 (LD_{100}); MOI 1 IP immediately following establishment of wound infection	Survival 80–100% 72 hours post-infection in each treatment group treated with cocktail and Kpn5 alone achieving similar survival	[196]
	Kpn12				
	Kpn13				
	Kpn17				
	Kpn22				
	Cocktail: Kpn5, Kpn12, Kpn13, Kpn17, Kpn22				

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
	Cocktail: KØ1, KØ2, KØ3, KØ4, KØ5	Mouse, burn wound infection	10^5 cfu B5055/mL SQ; MOI 1 IP (liposome- or un-encapsulated phage) 30 minutes post-infection	Encapsulated phages circulated systemically six times longer than un-encapsulated phages; 100% 7-day survival of animals treated 24 hour post-infection, liposome encapsulated, phage-treated mice compared with 0% 7-day survival of 24 hour post-infection, un-encapsulated phage-treated mice; 100% survival of encapsulated and un-encapsulated treatment groups when administered 30 minutes post-infection	[106]
	ΦNK5	Mouse, liver abscess	2×10^8 cfu NK-5 intragastric (IG); 2×10^5, 2×10^6, 2×10^7, 2×10^8 pfu IP or IG 0.5, 6 or 24 hours post-infection	IP phage 30 minutes post-infection yielded dose-dependent rescue, with 100% surviving following 10^7 pfu treatment and 30% surviving following 10^5 pfu treatment; similar dose-dependent effects observed in IG phage 30 minutes post-infection with 100% survival following 10^6 pfu; IP administration supported increased survival at both 6- and 24 h post-infection time points relative to IG dosing	[197]
	SS	Mouse, lung infection	10^8 cfu B5055 IN; MOI 200 IP administered concomitantly or 6 or 24 hours post-infection compared with phage (10^{10} pfu/mL) plus amikacin (3.75 mg/25 g)	No difference in pulmonary bacterial abundance between control and phage-treated mice, or between combination- and phage alone-treated animals	[198]
	Kpn1	Mouse, wound infection	10^7 cfu B5055/mL topically; MOI 10 topically 6 hours post-infection	Reduction in bacterial density on days 1–7 following cocktail treatment only	[38]
	Kpn2				
	Kpn3				
	Kpn4				
	Kpn5				
	Cocktail: Kpn1, Kpn2, Kpn3, Kpn4, Kpn5				
	Kpn5	Mouse, burn wound infection	10^8 cfu B5055 to full thickness burn (LD_{100}); MOI 200 topically as single dose immediately post-infection, compared with silver nitrate or gentamicin daily beginning 24 hours post-infection	7 day survival 57% and 17% following 0.5% and 0.0005% silver nitrate treatment, respectively; 53% and 13% with 1 g/L and 7 mg/L gentamicin, respectively; 63% with phage; survival in phage-treated *versus* high dose silver nitrate or gentamicin not different	[199]
	NTUH-K2044-K1–1	Mouse (BALB/cBy1), liver abscess	3×10^2 cfu NTUH K2044 IP; 1×10^8 pfu IP 16 or 24 hours later	Increase in TNF-α and IL-6 in blood, liver and spleen; survival increased in mice treated with phage 16- or 24	[200]

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
				h post-infection compared with controls	
	SS	Mouse, lung infection	10^8 cfu B5055 IN; IP phage (MOI 200) administration before, after, or concurrent with bacterial inoculation; or combination of IP phage (10^{10} pfu/mL) plus amikacin (3.75 mg/25 kg) concurrent with bacterial inoculation	No effect of phage alone or with amikacin	[198]
Mycobacterium tuberculosis	D29	Mouse, lung infection	Low inoculum (50–100 cfu) or ultra-low inoculum (5–10 cfu) H37Rv inhalation; ≈10^7 pfu/mouse via inhalation 30 minutes prior to bacterial challenge	Significant decrease in lung bacterial load at 24 hours and 3 weeks post-infection, but no difference in splenic bacterial load at 3 weeks between low inoculum and untreated groups; while significant decrease was observed at 24 hours post-infection in lungs of treated animals receiving ultra-low inoculum relative to bacterial load *versus* untreated animals	[201]
Mycobacterium ulcerans	D29	Mouse, Buruli ulcer	5.5×10^{10} cfu 1615 isolate footpad injection; 8×10^{10} pfu SQ 33 days post-infection	Decrease in bacterial abundance in phage-treated animals 68 days post-infection	[202]
