Cell Host & Microbe
Review

Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria

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Phage therapy, long overshadowed by chemical antibiotics, is garnering renewed interest in Western medicine. This stems from the rise in frequency of multi-drug-resistant bacterial infections in humans. There also have been recent case reports of phage therapy demonstrating clinical utility in resolving these otherwise intractable infections. Nevertheless, bacteria can readily evolve phage resistance too, making it crucial for modern phage therapy to develop strategies to capitalize on this inevitability. Here, we review the history of phage therapy research. We compare and contrast phage therapy and chemical antibiotics, highlighting their potential synergies when used in combination. We also examine the use of animal models, case studies, and results from clinical trials. Throughout, we explore how the modern scientific community works to improve the reliability and success of phage therapy in the clinic and discuss how to properly evaluate the potential for phage therapy to combat antibiotic-resistant bacteria.

Introduction

Soon after Alexander Fleming's 1928 discovery of penicillin and the beginning of Western medicine's widespread use of antibiotics in the 1940s, Fleming himself warned that misuses of these drugs could result in antibiotic-resistant bacteria (Fleming, 1945). As predicted, clinical reports of antibiotic resistance followed, such as the evolution of resistant *Mycobacterium tuberculosis* in early clinical trials for streptomycin efficacy in treating tuberculosis (Marshall et al., 1948). Nevertheless, the discovery and development of novel antibiotics flourished for many decades (Spellberg et al., 2008). However, in the latter 20th century, antibiotic discovery slowed, and the alarming increase in rates of antibiotic resistance signaled that the golden age of antibiotics had perhaps ended. Indeed, aside from three new antibiotic classes discovered between 2005 and 2018, no novel drug classes have been developed since the 1980s (Samson, 2005; Hover et al., 2018; Ling et al., 2015). Similar mechanism of action among these newer drugs has led to potential evolution of cross-resistance in bacteria. While synthetic modifications to some pre-existing antibiotics have temporarily extended their clinical usefulness (Fair and Tor, 2014), this approach has also selected for broader resistance mechanisms, such as extended spectrum beta lactamases (Heinz et al., 2018), adaptive changes that are perhaps more easily evolved compared to *de novo* resistance mechanisms.

In 2017, the World Health Organization (WHO) highlighted the particular threat of Gram-negative pathogens resistant to multiple antibiotics (WHO, 2017). Discovery, design, and development of new and alternative antibacterial therapies are crucial. This review concerns the therapeutic use of bacteriophage (phage): viruses that exclusively infect bacteria and can act as bactericidal agents. This approach of "phage therapy" is an old idea that is recently regaining popularity. Efforts are buoyed by development of easier methods for engineering phage for different purposes in biotechnology (Pires et al., 2016). Also, the extreme biodiversity of phage in nature (Brüssow and Hendrix, 2002; Wasik and Turner, 2013) can be leveraged for "bioprospecting": discovery and development of naturally evolved phage with properties that are ideal for phage therapy use (e.g., Chan et al., 2018). Below we examine the past, present, and future uses of phage therapy, especially addressing how this newly energized field may proceed with modern, rational therapeutic approaches.

Phage Biology

During a lytic infection cycle (Figure 1) a phage will (1) attach to receptor(s) on the surface of a bacterium; (2) deliver the genomic content into the bacterium; (3) undergo viral replication in the cytosol via bacterial transcription, translation, and replication; and (4) upon formation of new phage particles, escape the cytoplasm through lysis of the bacterium. This process is then repeated by the new phage particles as they infect additional susceptible cells. This highlights a long-understood benefit of phage therapy: utilizing lytic viruses as self-amplifying "drugs" that target and kill susceptible cells may be more efficient than applying antibiotics that are incapable of self-amplification.

Obligately lytic (or "virulent") phage seem to be the best candidates for development of phage therapy (hereafter we refer to such phage simply as "lytic"). However, for completeness we briefly remind readers that lysogenic (or temperate) phage are also prevalent in nature. Lysogenic phage integrate into the host genome and are inherited by daughter cells during binary fission; however, at a later time, under environmental perturbation or other physiological stressor, lysogenic phage excise from the bacterial genome and enter a lytic infection cycle. While lysogenic phage might be preferred in certain biotechnology applications, lytic phage are more akin to antibiotic drugs lethal to bacteria, which suggests an easier path to approval for treating bacterial infections.

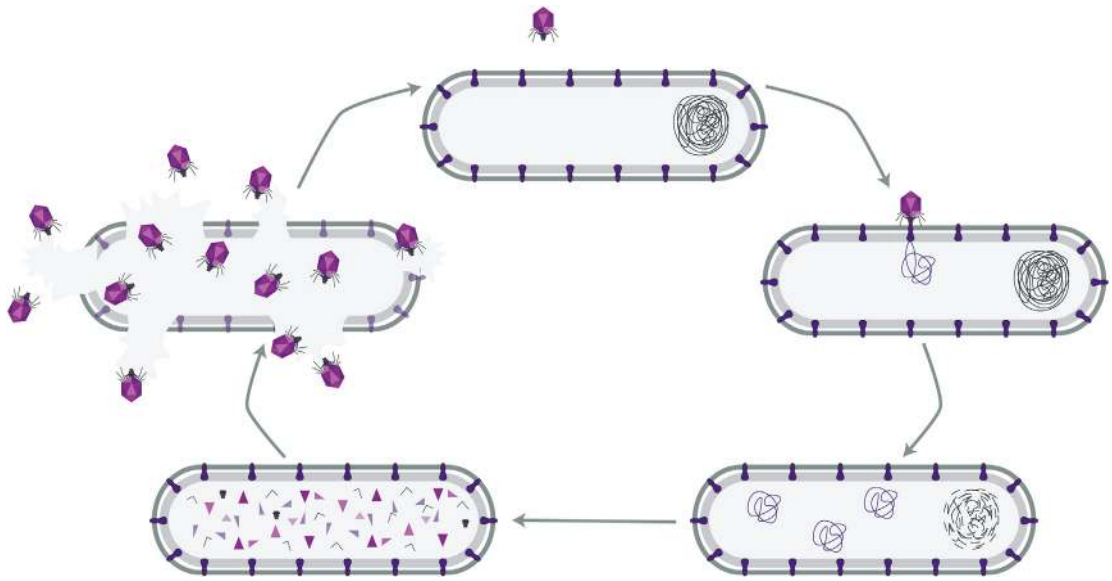

Figure 1. Lytic Phage Infection Cycle
A cycle of lytic phage replication begins when the virus recognizes and irreversibly binds to a receptor (protein or sugar) on the surface of a bacterial cell. The phage delivers its genomic content into the cytoplasm of the bacterial cell. Typically, host resources, including proteins and genomes, are repurposed to fuel phage replication. Replication, transcription, and translation of the phage genome begins usually through redirecting host metabolism to the production of new phage particles. Upon assembly of new phage particles, lysis of the bacterial cell allows newly replicated phage particles to escape the cytoplasm and go on to infect other susceptible bacteria.