Pseudomonas aeruginosa	vB_PsaP PAT14	Rat, foreign body osteomyelitis	Biofilm-coated IV catheter placed into tibial medullary canal; 16 days post-infection, 10^7 pfu local phage administration for 3 days alone or in combination with IP imipenem (120 mg/kg) and amikacin (25 mg/kg) daily for 14 days	Antibiotic- and phage-antibiotic-treatment decreased bacterial abundance without affecting biofilm thickness	[203]
	PAK_P1, PAK_P2, PAK_P3, PAK_P4, PAK_P5, LBL3, LUZ19, PhiKZ	Mouse, lung infection	1×10^7 cfu PAK-lumi IN; Phage IM 2 hours post-infection	Survival: 75–100% (PAK_P1–5), 50% (LBL3), 37% (LUZ19), 15% (PhiKZ); PBS control achieved survival levels “similar” to those of PhiKZ	[204]
	Cocktail (CT-PA): Pa193, Pa204, Pa222, Pa223	Sheep, sinusitis	2 mL of 10^8 cfu Aus20 (clinical isolate)/mL frontal sinus inoculation; Nasal rinse twice-daily for 7 days post-infection of 10^8, 10^9, or 10^{10} pfu/mL or saline	Phage dose-dependent antibacterial effect *versus* untreated controls, but no differences among groups treated with different phage concentrations	[205]
	Cocktail: vB_PaeP_PYO2, vB_PaeP_DEV, vB_PaeM_E215, vB_PaeM_E217	Zebrafish embryos, systemic infection	30 cfu PA01 in duct of Cuvier; 2 μL of 5×10^8 pfu/mL and/or ciprofloxacin (100 mg/mL) 30 minutes or 7 hours post-infection	Antibiotic and phage treatment alone reduced mortality *versus* untreated embryos; phage-antibiotic synergy observed; treatment at 30 minutes and 7 hours post-infection exhibited similar effects	[206]
	KPP10	Mouse, systemic infection	10^8 cfu/mL D4 PO	Significantly higher survival among 1 day post-infection treated mice than infected,	[207]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
			10^{10} pfu PO 1 day before, or 1or 6 days after infection	untreated controls, while treatment 1 day pre-infection or 6 days post-infection did not significantly rescue animals versus infected, untreated controls; reduction in fecal shedding of bacteria with phage delivered 1 or 6 days post-infection; reductions in bacterial organ colonization and IL-1β, IL-6, TNF-α inflammatory cytokines 1 day post-infection	
			2.4–300×10^6 cfu D4 IP; 10^{10} pfu administered IP 1 day prior, 6 hours after, or concurrently with bacterial inoculation	Phage administered concurrently with bacterial inoculum (but not 1 day prior or 6 hours post-infection) statistically improved survival rates *versus* infected, untreated controls	
	PAK_P1	Mouse, lung infection	*P. aeruginosa* PAKlumi 10^5 or 10^7 cfu IN; MOI 10 2 hours after bacterial challenge or MOI 100 4 days before bacterial challenge	Administration 2 hours post-infection rescued 100% of immunocompetent mice; administration 4 days pre-infection rescued 100% of immunocompetent and >90% of lymphocyte-deficient mice; neutropenic mice had 0% survival with phage treatment	[208]
	MPK1	*Drosophila melanogaster*, systemic infection	10^7 cfu PA01/mL injection; 5×10^7 pfu PO	Administration of MPK1 and MPK6 to infected *D. melanogaster* delayed death compared to controls	[209]
	MPK6	Mouse, systemic infection	2×10^6 PA01 cfu IP; 10^6–10^7 pfu IP or IM 6–12 hours post-infection	Approximately 1–4 $\log_{10}$ reduction in bacterial loads in lung, spleen and liver 24 hours post-infection wth IP or IM with MPK1 or MPK6 compared with controls	
	ΦKZ	*G. mellonella*, systemic infection	5×10^5 cfu PAO1 IP; Phage injection immediately following infection and every 12 hours thereafter	All phage administrations resulted in prolonged time to death *versus* infected, untreated controls, with phage cocktail achieving longest mean survival	[210]
	Cocktail: 14/1, ΦKZ, PNM, PT7		5×10^5 cfu PAO1 IP; Cocktail injection immediately following infection and every 12 hours thereafter		