In the mid-20th century, bacteria and lytic phage were used in classic experiments to elucidate fundamentals of biology and genetics, including demonstration of the spontaneous nature of mutations, DNA as hereditary material, and triplet nature of the amino acid code (Luria and Delbrück, 1943; Hershey and Chase, 1952; Crick et al., 1961). Beyond these studies, it is increasingly recognized that phage biodiversity is immense. Importantly, whereas some phage are highly specific to a single species or even genotypic strain of bacteria (Rohwer et al., 2014), other phage have naturally broad host ranges or can easily mutate to infect bacterial genotypes and species other than the typical host (Duffy et al., 2007). The aforementioned first step of lytic phage infection is attachment to the receptor(s) on the cell surface. These receptor binding sites are commonly proteins or sugar moieties on the bacterial cell, which are recognized by phage proteins that are effectively responsible for phage host specificity (Figure 2). There are many well-characterized examples of phage binding, especially the morphological structures used for these purposes. For instance, phage T4 uses two sets of tail fibers, long and short, to bind to susceptible *Escherichia coli* bacteria (Furukawa and Mizushima, 1982); phage SPP1 uses a tail spike to attach to *Bacillus subtilis* (Vinga et al., 2012); some *Siphoviridae* phage use baseplate proteins to initiate infection of *Lactococcus lactis* (Bebeacua et al., 2013); and *Cystoviridae* phage such as phi-6 infect *Pseudomonas syringae* via attachment proteins embedded within an envelope that surrounds the nucleocapsid (Mindich et al., 1976). However, the immensity of phage biodiversity suggests that the basic biology of phage binding to bacterial proteins remains a vastly untapped science, ripe for new discoveries. The presence of phage-binding sites and their possible structural variation should affect specialized versus generalized ability for phage attachment. Nevertheless, in the following sections we highlight that historical efforts in phage therapy have not always investigated and characterized the receptor binding site(s) used by phage to initiate infection. Clearly, current development of phage therapy candidates could include the following: an investigation of the receptor binding site(s) used by phage when infecting bacteria, whether phage-imposed selection for changes in these structures alters therapy success, and how the evolution of bacterial resistance to therapeutic phage may affect bacterial fitness components, such as rates of cell division and expression of pathogenicity traits.

Early Phage Therapy

The discovery of phage is attributed to the independent work of two microbiologists: Frederik Twort in 1915 and Félix d'Hérelle in 1917. While Twort was the first to observe and describe the effects of a "transparent material" that inhibited bacterial growth (Twort, 1915), it was not until 1917 when d'Hérelle isolated an anti-Shigella microbe that the idea of an obligate parasite of bacteria was termed bacteriophage or "bacteria-eater" (d'Hérelle, 1917). Almost immediately after his discovery, d'Hérelle recognized the therapeutic potential of phage as a treatment for bacterial diseases. In 1919, he successfully used phage to treat chickens infected with *Salmonella gallinarum* (Ho, 2001; d'Hérelle and Smith, 1926). This success in animals soon led d'Hérelle to attempt treating human infections with phage. In 1921, five patients with bacillary dysentery were successfully treated with a phage that infects *Shigella dysenteriae* (Ho, 2001; Summers, 1993). In 1927, clinical trials treating cholera in India showed that mortality decreased from

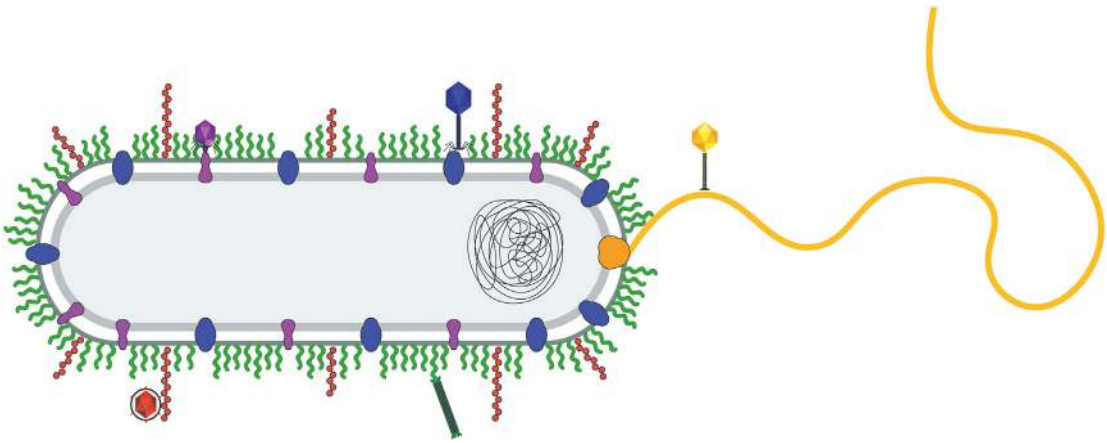

Figure 2. Examples of Bacterial Receptors for Phage Binding
Phage encode binding proteins that recognize and attach to sites on the surface of a bacterial cell. Many phage bind to protein structures on the bacteria such as pili (red; e.g., Mindich et al., 1976), flagella (yellow; e.g., Choi et al., 2013), porins (blue; e.g., Furukawa and Mizushima, 1982), or efflux pumps (purple; e.g., Chan et al., 2016). Phage have also been reported to bind to specific sugar moieties in LPS (green; e.g., Mindich et al., 1976).

62.8% in control groups to 8.1% in phage-treated groups (d'Hérelle et al., 1930). Furthermore, d'Hérelle noted that introducing anti-cholera phage into drinking wells of villages during an outbreak prevented additional infections from occurring (d'Hérelle et al., 1930).

Early-Identified Challenges

Soon after d'Hérelle's initial successes, many other scientists recognized the therapeutic and prophylactic potential of phage and began to target other infections, though with varying success. Among criticisms surrounding the design and quality of early phage-therapy trials, scientists started identifying some potential challenges of phage therapy. (1) The possible drawback of extreme phage specificity was recognized early on, indicating that a phage may not be useful without prior characterization of bacterial susceptibility. For example, in 1923, Beckerish and Hauduroy used phage successfully to reduce bacterial load in the blood of patients with typhoid fever (see Hadley, 1928), whereas a year later Smith (1924) unsuccessfully used phage on a similar patient population; Hadley (1928) speculated that Smith's failure stemmed from unknowingly using phage with a narrow host range. d'Hérelle himself acknowledged this weakness, attributing the success of his early trials to careful choice of phage capable of infecting the causative bacterial agent (Ho, 2001). (2) The early methods used to bulk manufacture therapeutic phage were likely heavily contaminated with lysed bacteria. With limited and unreliable filtering and purification steps, the possible beneficial effects of phage were difficult to separate from the confounding effects of contaminating bacterial antigens (Cowie and Hicks, 1932). (3) Early pharmacokinetic experiments showed that phage were rapidly removed from the body via the spleen, calling into question the sustained efficacy of phage over time (Krestownikowa and Gubin, 1925). (4) In 1943, Luria and Delbruck used selection by lytic phage to calculate spontaneous mutation rates of bacteria and in doing so demonstrated that bacteria are readily capable of evolving resistance to phage (Luria and Delbrück, 1943). (5) Lastly, early studies showed that *in vitro* laboratory experiments with phage and bacteria did not always match experimental outcomes observed *in vivo* (Riding, 1930; Eaton and Bayne-Jones, 1934; Krueger and Scribner, 1941). As these perceived problems were identified, interest in phage therapy waned relative to newly discovered antibiotics, and this trend away from phage therapy in the West was firmly cemented through the 1970s. This sentiment was in stark contrast to vested interests of physicians and scientists in the then USSR, Poland, and elsewhere, who continued to develop phage therapy in earnest; the legacy continues to be evident in locales such as the G. Eliava Institute of Bacteriophages in Tbilisi, Georgia. This work has been the focus of numerous prior reviews and will not be discussed here (such as Kutateladze and Adamia, 2010). However, as new antibiotic resistance mechanisms arose for every novel class and compound, and the incidence of antibiotic resistant infections increased globally, phage therapy was reconsidered by the West.