	14/1, PT7, ΦKZ, PNM		5×10^5 cfu PAO1 IP; Phage injection sequentially immediately following infection and every 12 hours thereafter		
	PAK-P1	Mouse, lung infection	1×10^7 cfu PAKlumi IN; MOI 0.1, 1 and 10 IN 24 hours before and 2,4, or 6 hours after infection	Phage resulted in dose-dependent increase in survival, with 100% animals receiving MOI 10 phage surviving to end of 12 day experiment and 80% receiving MOI 1 surviving to same point; treatment	[211]

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
				delays longer than 2 hours post-infection did not rescue all mice	
	CSV-31	Mouse, systemic infection	10^7 cfu YFN-58(clinical isolate) IP (lethal dose); 10^4, 10^8, 10^9 pfu IP 45 minutes after infection	Phage rescued 100% infected mice when administered as late as 5 hours post-infection at 10^9 pfu	[212]
	Cocktail (composition unspecified)	*G. mellonella,* systemic infection	10 or 100 cfu PAO1 hemolymph injection; Phage hemolymph injection 2 hours pre- (MOI 0.1, 1, 10, 100) or post- (MOI 0.1, 1, 10) infection	Survival improved in pre-treated (90% MOI=100 infected with 10 cfu and 80% MOI=100 infected with 100 cfu) *versus* post-treated (40% MOI=10 infected with 10 cfu and 20% MOI=10 infected with 100 cfu)	[213]
	GNCP	Diabetic mouse, systemic infection	3×10^8 cfu/mL IP; 3×10^{10}, 10^9, 10^8, 10^7, 10^6, and 0 pfu IP ± imipenem IP (30 mg/kg) 20 minutes after infection, or delayed 0, 1, 2, 3, 4, or 6 hours post-infection	Phage (3×10^6–3×10^8 pfu) rescued 90% diabetic and non-diabetic mice from lethal bacteremia *versus* 20% single-dose imipenem-treated diabetic mice; treatment delays up to 8 hours post-infection rescued fewer diabetic and non-diabetic animals, with 20+ hour treatment delay rescuing 10% non-diabetic mice and 0% diabetic mice	[214]
	Cocktail: 1 *P. aeruginosa* 24, *P. aeruginosa* 25, *P. aeruginosa* 7	Mouse, lung infection	2.5×10^6–5×10^8 cfu/mL IN; 1.2×10^9 pfu IN simultaneously with infection, 48 hours post-infection or 24 hours pre-infection	100% mice administered phage simultaneously with bacterial challenge cleared infection, while 5/7 and 6/8 treated mice cleared infection in groups administered phage pre- and post-infection, respectively; all infected, untreated mice exhibited systemic infection	[215]
	Cocktail: Pa1, Pa2, Pa11	Mouse, burn wound infection	2–3×10^2 cfu PA01^{Rif} SQ; 3×10^8 pfu IP, IM, or SQ concurrent with bacterial inoculation	6% infected, non-treated mice died, while 28%, 22% and 88% infected mice survived when treated with phage IM, SC, or IP, respectively	[216]
	Cocktail: ΦBHU49, ΦBHU61, ΦBHU83, ΦBHU89, ΦBHU98, ΦBHU2255, ΦBHU7799, ΦBHU10858, ΦBHU10956, ΦBHU10958, ΦBHU10976	Mouse, catheter biofilms	SQ biofilm-coated (10^6 cfu) catheter; Daily 10 μL 10^7 pfu/mL SQ beginning day of catheter placement for 10 days	Infected, treated mice exhibited decreased colonization *versus* untreated animals	[217]
	PA10	Mouse, systemic infection	10^4–10^7 cfu PA01 IP to immunocompetent or 10^3–10^5 cfu to neutropenic mice; IP phage (MOI 1, 10, or 100 in immunocompetent l mice; MOI 10 in neutropenic mice)	100, 100 and 80% immunocompetent mice treated with MOI 100, 10 or 1 phage, respectively, survived; 0% immunocompromised mice (treated and untreated) survived 48 hours post-infection	[218]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
			concurrent with bacterial inoculation		
	Cocktail: Phagoburn	Rat, endocarditis	10^8 cfu CHA; 10^{10} pfu/mL by bolus or continuous IV administration, IV ciprofloxacin bolus (20 mg/kg), or phage and ciprofloxacin administered by IV bolus 18 h post-infection	Phage-antibiotic combination resulted in bacterial clearance of 7/11 rats compared with 0/28 rats receiving phage or ciprofloxacin alone	[219]
Salmonella enterica	1 phage (unspecified)	Mouse, gastroenteritis	1.5×10^8 cfu/mL oral gavage; 2×10^9 pfu/mL at time of infection; 4, 7 and 10 days post-infection; or 4 days pre-infection; ciprofloxacin (0.5 g/mL thrice over 24 hours) evaluated alone and in combination with phage	*S. enterica* isolated from stool of untreated mice with all other groups exhibiting absence of *S. enterica* in stool as early as 7 days post-infection	[220]
Staphylococcus aureus	MR-10	Mouse, wound infection	10^5, 10^6, 10^7, 10^8 cfu ATCC 43300/mL hindpaw injection MOI 100 (10^8 pfu/mL), MOI 100 (10^8 pfu/mL) + 25 mg/kg PO linezolid or linezolid alone	Mice receiving phage alone or combination therapy scored similarly in assessment of lesion quality and localized edema with improvement *versus* untreated, infected controls	[221]
	Cocktail: 2003, 2002, 3A, K	Rat, lung infection	LD_{100} ($6–8 \times 10^9$ cfu) administered intratracheally following 4 hours mechanical ventilation; IV administration of teicoplanin (3 mg/kg), phage cocktail (10^9 pfu/mL of each of 4 phages), or combination teicoplanin and phage cocktail 2, 12, 48 and 72 hours post-infection	Each treatment group exhibited similar bacterial loads with significant reduction *versus* infected, sham-treated controls; non-significant differences in cytokine levels across test groups except for increase in IL-1β levels among infected and non-infected animals treated with phage *versus* non-phage treated, and increase in IL-6 levels in infected, untreated rats *versus* uninfected, sham-treated	[222]
	P-27/HP	Mouse, systemic infection	5×10^8 cfu IP; 10^7 pfu phage SQ 24 hours post-infection	Infected, untreated mice 4 $\log_{10}$ bacteria in spleen *versus* phage-treated mice 2 $\log_{10}$ cfu bacteria in spleen 3 days post-treatment	[223]
	Cocktail: (composition unspecified)	Rabbit, wound infection	Wounds inoculated with 100 μL 1.5×10^8 cfu /mL; 2×10^8 pfu topical phage immediately before (prevention) or after (treatment) bacterial challenge	Infected, phage-treated rabbits (both treatment and prevention groups) higher rate of wound healing and lower bacterial wound colonization than infected, untreated animals	[224]
	AB-SA01 cocktail	Mouse, lung infection	3×10^8 cfu Xen29 IN; 50 μL vehicle or 5×10^8 pfu IN 2 and 6 hours post-infection or SC vancomycin (110 mg/kg) 2, 6, and 12 hours post-infection	Phage-treated animals exhibited reductions in bacteria in lung *versus* untreated animals with comparable amounts to antibiotic-treated animals	[225]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
	Sb-1	Rat, foreign body osteomyelitis	Biofilm-coated IV catheter in tibial medullary canal; 16 days post-infection, local phage (10^7 pfu) for 3 consecutive days alone or with teicoplanin (20 mg/kg) IP daily for 14 days	Phage-antibiotic-treated group diminished bacterial load compared with control group and other treatment groups; biofilm thickness similar between treatment and control groups	[203]
	Stau2	Mouse, systemic infection	Bacterial challenge (OD_{600}=0.5) S23 IP; MOI 0.1, 1, 10, and 100 IP 0, 30, 60 minutes post-infection with assessment 7 days following infection	0/10 infected, untreated animals; 10/10 infected, treated receiving phage 0 minutes post-infection (MOI 100); 3/5 infected, treated receiving phage 30 minutes post-infection (MOI 100); 2/5 infected, treated receiving phage 60 minutes post-infection (MOI 100) survived	[226]
	SATA-8505	Mouse, skin and soft tissue infection	10^7 cfu USA 300 SQ to immunocompetent or immunosuppressed [chronic granulomatous disease (CGD) model] mice; MOI 1 and 10 IP immediately preceding infection	MOI 1 reduced lesion size in CGD but not wild-type mice *versus* respective untreated counterparts with no differences in cfu/lesion among either wild-type or CGD mice; MOI 10 did not influence lesion size among wild-type or CGD mice compared with, untreated counterparts; reduced cfu/ lesion among CGD but not wild-type treated mice	[227]