Smith and Huggins' Pioneering Studies

This interest was propelled forward in the 1980s with a series of well-designed experiments by Smith and Huggins. These experiments addressed many of the historic criticisms of phage therapy described above, while demonstrating safety and efficacy in animal models. Smith and Huggins began by showing that phage effectiveness *in vitro* could correlate with *in vivo* efficacy and ultimately chose phage R, which demonstrated the greatest *in vitro* virulence, for further characterization (Smith and Huggins, 1982). Phage R appears limited in host range, only infecting $K1^+$ *E. coli*, and likely uses the K1 capsule as a receptor. Through a series of lethal bacterial challenges in mice, Smith and Huggins demonstrated that a single dose of phage R was as effective as eight doses of streptomycin (Smith and Huggins, 1982). In the same experiment, they showed that bacterial lysate, free of phage, provided no therapeutic effect. After intramuscular inoculation of mice with no bacterial challenge, they observed that phage persisted in the inoculated muscle and spleen 28 days after the original inoculation, while it was cleared relatively rapidly from the liver and blood at 16 and 20 h post inoculation, respectively. Smith and Huggins noted that all phage-resistant mutants,

observed at a frequency of ~0.01, were $K1^-$ variants that had been previously shown to be avirulent. In a single paper, Smith and Huggins were able to address many previous criticisms. Furthermore, they observed phage therapy to be potentially more effective than chemical antibiotics.

Smith and Huggins further investigated factors that could influence the effectiveness of phage therapy in an *E. coli* diarrhea model in calves. They cleverly employed a rational multi-phage approach to combat the emergence of resistant mutants (Smith and Huggins, 1983). Specifically, after choosing lytic phage B44/1 that only infected $K85^+$ strains of *E. coli*, they isolated phage-resistant mutants *in vitro*. Subsequently, they chose a second phage, B44/3, for its ability to infect bacteria that were resistant to the first phage B44/1, and additionally selected for B44/3-resistant mutants that were susceptible to phage B44/1 infection. By examining phage resistance prior to the therapeutic use of these phage, Smith and Huggins were able to anticipate the evolution of phage resistance and use a dual-phage approach that might limit the emergence of phage-resistant bacteria. In 1987, Smith and Huggins examined the stability of phage during orally administered therapy and observed that poor phage stability in the acidic environment of the stomach could be countered by administering calcium carbonate prior to phage (Smith et al., 1987). These revolutionary studies by Smith and Huggins determined that the many perceived criticisms of phage therapy were unfounded or less concerning than believed, paving the way for new and rational approaches to phage therapy.

Other scientists have since re-examined and expanded on Smith and Huggins' studies. Rapid clearance of phage *in vivo* was originally deemed a negative aspect of phage therapy, but Merril et al. (1996) demonstrated that it is possible to select for phage variants that are long circulating in blood. Following Smith and Huggins' favorable results in phage treatment of mice infected with *E. coli*, Soothill (1992) demonstrated efficacy of phage treatment in mice infected with either *Pseudomonas aeruginosa* or *Acinetobacter baumanii*. In 2002, Bull and Levin et al. revisited Smith and Huggins' original experiments comparing the efficacy of K1-antigen-targeting phage versus a non-K1-targeting phage against *E. coli* in mice (Bull et al., 2002). The K1-targeting phage was observed to protect 100% of mice treated immediately, while the non-K1 targeting phage resulted in 60% mortality. In a second experiment, phage therapy resulted in 9% mortality compared to streptomycin, which resulted in 54% mortality. These data confirm Smith and Huggins' results showing that K1-targeting phage are more effective in treatment than non-K1-targeting phage or antibiotic treatment (Bull et al., 2002).

Phage Therapy: A Renewed Approach

The ability to characterize and test phage as antibacterial therapies has advanced immensely. The current era offers inexpensive whole-genome sequencing, automated technology for measuring the growth of microbes, and efficient high-throughput methods for screening hundreds or even thousands of samples simultaneously. Meanwhile, it is increasingly recognized that modern clinical trials should be carefully designed to be safer, to be more inclusive, and (if possible) to generate valuable data compared to earlier attempts. An appropriately conducted phage therapy trial should be double blinded and placebo controlled with large diverse cohorts, and perhaps designed to generate relevant longitudinal data from clinical isolates. For example, researchers could conduct follow-up lab studies and whole-genome sequencing of phage and/or bacteria taken during treatment, to test a myriad of basic and clinical microbiology as well as evolutionary hypotheses. Also, our increased understanding of the human microbiome and its interactions with human immunology warrant closer investigation of possible phage and immune system interactions in clearing infections.

However, one obvious limitation to phage therapy is the inevitable evolution of phage resistance in bacteria (Labrie et al., 2010). Modern approaches to phage therapy should both acknowledge and capitalize on this certainty. Evolutionary biology describes how genetic trade-offs should be widely observed in biological systems; organisms sometimes evolve one trait that improves fitness (a relative advantage in survival or reproduction) while simultaneously suffering reduced performance in another trait (Stearns, 1989; Turner and Chao, 1998; Messenger et al., 1999; Dessau et al., 2012; Goldhill and Turner, 2014; Sexton et al., 2017). Phage therapy would thus benefit from utilizing certain phage which select for the target bacterial pathogen to suffer specific genetic trade-offs (Chan et al., 2016). In particular, if the proximate binding of a lytic phage is known to associate with a virulence factor or mechanism for antibiotic resistance in the target bacteria, this should exert strong selection for the bacteria to mutate or downregulate the phage-binding target(s). This approach should be especially useful in the case of opportunistic bacterial pathogens, because the bacteria could evolve reduced virulence or antibiotic resistance and still thrive in a different ecological setting (e.g., soil) as opposed to "arms-race" selection for escalating virulence in an obligate pathogen such as in response to vaccine pressure (e.g., Marek's disease virus in chickens; Nair, 2005). Thus, this approach to phage therapy should be doubly effective; success is achieved when phage lyse the target bacterium, but also when bacteria evolve phage resistance because they suffer reduced virulence or increased sensitivity to antibiotics. In the following sections we return to this paradigm of phage-imposed genetic trade-offs.

A phage that requires a virulence factor to attach to and infect a bacterium may select against the expression of that virulence factor (Figure 3). Selection against virulence factors could be multiply effective, as some virulence factors such as capsules have been shown to hide antigenic sites (Foster, 2005), provide some degree of antibiotic resistance (Geisinger and Isberg, 2015), and prevent phagocytosis by macrophage (Foster, 2005). Phage that use components of LPS as receptors select against the expression of these components typically resulting in "rough" colony-forming mutants through phase variable expression of LPS, point mutations, or even large chromosomal deletions in LPS biosynthesis genes (Seed et al., 2012; Kim and Ryu, 2011, 2012; Filippov et al., 2011; Le et al., 2014). While resistant to LPS targeting phage, these bacterial mutants are typically reduced in both fitness and virulence (León and Bastías, 2015). Selection against other virulence factors that can serve as phage receptors such as adhesins, pili, or secretion systems could prevent

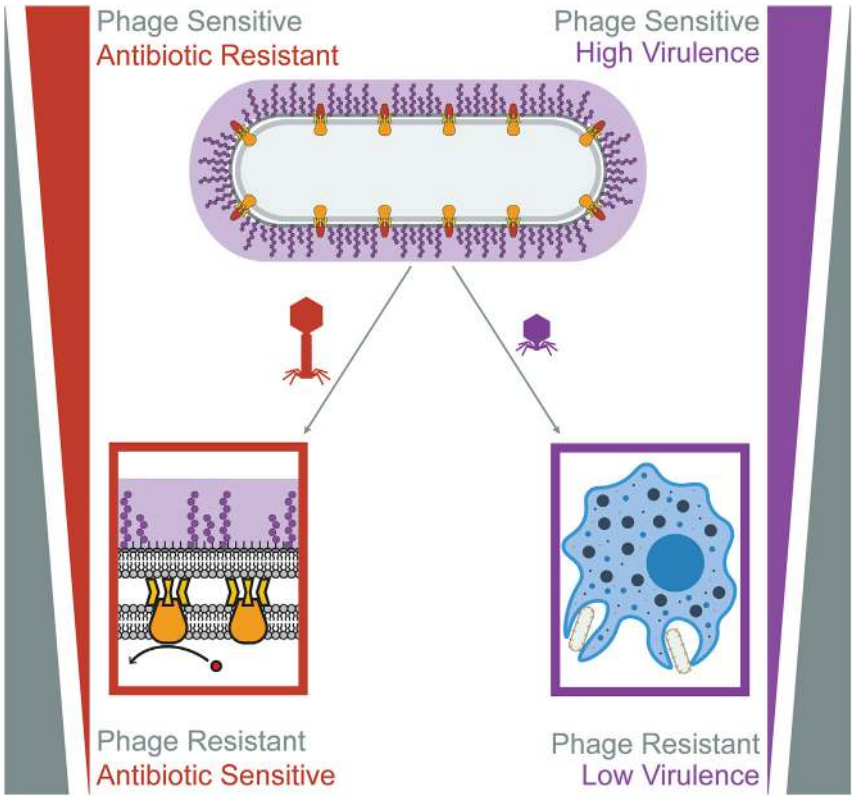

Figure 3. A Renewed Approach to Phage Therapy: Phage Selection against Virulence or Antibiotic Resistance

Certain lytic phage may be more effective in phage therapy, because they kill target bacteria while simultaneously imposing strong selection against bacterial virulence or antibiotic resistance when bacteria mutate to avoid phage attack. Phage that use antibiotic efflux pumps as receptors (red) can select for phage-resistant bacterial mutants with impaired efflux pumps; these phage-resistant bacterial mutants are more sensitive to antibiotics (Chan et al., 2016). Phage that bind to structural virulence factors such as a capsular antigen (purple) can select for phage-resistant bacterial mutants that lack the capsule (Smith and Huggins, 1982); these non-capsulated phage-resistant mutants are less virulent because they are more easily engulfed by phagocytic cells (Foster, 2005).

bacterial attachment and invasion of epithelial cells (Kaper et al., 2004; Bishop-Lilly et al., 2012; Choi et al., 2013; Davison et al., 2005).

Similarly, phage that attach to an antibiotic efflux pump to infect may select against the expression of the efflux pump, rendering the bacteria more sensitive to antibiotics that were previously effluxed (Figure 3). For example, phage TLS selected for *tolC* and *rfa* mutants in *E. coli* at a typical frequency of 10^{-5} to 10^{-6} (German and Misra, 2001). The TLS-resistant mutants with altered TolC were hyper-sensitive to novobiocin. Additionally, when phage-resistant mutants were selected in the presence of novobiocin, the frequency of recovered mutants decreased 1,000-fold. More recently, it was demonstrated that phage OMKO1 associates with the outer membrane protein M (OprM) of MexAB- and Mex-XY-OprM efflux pumps of the opportunistic pathogen *P. aeruginosa* (Chan et al., 2016). This interaction selects for phage-resistant mutants that are sensitive to antibiotics, as a "genetic trade-off." Chan et al. demonstrated that phage-resistant mutants, in both lab strains and clinical isolates of *P. aeruginosa*, were more sensitive to antibiotics, including ceftazidime. This was likely due to mutations or deletions in the operon encoding for the multidrug efflux pump resulting in nonfunctional gene products. Hypothetically, this promising result might also occur in other bacterial pathogens with similar modes of achieving broad antibiotic resistance via homologous or convergent efflux pump mechanisms. Overall, thoughtful consideration of the inevitable evolution of phage resistance during treatment could greatly benefit phage therapy efforts.

Animal Models for Efficacy

Animal studies can help bridge the gap between *in vitro* studies and actual clinical application of phage therapy. Unfortunately, most animal models investigate acute infections, which may not be the ideal analog for phage therapy targeting chronic infections in humans. Many of these studies observe best results when phage are applied simultaneously with the bacterial challenge, which will not necessarily be applicable in the clinic. In many cases, no measures were taken to check for the *in vivo* evolution of phage resistance by bacteria. Also, the comparison of phage treatment to antibiotic treatment or even a combination of phage and antibiotic treatments is only beginning to be investigated in animal models. Nevertheless, animal models provide vitally useful data on efficacy and safety of phage therapy in living hosts and are crucial for further development of the approach.

Systemic Infections

Several studies have investigated the efficacy of phage therapy for treatment of systemic infections. In a gut-derived model of *P. aeruginosa* sepsis, Watanabe et al. (2007) observed 67% survival of infected mice when phage therapy was administered orally 1 day post-infection. Capparelli et al. (2007) observed that successful protection of mice with a systemic *Staphylococcus aureus* infection depended on phage dose; Biswas et al. (2002) observed similar results of dose-dependent success in a mouse model of vancomycin-resistant *Enterococcus faecium* bacteremia. In a systemic disease model of *Vibrio vulnificus*, successful control of disease was only achieved when bacterial infection and phage treatment were administered simultaneously (Cerveny et al., 2002). The determinants of success for phage therapy to treat systemic infections are likely dependent on multiple factors which need to be thoroughly examined prior to the widespread use of phage as a treatment for sepsis in humans.