	M^{Sa}	Mouse, systemic infection	10^6–10^9 cfu A170 SQ, or 5×10^6 cfu A170; 10^7–10^9 pfu SQ concurrent with bacterial inoculation, or 10^9 pfu IV 10 days post-infection	Dose-dependent effect of phage with 100% survival in 10^9 pfu-treated mice *versus* 40% of 10^8 pfu-treated, concurrently infected, mice; 100% of 10 day post-infection treated mice exhibit sterility of blood, spleen, kidneys, and heart on day 20 post-infection *versus* 0% of infected, untreated mice	[228]
	M^{Sa}	Mouse, local infection	SQ abscess induction with 10^7 cfu A170; SQ 10^9 pfu concurrent with bacterial inoculation or 4 days post-infection	Concurrent phage administration prevented abscess formation in 100% of treated mice; 4 day post-infection phage minimized abscess biomass and bacterial colonization	[228]
	S13	Mouse, systemic infection	6.4×10^8 cfu SA27/mL IN; 10^{10} pfu/mL IP 6 hours after infection	Phage-treated animals higher survival at day 14 post-infection and decreased bacterial loads in spleen and liver at day 2 post-infection compared with controls	[229]
	A5	Mouse (CBA mice administered busulfan PO and cyclophosphamide IP and syngeneic bone marrow transplant), systemic infection	1×10^7 cfu L IV 1×10^6 pfu IP 4 days post-bone marrow transplant, 30 minutes prior to bacterial challenge	Phage-treated mice had decreased splenic and hepatic bacterial loads *versus* controls	[230]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
	Cocktail: SA-BHU1, SA-BHU2, SA-BHU8, SA-BHU15, SA-BHU21, SA-BHU37, SA-BHU47	Rabbit, osteomyelitis	5×10^{6} cfu/mL femur osteomyelitis; 5×10^{12} pfu/mL beginning 3 weeks or 6 weeks post-infection with 4 doses locally administered 48 hours apart	Wound swabs culture-negative at conclusion of phage dosing (3 weeks post-infection) in 3 week post-infection treatment group and at conclusion of phage dosing (8 weeks post-infection) in 6 week post-infection treatment group	[231]
	Unspecified	Rabbit, wound infection	10^{6} cfu UAMS-1 or UAMS-929 into wound; 3 days post-infection debridement only, phage only (MOI 1), or phage (MOI 1)-debridement combination every other day for 6–12 days	Reductions in bacterial counts in debridement-phage combination group only	[232]
Vibrio cholerae	ICP1	Mouse, gastroenteritis	5×10^{5} cfu AC53 PO; 10^{6}–10^{7}pfu PO 3 hours before infection	Bacterial reductions of at least 2 $\log_{10}$ with phage	[233]
	ICP2				
	ICP3				
	Cocktail: ICP1, ICP2, ICP3		5–9×10^{5} cfu or 1×10^{8} cfu AC53 PO; 3×10^{7}–3×10^{8} pfu PO 6, 12, or 24 hours before infection	Reductions in *V. cholerae* with treatment 6 and 12 hours prior to low-dose challenge and 6, 12, and 24 hours prior to high-dose challenge	
	Cocktail: ICP1, ICP2, ICP3	Rabbit, gastroenteritis	5×10^{8} cfu AC53 PO; 10^{9} pfu PO 3 or 24 hours before infection	Reductions in *V. cholerae* with treatment 3 and 24 hours prior to challenge	
	Cocktail: ATCC 51352- B1, -B2, -B3, -B4, -B5	Rabbit, gastroenteritis	1×10^{9} cfu ATCC 51352/mL PO; 1×10^{8} pfu/mL (total MOI 0.1) 6 or 12 hours before or 6 or 12 hours after infection	Phage pre-treatment no effect on fecal shedding of *V. cholerae*; phage treatment 6 hours post-infection led to reduction in f *V. cholerae* shedding between 12 and 60 hours post-infection, while phage treatment 12 hours post-infection generated no bacterial reduction over this timespan; infected, untreated controls developed severe diarrhea, pre-treated rabbits developed moderate diarrhea, and post-treated rabbits developed no diarrhea	[234]
	Cocktail: ATCC 51352-B1, -B2, -B3, -B4, -B5	Mouse, gastroenteritis	1×10^{9} cfu ATCC 51352/mL PO; 1×10^{8} pfu/mL daily PO, ciprofloxacin (40 mg/kg) daily, or reduced osmolarity oral rehydration solution daily starting 24 hours after infection for 3 days	Ciprofloxacin reduced bacterial counts by more than 2 $\log_{10}$ compared with phage cocktail; both decreased IL-6 and TNF-α levels	[235]