Local Infections

Phage therapy for localized infections (e.g., otitis, urinary tract infections, infected burns) is recognized for its potential to entirely circumvent the use of chemical antibiotics. Furthermore, use of

chemical antibiotics for surgical and hospital-acquired infections is limited, as these often constitute the strains with greatest antibiotic resistance. Watanabe et al. (2007) observed 92% survival of mice with an intraperitoneal *P. aeruginosa* infection treated simultaneously with phage. A similar study of *S. aureus* abscesses in mice by Capparelli et al. (2007) enumerated the reduction in bacterial load resulting from phage therapy and observed that phage applied concurrently with bacteria prevented the formation of abscesses. When administered 4 days after bacterial challenge, a single dose of phage resulted in a 100-fold reduction in bacterial load, whereas multiple doses of phage resulted in a 10,000-fold reduction (Capparelli et al., 2007). In a mouse model of *P. aeruginosa* infection of burn wounds, phage treatment improved survival rate from 6% in the untreated controls to 88% when phage were administered via intraperitoneal injection 72 h post-infection (McVay et al., 2007). In contrast, phage treatment only resulted in 22% or 28% survival when administered subcutaneously or intramuscularly. Further pharmacokinetic studies demonstrated that phage delivered intraperitoneally persisted at higher levels in the liver, spleen, and blood than phage delivered intramuscularly or subcutaneously (McVay et al., 2007). Finally, a murine model was used to investigate the ability of phage to treat an *E. coli* urinary tract infection (Dufour et al., 2016). Phage administered intraperitoneally 24 h after bacterial challenge resulted in a 100-fold reduction in bacterial load in the kidneys 48 h after phage treatment. The same phage resulted in a significant reduction in bacterial load in an *E. coli* pneumonia model but was ineffective in an *E. coli* model of sepsis.

Gastrointestinal Infections

Applying phage therapy to gastrointestinal bacterial infections could potentially reduce or prevent colonization of virulent bacteria without disrupting the natural gut flora. Galtier et al. (2017) observed that a preventative treatment of phage, 4 days after an adherent-invasive *E. coli* challenge, was able to reduce bacterial colonization in the gut of dextran sodium sulfate-treated mice and prevented the progression of colitis symptoms. In an insect model of *Clostridium difficile* colonization, prophylactic treatment with phage 2 h prior to bacterial challenge resulted in 100% survival, while simultaneous administration of phage and bacteria resulted in 72% survival and phage administration 2 h post bacterial challenge resulted in 30% survival (Nale et al., 2016). Yen et al. (2017) observed that prophylactic treatment with a phage cocktail was able to reduce *V. cholerae* colonization in the small intestine of infant mice when phage were provided 3 and 6 h prior to bacterial challenge. However, phage-resistant bacterial mutants were recovered after treatment, and effects of phage treatment were reduced when administered more than 6 h before bacterial challenge and when mice were challenged with a higher dose of *V. cholerae* (Yen et al., 2017). While the result of prophylactic treatment of gastrointestinal infections with phage is generally favorable, more studies that provide treatment after bacterial challenge, such as Galtier et al. (2017), are needed, as prophylactic treatment is not always possible in the clinic.

Lung Infections

Phage therapy for the treatment of lung infections, particularly chronic lung infections which are common in those with cystic fibrosis (CF), has seen renewed interest recently with the increase in MDR bacteria associated with the lung. Waters et al. (2017) observed complete eradication of a CF strain of *P. aeruginosa* in mice when two doses of phage were administered intranasally to infected mice 24/36 or 48/60 h after infection. Treatment at 144/156 h post-infection resulted in complete eradication of infection in 70% of mice and a significant reduction in the remaining 30%. In another CF lung infection model, phage treatment significantly improved the survival rate of mice when administered intranasally at 2 h post-infection (Morello et al., 2011). Interestingly, a high dose of phage administered 4 days prior to bacterial challenge provided complete protection to mice, indicating that prophylactic treatment with phage could prevent chronic infections. Semler et al. (2014) investigated different routes of administration of phage in a mouse model of *Burkholderia cepacia* complex respiratory infection. A 100-fold decrease in bacterial load was observed when phage was administered via nebulization, while no decrease was observed when administered via intraperitoneal injection (Semler et al., 2014). Promising results for both prophylactic and curative treatment of lung infections with phage indicate that these types of infections may be a reliable target for effective phage therapy.

Antibiotic and Phage in Combination

While there have been many *in vivo* studies on the efficacy of phage therapy, not many recent studies have compared the *in vivo* efficacy of phage therapy to that of antibiotics or even combined phage and antibiotic treatment. Huff et al. (2004) investigated the efficacy of traditional antibiotics, phage treatment, or a combination of both in a head-to-head trial in an *E. coli* challenge in broiler chickens. The standard of care treatment, enrofloxacin (fluoroquinolone), reduced mortality from 68% in untreated birds to 3%, while phage treatment alone reduced mortality to 15%. A combination therapy of phage and enrofloxacin resulted in no mortality. Similarly, Oechslin et al. (2017) observed that phage in combination with ciprofloxacin resulted in a 10,000-fold greater reduction in bacterial load as compared to phage or ciprofloxacin treatment alone in rats with experimental endocarditis due to *P. aeruginosa*. Furthermore, they noted that this particular combination of phage and antibiotics resulted in synergistic killing of *P. aeruginosa* both *in vitro* and *in vivo* (Oechslin et al., 2017). As the future of phage therapy will likely be that of combined therapy with chemical antibiotics, additional studies examining potential synergy between phage and antibiotics both *in vitro* and *in vivo* are needed.

Compared to phage therapy studies in *in vivo* animal models, there have been relatively few reports on the clinical use of phage and even fewer controlled clinical trials. As summarized in Table 1, below we describe some notable case studies and clinical trials that have been performed; the lists are not exhaustive, and other examples can be found in the literature (e.g., Jennes et al., 2017; Hoyle et al., 2018).

Case Reports of Emergency Phage Therapy

Case 1: Pseudomonas *Sepsis Case Report*

A child with DiGeorge syndrome and congenital heart disease presented with *P. aeruginosa* bacteremia following multiple surgeries that included insertion of a pacemaker (Duplessis et al.,

Table 1. Case Reports and Clinical Trials

Case Reports

	Infection	Complicating Conditions	Antibiotic Courses	Antibiotic Resistance or Allergies	Phage Dose and Application	Duration of Phage Treatment	Outcome
Case 1 (Duplessis et al., 2017)	*Pseudomonas aeruginosa* bacteremia	DiGeorge syndrome and congenital heart disease with pacemaker	Meropenem, tobramycin, aztreonam, polymyxin B, and colistin	Meropenem, tobramycin, aztreonam, polymyxin B, colistin, Cephalosporins, and fluoroquinolones	3.5×10^5 PFU delivered intravenously every 6 h	Initial treatment for 36 hours (six doses total), treatment resumed 11 days later	Blood cultures negative after phage treatment; reverted to positive following cessation of phage administration
Case 2. (Khawaldeh et al., 2011)	*Pseudomonas aeruginosa* urinary tract infection (2 years)	Intra-abdominal resection and irradiation for adenocarcinoma, bilateral ureteral stent placement	Gentamicin, ceftazidime, ciprofloxacin, and meropenem	None reported	2×10^7 PFU directly instilled into the bladder every 12 h	10 days (meropenem and colistin initiated on day 6)	Urine samples sterile following phage therapy and a 30-day course of meropenem
Case 3 (LaVergne et al., 2018)	*Acinetobacter baumanii* surgical site infection	Craniectomy	Combination of colistin, azithromycin, and rifampin	Intermediate sensitivity to colistin, with resistance to all other tested antibiotics	8.56×10^7 PFU delivered intravenously every 2 h	8 days (98 doses total)	Initial improvements observed; bacterial load not measured
Case 4 (Schooley et al., 2017)	*Acinetobacter baumanii* infected pseudocyst (3 months)	Necrotizing pancreatitis	Azithromycin, colistin, and rifampin	Cephalosporins, meropenem, gentamicin, amikacin, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, and colistin	5×10^9 PFU delivered intravenously every 6 h	84 days (336 doses total), minocycline added on day 2	Clinical improvement and resolution of infection
Case 5 (Chan et al., 2018)	*Pseudomonas aeruginosa* infected aortic graft (3 years)	Aorto-cutaneous fistula	Ceftazidime and ciprofloxacin	Ciprofloxacin	1×10^8 PFU delivered topically on fistula	Single dose	Cultures negative four weeks post treatment; no recurrence of infection after >2 years