	JSF4	Mouse, gastroenteritis	Human cholera (10^{4}–10^{5} cfu) stools - ID_{50} analysis and competition assay; Phage-containing and phage-free human stool	Infectious dose (ID_{50}) 10-fold higher in mice inoculated with phage-containing stools *versus* phage-free stools	[236]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Phage	Disease model	Administration (Bacteria [strain(s), if specified]; Phage)	Findings	Reference
	Cocktail: ATCC 51352-B1, -B2, -B3, -B4, -B5	Rabbit, gastroenteritis	1×10^9 cfu MAK 757 intrajejunally; 1×10^8 pfu concurrently administered intrajejunally with bacterial inoculum	Administration phage cocktail with bacteria reduced bacterial load, prevented symptom progression and minimized histopathologic findings (intestinal villi morphology, immune cell invasion)	[237]
	Phi_1	Rabbit, gastroenteritis	5×10^8 cfu O1 1051 Sm^R in $NaHCO_3$ Ranitidine (5 mg/kg IP) followed by bacterial challenge and 1×10^9 pfu PO	11 of 17 control animals and 0 of 19 experimental animals symptoms by 24 hours post-infection; decrease in intestinal and cecal fluid bacterial load in phage-treated *versus* control animals	[238]
Vibrio parahaemolyticus	pVp-1	Mouse, gastroenteritis	2×10^6–10^7 cfu CRS 09–17 (clinical isolate) IP or PO (LD_{50}); 2×10^8 pfu PO or IP treatment 1 hour post-infection	56% (IP) and 52% (PO) infected, untreated mice died by 36 hours post-infection, while 8% and 16%, respectively, died in treated groups	[239]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2.

Experimental animal and moth larvae model lysin efficacy studies published between January 1, 2007 and October 21, 2019.

Bacterium	Lysin	Disease Model	Administration (Bacteria; Lysin)	Findings	Reference
Acinetobacter baumannii	PlyF307	Mouse, subdermal catheter biofilms	Subdermal catheter with 3 day-old biofilm; Endolysin (250 μL) administered to catheter intraluminally at 24 and 28 hours post-infection; catheters removed 3 hours post-treatment	2 log reduction in bacterial density on lysin-treated *versus* lysin-untreated catheters	[124]
	PlyF307	Mouse, systemic infection	IP lethal dose (10^8 cfu); 1 mg PlyF07 IP 2 hours post-infection	50% lysin-treated *versus* 10% control mice survived 14 days	[124]
	K2	*Galleria mellonella*, systemic infection	Proleg injection (10^6 cfu NIPH 2061/5.5 μL) Proleg injection phage-pretreated (0.25, 0.5, or 3 μg) NIPH 2061 cells or phage (0.25, 0.5, or 3 μg) 30 minutes post-infection	90% survival of phage-treated larvae at 20 hours and 60% survival at 42 hours post-infection	[240]
		Mouse, systemic infection	IP administration 10^7 cfu following chemically-induced immunosuppression; IP lysin administration 1 hour post-infection	Pretreated mice survival 53%, 69%, and 88% (0.25, 0.5, or 3 μg/mL, respectively) at 72 hours post-infection. Treated mice survival 15%, 56%, and 70% (0.25, 0.5, or 3 μg/mL, respectively) at 72 hours post-infection	
	Ply6A3	Mouse, systemic infection	IP minimum lethal dose ($1{\times}10^9$ cfu AB32/mL); IP administration Ply6A3 (1 mL, 2 mg/mL) or phage PD6A3 (1 mL, 10^9 pfu/mL); uninfected mice received 14-phage cocktail (1 mL, 10^9 pfu/mL), Ply6A3 (1 mL, 2 mg/mL), phage PD6A3 (1 mL, 10^9 pfu/mL), or combined PD6A3 phage and Ply6A3 lysin	70% survival among Ply6A3-treated mice, 70% survival among combination PD6A3 phage and Ply6A3-treated mice, 60% survival among PD6A3 phage-treated mice, 50% survival among phage cocktail-treated mice, and 0% among infected, shame-treated mice at 7 days post-infection; decrease in WBC counts among all treatment groups *versus* infected, sham-treated; decrease in WBC counts among lysin Ply6A3 and combination PD6A3 phage and Ply6A3-treated animals *versus* PD6A3 alone and phage cocktail-treated mice; increase in IL-10 and procalcitonin levels among infected, untreated controls while the same values remained at baseline in uninfected mice, PD6A3-treated mice, and Ply6A3-treated mice	[116]