Clinical Trials

	Infection	Trial	Treatment Group	Placebo Group	Phage Dose and Application	Outcome
Trial 1 (Wright et al., 2009)	*Pseudomonas aeruginosa* otitis	Placebo controlled, double blind for safety and preliminary effectiveness	12 individuals received phage cocktail	12 individuals received a single dose of glycerol-PBS buffer	10^9 PFU delivered intra-aurally (single dose)	Three individuals from each group had undetectable levels of *P. aeruginosa* at the end of the trial
Trial 2 (Sarker et al., 2016)	*Escherichia coli* diarrheal diseases	Placebo controlled, double blind for safety and efficacy	40 individuals received phage cocktail M, 39 individuals received phage cocktail T	41 individuals received oral rehydration solution	1.4×10^9 PFU cocktail M or 3.6×10^8 PFU cocktail T delivered orally in oral rehydration solution three times per day for 4 days (12 doses)	No significant difference between phage treatment group and placebo group
Trial 3 (Jault et al., 2018)	*Pseudomonas aeruginosa* burn wound infection	Placebo controlled, blinded trial for safety and efficacy	12 individuals received a phage cocktail	13 individuals received standard of care 1% sulfadiazine silver	2×10^7 PFU (expected) 200–2,000 PFU (actual) applied topically one time per day for 7 days (seven doses)	Trial halted due to insufficient efficacy; this was likely due to significantly lower applied dose of phage than expected

Details and summaries are provided for each of the case reports and clinical trials discussed in the main text.

2017). Anti-pseudomonal antibiotics initially controlled the infection but ultimately failed. Adverse reactions to cephalosporins and fluoroquinolones further limited antibiotic options. Phage provided by the U.S. Navy were screened for lytic activity against the infectious strain, and a cocktail of two phage was created. After intravenous phage administration, blood cultures fluctuated between positive for *P. aeruginosa* and below the limit of detection for several days. Phage therapy was resumed on day 11, following a temporary cessation due to decompensation attributed to progressive heart failure, which coincided with 4 days of blood cultures negative for *P. aeruginosa*. In this case, phage therapy appeared to reduce the infection in the blood, though it was apparently ineffective at source control, as blood cultures reverted to positive upon termination of therapy.

Case 2: Urinary Tract Infection Case Report

Khawaldeh et al. (2011) reported treatment of a *P. aeruginosa* urinary tract infection associated with a bilateral ureteral stent. Following cessation of antibiotic therapy, the infection consistently recurred within a week. Libraries of phage from the Eliava Institute were screened against the bacterial isolate, and a suitable commercial phage product was identified. This phage cocktail contained phage with activity against *Streptococcus pyogenes*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus vulgaris*, and *Proteus mirabilis*. On day 6 of the treatment, antibiotic therapy with meropenem and colistin was initiated. Khawaldeh et al. (2011) reported 10-fold reduction of bacteria in the urine after 5 days of phage treatment. Two days of subsequent antibiotic treatment resulted in apparent clearance of the infection, at which point culturable *P. aeruginosa* was below the limit of detection. Following completion of the 30-day course of meropenem, both stents were removed, and one was replaced. Urine samples remained sterile for 1 year after treatment, at which point observations were concluded.

Case 3: Surgical Site Infection Case Report

LaVergne et al. (2018) reported treatment of a postoperative *A. baumanii* infection in an individual following a craniectomy. Strains of *A. baumannii* isolated from the infection were resistant to almost all antibiotics and antibiotic combination therapy. Phage provided by the U.S. Navy were screened for lytic activity against the infectious strain, and a cocktail of five phage was chosen for therapeutic use. Five minutes after phage administration, the concentration of phage in blood was approximately 100 PFU/mL. This was approximately 100-fold lower than expected if the phage was simply diluted into an average human blood volume. Ten minutes after administration, phage were undetectable. Despite initial improvements, the lack of significant improvement led to withdrawal of care. Unfortunately, bacterial load was not measured during the course of treatment, and it is difficult to attribute any clinical improvement to the administration of phage. It appears that in this case phage were actively removed from the blood, either through adsorption to bacteria *in vivo* or active removal by the body.

Case 4: Pancreatitis Case Report

Phage therapy was utilized in a case of necrotizing pancreatitis complicated by an MDR *A. baumannii*-infected pseudocyst (Schooley et al., 2017). Phage from several institutions were screened against the isolated infectious strains, and two cocktails, each consisting of four different phage, were formulated. The first cocktail was administered via lavage at the site of the pancreatic pseudocyst. The following day, the second cocktail was administered intravenously. Resistance emerged against all of the phage used in both cocktails by 8 days after the first administration of phage, suggesting phage selection occurred *in vivo*. A third phage cocktail was formulated that was active against the resistant strains and was again administered intravenously. Resistance to the original cocktails correlated with increased presence of encapsulated bacteria, further suggesting bacterial response to phage administration. Phage therapy was continued for approximately 12 weeks, over the course of which clinical improvement was observed and the infection was eventually resolved.

Case 5: Aortic Graft Infection Case Report

Surgical intervention to repair an aortic aneurysm with a Dacron graft resulted in a *P. aeruginosa* infection that was refractory to standard treatment (Chan et al., 2018). This chronic infection resulted in the formation of an aorto-cutaneous fistula with purulent discharge. Infection control was attempted with intravenous ceftazidime followed by oral ciprofloxacin. Resistance to ciprofloxacin evolved during the course of treatment, debridement and irrigation were unsuccessful in resolving the infection, and surgical replacement of the graft was not an option. After 3 years of suppressive antibiotic therapy which failed to eradicate the infection, other options for infection control were considered. A recent report of *P. aeruginosa* phage OMKO1 that had demonstrated synergy when used in combination with ceftazidime (Chan et al., 2016) was screened for lytic activity against the strain. Instillation of a single dose of phage OMKO1 and ceftazidime was applied topically at the site of fistular discharge while continuing the existing therapy of intravenous ceftazidime. Four weeks after the administration of phage, partial graft excision and replacement was required following bleeding from the fistula. Cultures taken at the time of surgery were negative for *P. aeruginosa*, and the course of ceftazidime was discontinued. Two years after phage treatment, there was no recurrence of the infection in the absence of any antibiotic therapy. The favorable outcome of this case underscores the rational choice of phage and route of administration for this particular infection; thoughtful selection of a phage that had previously demonstrated synergy with the clinically relevant antibiotics, applied in proximity to the source of infection undoubtedly contributed to the positive outcome.