Escherichia coli	K1E K1F K1H K5 K30gp41	Mouse, systemic infection	1–$4{\times}10^8$ cfu IM; Capsule depolymerase (0, 2, 5, 20 μg) IM 30 minutes post-infection; Toxicity assessed using IM depolymerase (100 μg)	20 μg depolymerase (save K1E) rescued most mice from death; toxicity studies revealed no changes in survival (100%), behavior, or body weight of lysin-treated *versus* lysin-untreated controls	[241]

Bacterium	Lysin	Disease Model	Administration (Bacteria; Lysin)	Findings	Reference
	K30gp42				
Pseudomonas aeruginosa	PlyPa03 PlyPa91	Mouse, skin infection	Topical application of PA01 (5×10^6 cfu/mL); Topical treatment 20 hours post-infection with PlyPa03 (200 or 300 μg) or PlyPa91 (100 μg)	Dose-dependent reduction in bacterial load (>2 log in PlyPa03-treated group and 1 log in PlyPa91-treated group)	[125]
	PlyPa91	Mouse, lung infection	Two sequential intranasal administrations of PA01 (50 μL 10^8 cfu/mL each); Two doses (two intranasal or one intranasal and one intratracheal; each 50 μL [1.8 mg/mL]) of PlyPa91 administered 3 or 6 hours post-infection	10-day survival rates 0% infected, untreated controls; 20% infected mice treated with two intranasal phage doses; 70% infected mice treated with one intratracheal and one intranasal dose	[125]
Staphylococcus aureus	Ply187	Mouse, endophthalmitis	500 cfu intravitreally; Endolysin intravitreally 6 and 12 hours post-infection	Improvement in 24 hour post-infection clinical score of lysin-treated eyes at 6 and 12 hours post-treatment *versus* controls; bacterial abundance reduced 24 hours post-treatment in 6 hour post-infection treated mice (3.6×10^3 cfu/eye) and 12 hours post-infection treated mice (2.9×10^4 cfu/eye) *versus* untreated mice (1.0×10^5 cfu/eye)	[242]
	Exebacase	Mouse, systemic infection	10^6–10^9 cfu IP; Lysin IP (0.25–5.0 μg/mL) 2 hours post-infection with or without vancomycin or daptomycin	Lysin associated with 2 $\log_{10}$ reduction in bacteria in blood; increased efficacy with combination of lysin and vancomycin or daptomycin; fourfold maximal increase in lysin MIC	[128]
	P-27/HP	Mouse, systemic infection	5×10^8 cfu IP; Lysin (250 μg SQ) 24 hours later	Infected, untreated mice 4 $\log_{10}$ bacteria in spleen *versus* endolysin-treated mice 2 $\log_{10}$ cfu bacteria in spleen 4 days post-treatment	[243]
	MR-10	Mouse, burn wound infection	10^5–10^8 cfu/mL SQ; Lysin (50 μg SQ) and/or minocycline (50–100 mg/kg PO) 3 hours post-infection	Infected, untreated mice 100% mortality (7 days); animals treated with either agent alone 35% mortality by day 5; animals treated with high-dose minocycline or combination therapy no mortality (7 days)	[244]
	MR-10	Mouse, burn wound infection	10^7–10^9 cfu topical; Lysin (50 μg SQ) and/or minocycline (50–100 mg/kg PO) 3 hours post-infection	Combination therapy-treated and high-dose minocycline-treated animals nearly total wound closure 12 days post-treatment *versus* infected, untreated control animals (28%), low-dose minocycline treated (43%), and lysin only-treated (49%) animals	[244]
	80α Phi11 LysK P68 2638A	Mouse, systemic infection	4×10^7 cfu IP; Endolysins (200 μg) IP 30 minutes post-infection	Six of the eight PGH compounds administered intraperitoneally (200 μg) 30 minutes after microbial challenge exhibited similar activity to vancomycin, with the six compounds and vancomycin all effecting 100% survival	[131]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bacterium	Lysin	Disease Model	Administration (Bacteria; Lysin)	Findings	Reference
	Twort				
	PhiSH2				
	WMY				
	Exebacase	Rat, osteomyelitis	Intratibial injection of 10 μL arachidonic acid (50 μg/mL) and 50 μL *S. aureus* (10^7 cfu/ mL); IP administration of daptomycin (60 mg/kg twice daily doses for 4 days), IV Exebacase (single dose of 40 mg/kg), or daptomycin and Exebacase combination therapy, all beginning one week post-infection	Reduction in bacterial loads in all treatment groups compared with infected, untreated controls 4 days post-treatment; reduction in bacterial loads among combination treated animals *versus* animals treated with Exebacase or antibiotic alone	[127]
	S25–3lys-his	Mouse, impetigo	2×10^8 cfu administered topically to inner pinna; Topical administration of 2 μL (1 mg/mL) lysin immediately post-infection	Lysin-treated animals had decreased bacterial densities, pustule surface area (but not pustule quantity), and epidermal invasion and increased species richness of pinna epidermal microbiome *versus* infected, untreated pinna	[245]
Streptococcus pneumoniae	PL3 chimeric N-acetylmuramoyl-l-alanine amidase	Zebrafish embryos, systemic infection	Embryos cultured with 10^8 cfu/mL D39; Single dose of PL3 (15 or 20 μg) post-infection (timing unspecified)	0% mortality with single dose 20 μg PL3 *versus* 40% in untreated, infected embryos	[129]
	ClyJ	Mouse, systemic infection	2.68×10^7 cfu IP (LD_{100}); IP ClyJ (0.3 or 0.4 mg) or penicillin G (0.25 mg) 1 hour post-infection	0, 90, 100 and 40%, survival among untreated, low- and high-dose phage- and antibiotic-treated animals, respectively, at 10 days post-treatment	[246]
	Cpl-711	Zebrafish, systemic infection	10μL 10^6 cfu/mL strain 48 IP; 1 hour post-infection, subtherapeutic doses of Cpl-711 (10 μL 0.5 μg/mL) or PL3 (10 μL 0.15 μg/mL) administered IP, alone or in combination (10 μL 0.125 μg/mL Cpl-711, 10μL 0.08 μg/mL PL3)	72 hours post-infection, 28% survival of infected, sham-treated animals; 44% survival of Cpl-711-only treated animals; 50% survival of PL3-only treated animals; 78% survival of Cpl-711 and PL3 combination-treated animals; 100% survival of uninfected, lysin-treated animals	[115]
	PL3				

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3.

Phage and phage lysin therapy in human clinical trials published between January 2007 and March 2019.

Trial Start	Trial Registration Number	Ref.	Phage/Lysin Formulation (Investigator/ Sponsor)	Bacteria Targeted	Treatment Indication	Phase	Outcome
2006		[21]	WPP-201 cocktail (Southwest Regional Wound Care Center)	*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	Infected venous leg ulcers	I	No adverse effects observed
2009		[61]	T4 coliphage cocktail: AB2, 4, 6, 11, 46, 50, 55, JS34, 37, 98, D1.4 (Nestlé)	*E. coli*	Dysentery	I/II	No adverse effects or benefits noted; study prematurely discontinued
2009	EudraCT 2004–001691–39	[247]	Biophage-PA (Biocontrol Ltd)	*P. aeruginosa*	Otitis externa	I/II	No adverse effects noted; at 42 days post-treatment, phage-treated cohort exhibited reduced median bacterial abundance compared to placebo group
2015		[21]	PhagoBurn (Pherecydes Pharma)	*P. aeruginosa, E. coli*	Infected burn wounds	I/II	No adverse effects noted; time to "sustained bacterial reduction" longer in phage treatment arm; study prematurely discontinued
2017		N/A	SAL200 (Tonabacase) (iNtRON Biotech)	*S. aureus*	*S. aureus* bacteremia	II	Ongoing
2017		[137]	Exebacase (ContraFect Corp)	*S. aureus*	Bacteremia, including endocarditis (single intravenous dose added to standard of care antibiotics)	II	Well tolerated; higher clinical response rate compared to standard of care antibiotics alone in methicillin-resistant but not methicillin-susceptible *S. aureus* subgroups
2017		N/A	Pyophage (Tzulakidze National Center of Urology)	*Enterococcus* species, *E. coli, Proteus mirabilis,* staphylococci, streptococci, *P. aeruginosa*	Urinary tract infections	II/III	Results pending (personal communication, Thomas Kessler)
2019		N/A	EcoActive (Intralytix, Inc.)	Adherent invasive *E. coli* (AIEC)	Exacerbation of inflammation in Crohn disease secondary to AIEC	I/II	Recruitment ongoing
2019		N/A	PhagoPied (Pherecydes Pharma)	*S. aureus*	Diabetic wounds	I/II	Not yet recruiting

Table reproduced from [174]