Clinical Trials of Phage Therapy

Trial 1: Otitis Clinical Trial

In 2009, a clinical trial was designed to investigate the safety and preliminary efficacy of phage therapy for treating chronic *P. aeruginosa* otitis. Wright et al. (2009) utilized a cocktail of six phage with lytic activity against *P. aeruginosa* in individuals with chronic otitis. While the authors report a significant accumulated reduction of bacterial counts in the phage treatment group and no significant accumulated change of bacterial counts in the placebo group, three individuals from each group had undetectable levels of *P. aeruginosa* by day 42. Phage were isolated for an average of 23 days from individuals in the phage treatment group, suggesting either that phage were cleared upon resolution of the infection, phage were unable to reach the site of

Table 2. Comparing and Contrasting Antibiotics and Phage

Antibiotics	Lytic or "Virulent" Phage
Activity and Mechanism of Action	
Bacteriostatic or Bacteriacidal	Bacteriacidal
Typically disrupts ONE bacterial process	Disrupts MANY/ALL bacterial processes
Broad spectrum more common than narrow spectrum	High degree of species or strain specificity
Disruption of microbiome	Only disrupts target bacteria
Not very effective against biofilms	Penetration and destruction of biofilms
Clinical Use	
Minimal identification of bacteria	Phage tested against target bacteria
Short time between diagnosis and treatment	Longer time between diagnosis and treatment
Constant dosing to maintain inhibitory concentrations	Self-amplifying while target bacteria are present
Potential for immune recognition	Potential for immune recognition
Widely accepted; used as treatment for infections	Pushback on clinical application; only used on a compassionate care basis
Diffusion through membranes allows for treatment of intracellular bacteria	Unable to penetrate eukaryotic cells
Discovery, Production, and Manufacturing	
Slow discovery process	Rapid discovery process
Production methods in place to ensure safety of products	Steps must be taken to ensure removal of contaminating bacterial antigens generated during production
Regulations for production and manufacturing in place	Require new regulations for production and manufacturing
Resistance	
Resistance inevitable	Resistance inevitable, but phage can co-evolve to infect resistant bacteria
Resistance frequently accompanied by compensatory mutations	Resistant mutants may result in lower fitness via reduced virulence or antibiotic sensitivity

A rational approach to phage therapy has many potential benefits that cannot be achieved with antibiotics alone. However, there are also limitations to phage therapy in comparison to traditional antibiotics. While many of these differences historically have been considered limitations to using phage therapy, in some circumstances the perceived drawbacks may instead be leveraged as benefits. In the past, both types of therapies have typically been investigated alone; however, with many identified differences, a combination approach utilizing both therapies may prove to be the most efficacious in the long run.

infection, or the bacteria became phage resistant. There were no serious adverse events reported in either group, indicating the safety of phage therapy for the treatment of otitis.

Trial 2: Diarrheal-Disease Clinical Trial

A clinical trial was conducted in Bangladesh to test safety and efficacy of two different phage cocktails that target pathogenic *E. coli* in diarrheal diseases (Sarker et al., 2016). Individuals presenting with acute onset of dehydrating diarrhea were admitted to the study. Phage treatment consisted of oral administration of one of two different phage cocktails that had been previously characterized: a T4-like phage cocktail (T) containing 11 phage and a commercial phage cocktail (M) from Russia consisting of at least 17 different phage in oral rehydration solution. Standard treatment of oral rehydration solution was given to the placebo group. There were no adverse effects reported and no significant differences between the phage treatment and placebo groups. It was unclear if the phage were lytic against the specific *E. coli* strains causing disease, and no actions were taken to buffer the stomach prior to the administration of phage. It is possible, therefore, that significant numbers of phage particles were unable to survive the low-pH environment of the stomach and that the surviving particles were unable to amplify due to the lack of an appropriate host (Sarker et al., 2017).

Trial 3: PhagoBurn Burn Wound Clinical Trial

The most recent clinical trial to date, PhagoBurn, evaluated the safety and efficacy of phage therapy to treat *P. aeruginosa*-infected burn wounds (Jault et al., 2018). A phage cocktail of 12 phage with lytic activity against *P. aeruginosa* was added to an alginate template that was applied directly to the wound. The control group received standard of care treatment which consisted of 1% sulfadiazine silver applied topically. The average time to sterilization for the phage treated group and control group was 144 h and 47 h, respectively. These unexpectedly poor results could be explained by administration of a lower phage dose than intended. The concentration of the phage cocktail had dropped over the course of the study, and a dose of 200–2,000 PFU was used instead of the expected 2×10^7 PFU dose. With concentrations of phage 4 to 5 orders of magnitude lower than expected, it is possible that the lack of efficacy can be attributed to this unintended change in the treatment protocol.

Perspectives and Future Directions

Will Phage Ever Replace Antibiotics?

A rational approach to phage therapy has many potential advantages over a traditional chemical antibiotic approach (Table 2). (1) The non-lethal nature of some bacteriostatic antibiotics may

permit antibiotic resistance to evolve more easily, as well as accommodate the emergence of persistor cells that are genotypically equivalent to wild-type bacteria yet physiologically capable of withstanding antibiotic exposure (Kudrin et al., 2017). On the other hand, lytic phage are always bactericidal, lysing cells at the completion of a replication cycle. Furthermore, phage (unlike antibiotics) hijack many essential cellular processes, including DNA replication, transcription, and translation upon infection, and are perhaps harder targets for evolution of bacterial resistance (Loc-Carrillo and Abedon, 2011). (2) The popular broad-spectrum antibiotics may disrupt the normal balance of the microbiome, which might otherwise provide a protective effect by occupying niche sites that prevent or constrain bacterial pathogens from invading the body (Theriot & Young, 2015). (3) Phage can be specific to species and even single strains of bacteria, making them an ideal therapeutic to selectively target and kill pathogens (Loc-Carrillo and Abedon, 2011). The clinical use of phage will likely require preliminary laboratory assays to identify susceptibility of strains to therapeutic phage. Phage susceptibility could be determined in parallel with antibiotic sensitivity to ensure a better match between the proposed drug (phage) and the target bacterial strain. Relatedly, this may explain why the above-described case reports have been generally more successful than clinical trials to date (see case studies and clinical trials summarized in Table 1). (4) In addition, while antibiotics must be continually dosed to clear infection, phage are able to amplify at the site of infection (suggesting fewer doses should be needed) and will be cleared from the body when the susceptible bacteria are gone. (5) Resistance to antimicrobials is inevitable (Luria and Delbrück, 1943). However, unlike antibiotic therapy, phage therapy can take advantage of this outcome via careful choice of therapeutic phage that select for resistant bacterial mutants with lower fitness, especially reduced virulence, or impaired antibiotic efflux (see above section, Phage Therapy: A Renewed Approach). (6) Furthermore, as phage co-evolve with bacteria over time, it is possible that the administered phage population will evolve to infect the phage-resistant bacteria (an arms race), which is not possible for antibiotics. (7) Phage treatment of biofilms may prove more promising than antibiotic treatment of biofilms (Chan et al., 2018); however, this difference may depend on the target bacterium, and the general benefits of using phage to treat biofilms merits further investigation (Darch et al., 2017). (8) Finally, while novel antibiotic discovery has stagnated in recent years, discovery of new phage has proven expeditious due to the vast biodiversity of phage in nature that have useful properties in biotechnology (Fair and Tor, 2014; Loc-Carrillo and Abedon, 2011).

However, even with this more rational phage therapy approach, phage still have some limitations compared to traditional chemical antibiotics that need to be addressed before phage therapy can be fully accepted in modern clinical practice (Table 2). (1) Phage will likely not be an appropriate therapeutic for all infections. While some antibiotics are capable of treating intracellular bacterial pathogens, phage do not have a reliable mechanism of entry into eukaryotic cells. (2) Also, there have been decades of research performed on antibiotics and their interactions with the immune system, whereas abundant analogous research with phage has yet to be completed (Loc-Carrillo and Abedon, 2011; Roach et al., 2017). (3) Since phage can be found everywhere, including within the human microbiome, neutralizing antibodies against certain phage typically associated with humans may be a general obstacle for phage therapy. (4) Because a phage population can undergo rapid exponential growth, widespread lysis of target bacteria can potentially release bacterial antigens that could be dangerous, particularly if the phage is administered internally; thus, endotoxin removal from preparations of phage lysates intended for therapy, as well as generation of endotoxins during therapy, are valid concerns. (5) Finally, regulatory hurdles represent a significant barrier to the implementation of phage therapy in modern medicine; unlike the well-established path to approval for antibiotics, this path is currently being paved for phage, and therefore very little useful precedence exists.

Realistically, therapeutic use of phage may never completely replace administration of chemical antibiotics and may be inappropriate under some clinical conditions, suggesting that adjuvant approaches should be closely studied. A mixed therapy of phage and antibiotics could be an ideal combination that capitalizes on each treatment's differing strengths (Table 2). Bedi et al. (2009) observed an additive effect when phage and antibiotics were used to treat a *Klebsiella pneumoniae* biofilm. Knezevic et al. (2013) investigated the potential for phage-antibiotic synergism and observed synergy between *P. aeruginosa* phage and subinhibitory concentrations of ceftriaxone, but not with gentamicin, ciprofloxacin, or polymyxin B. They proposed that the mechanism of action of the antibiotic must not interfere with critical processes in phage replication to see a synergistic effect. While not all phage and antibiotic combinations appear to be synergistic, and the mechanisms behind synergism are still being explored, precision medicine is currently in vogue, and phage therapy shows promise as a "personalized" approach for at least some clinical cases. As with any drug, the ideal circumstance is that phage therapy should be developed to reduce off-target effects and to minimize disruption of helpful microbiome communities to the extent possible.

Other Considerations for Phage Therapy

One method employed to expand phage host range and subvert the criticism of narrow spectrum is to combine multiple phage to create phage cocktails. Traditionally, this has been perceived as a benefit, allowing the cocktail to be used against different strains or species of bacteria, and presumably decreasing the likelihood that mutations against all of the phage will simultaneously occur. Recent case studies show that in principle either a single phage or phage-cocktail approach might work (see Clinical Cases) (Chan et al., 2018; Schooley et al., 2017). However, a conceivable drawback of phage cocktails is their ability to select for "broad-spectrum" mechanisms of phage resistance, such as the production of a capsule that surrounds the cell, preventing phage binding (Schooley et al., 2017). For these reasons, a rational approach to designing cocktails is warranted, involving consideration of mechanism(s) whereby phage resistance can evolve, potential for bacteria to develop cross-resistance to multiple phage, and confirmation that the various phage in a cocktail do not

compete with one another to reduce the overall efficacy. At the least, we see relevance for a more dedicated merger between phage-cocktail formulation and core principles and theories of evolution and ecological competition.

A related subject is the mode of delivery for phage in therapy and whether this choice would impact relative ratios of phage to bacteria at the site(s) of infection, and/or ratios among phage in a cocktail as they are administered versus the times when they actually encounter the infecting bacteria. The self-amplifying nature and lethality of lytic phage suggest that this therapy should often avoid the analogous consequence of bacterial evolution of resistance to chemical antibiotics delivered at too low of concentration. However, the density of phage particles relative to target bacterial cells may greatly impact timing and quality of phage therapy outcome, and mode of delivery necessarily affects these key ratios. Therefore, the route of administration for phage therapy should consider the most likely method to deliver the highest concentration of phage particles to the site of infection, and therapeutic approaches might consider low initial doses of phage that are adjusted over time. Phage studies have not focused very closely on the therapy benefits versus costs of changes in the multiplicity of infection (ratio of phage particles to target susceptible bacterial cells) over time, either via administration or by estimating changes within the treated human or animal model; this seems like a relevant focus for phage therapy research moving forward.

Summary

Renewed interest in phage therapy in the West, and its continued development in countries such as Poland, Russia, and Georgia, marks a time for optimism for a viable alternative (or adjunct) to antibiotic therapy. Phage therapy, like any medical treatment, has benefits, costs, and limitations in usefulness that merit close scrutiny (Bull and Gill, 2014). Overall, we can identify several intriguing questions and topics that should be addressed, especially as greater numbers of clinical trials on phage therapy are planned and executed. Is it possible to discover or engineer individual phage strains that broadly infect genotypes of a target pathogen, meriting their approval as standalone "drugs"? Or would additional experiments on phage cocktails provide convincing evidence that phage mixtures should be the standard of care? Bioprospecting for phage is likely to continue yielding candidates with useful biologically properties, such as ability to select for reduced virulence and re-sensitization of bacteria to antibiotics. But what is the probability that bacteria can evolve simultaneous resistance to both phage and antibiotics at no cost? How can the process of bioprospecting for phage be made more efficient? Could developing genomics and bioinformatics analyses as well as computer algorithms help identify potential phage candidates and predict phage binding to cell receptors, strictly through high-throughput sequencing? Would reliable models such as non-pathogenic *E. coli* yield sufficiently useful data to predict utility of phage therapy in pathogens that are much harder to culture in the laboratory or where we lack animal models for acute and chronic bacterial diseases? How is phage therapy observed or theoretically predicted to interact with the immune system and human microbiomes (Roach et al., 2017; Leung and Weitz, 2017; Torres-Barceló and Hochberg, 2016) to either enhance or suppress this approach in resolving systemic and biofilm infections? How would these interactions differ in sites as different as the respiratory and gastrointestinal systems? This non-exhaustive list highlights many hypotheses regarding phage therapy that should be the focus of future basic research. Fortunately, this is an opportune time for basic researchers, clinicians, and physicians to work together to address open questions through rigorous experiments in the laboratory, *in vivo* models, and clinical cases to reward phage therapy with the renewed interest and greater examination that it deserves.

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