

A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens

Xavier Wittebole^{1,*}, Sophie De Roock², and Steven M Opal^{3,4}

¹Critical Care Department; St Luc University Hospital; Université Catholique de Louvain; Brussels, Belgium; ²High Care Burn Unit; Military Hospital; Brussels, Belgium;

³The Infectious Disease Division; Memorial Hospital of RI; Providence, RI USA; ⁴The Alpert Medical School of Brown University; Providence, RI USA

Keywords: sepsis, septic shock, multidrug resistant pathogens, bacteriophage therapy, phage therapy

The seemingly inexorable spread of antibiotic resistance genes among microbial pathogens now threatens the long-term viability of our current antimicrobial therapy to treat severe bacterial infections such as sepsis. Antibiotic resistance is reaching a crisis situation in some bacterial pathogens where few therapeutic alternatives remain and pan-resistant strains are becoming more prevalent. Non-antibiotic therapies to treat bacterial infections are now under serious consideration and one possible option is the therapeutic use of specific phage particles that target bacterial pathogens. Bacteriophage therapy has essentially been re-discovered by modern medicine after widespread use of phage therapy in the pre-antibiotic era lost favor, at least in Western countries, after the introduction of antibiotics. We review the current therapeutic rationale and clinical experience with phage therapy as a treatment for invasive bacterial infection as novel alternative to antimicrobial chemotherapy.

Introduction

Upon the introduction of the new *Bacteriophage* journal, launched in early 2011, Alexander Sulakvelidze defined bacteriophages as “the most ubiquitous organisms on Earth, playing a significant role in maintaining microbial balance on this planet”.¹ Indeed, bacteriophages or phages are everywhere where their bacterial host is present; it has been established that the population number of phages in aquatic systems lies within the range of 10^4 to 10^8 virions per ml and about 10^9 virions per g in the soil,² with an estimated total number of 10^{32} bacteriophages on the planet.³ Initially described almost a century ago by William Twort, and independently discovered shortly thereafter by Félix d’Herelle (considered by many as the founder of bacteriophages and its therapeutic implication: the phage therapy), phages are small viruses displaying the ability to kill bacteria while they do not affect cell lines from other organisms. Because of the specificity of cellular target hosts, application of phages has been proposed

since its inception as a therapy to treat acute and chronic infections with initial successes first described in the disciplines of dermatology, ophthalmology, urology, stomatology, pediatrics, otolaryngology, and surgery.^{4–6} The initial fervor over phage therapy as a treatment for bacterial diseases in the pre-antibiotic era was understandably enormous. Indeed, the only therapy available in the 1920s and most of the 1930s was serum therapy for selected pathogens such as pneumococci and diphtheria. The use of bacteriophages was even described with considerable fanfare when the main protagonist in the Sinclair Lewis’s Pulitzer Prize-winning novel, *Arrowsmith*, used this treatment to fight a bubonic plague outbreak on a Caribbean island.

This concept of the therapeutic use of phages to treat bacterial infection was, however, highly controversial from the very beginning and not widely accepted by the public or medical community alike. Early studies were widely criticized for lack of appropriate controls and inconsistent results. The lack of reproducibility and many conflicting results obtained in the various published studies led the Council on Pharmacy and Chemistry of the American Medical Association to conclude that the evidence for the therapeutic value of lytic filtrates was for the most part contradictory, unconvincing, and recommended additional research to confirm its purported benefits.^{7–9} The emergence of age of antibiotic chemotherapy with the introduction of sulfa drugs in the 1930s and later penicillin in the 1940s further dampened enthusiasm on phage research and therapy was largely relegated to medical history in the western countries. However, phage therapy remained an active area of research and development in the former USSR, Poland, and to a lesser extent India. Remarkably, over the last decade, the emergence of multi-drug resistant bacteria has led investigators to re-consider this century-old approach and take a fresh look at phage therapy as a “new” and potentially viable treatment option for difficult to treat bacterial pathogens.

In this review, we will discuss the origins of phage therapy and the biology and lifecycle of phage, along with a summary of the experimental and clinical data in support of phage therapy as a treatment for multidrug-resistant (MDR) bacterial infection and sepsis. Whether phage therapy will ever reach its full therapeutic potential in the modern intensive unit setting remains to be seen, yet its practical utility as an alternative to antibiotics to

*Correspondence to: Xavier Wittebole;

Email: xavier.wittebole@uclouvain.be

Submitted: 06/27/2013; Revised: 07/30/2013; Accepted: 07/31/2013

<http://dx.doi.org/10.4161/viru.25991>

treat human sepsis from pathogens carrying multiple antibiotic resistance genes is now being seriously re-considered.

Historical Background

In 1896, Ernest Hanbury Hankin, a British bacteriologist working as the Chemical Examiner and Bacteriologist to the Government of the United Provinces and of the Central Provinces of India, demonstrated that the waters from the Indian rivers Ganga and Yamuna contained a biological principle that destroyed cultures of cholera-inducing bacteria. This substance could pass through millipore filters, known to be able to retain larger microorganisms such as bacteria. He published his work in French in the *Annals of the Pasteur Institute*.¹⁰ In 1915, while he was studying the growth of vaccinia virus on cell-free agar media, Frederick Twort, a British microbiologist, noted that “pure” cultures of bacteria may be associated with a filter-passing transparent material which may entirely break down bacteria of a culture into granules.¹¹ This “filterable agent” was demonstrated in cultures of micrococci isolated from vaccinia: material of some colonies which could not be sub-cultured was able to infect a fresh growth of micrococcus, and this condition could be transmitted to fresh cultures of the microorganism for almost indefinite number of generations. This transparent material, which was found to be unable to grow in the absence of bacteria, was described by Twort as a ferment secreted by the microorganism for some purpose not clear at that time.

Two years after this report, Félix d’Herelle independently described a similar experimental finding, while studying patients suffering or recovering from bacillary dysentery. He isolated from stools of recovering shigellosis patients a so-called “anti-Shiga microbe” by filtering stools that were incubated for 18 h. This active filtrate, when added either to a culture or an emulsion of the Shiga bacilli, was able to cause arrest of the culture, death and finally lysis of the bacilli.¹² D’Herelle described his discovery as a microbe that was a “veritable” microbe of immunity and an obligate bacteriophage. He also demonstrated the activity of this anti-Shiga microbe by inoculating laboratory animals as a treatment for shigellosis, seeming to confirm the clinical significance of his finding by satisfying at least some of Koch’s postulates.

Beyond the actual discussion on origins of d’Herelle himself (some people stating he was born in Paris while others claim he was born in Montreal), the initial controversy was driven mainly by Bordet and his colleague Gartig at the Institut Pasteur in Brussels. These authors offered competing claims about the exact nature and importance of the fundamental discovery.¹³⁻¹⁵ While Twort, due to a lack of funds and his enlistment in the Royal Army Medical Corps, did not pursue his research in the same domain, d’Herelle introduced the use of bacteriophages in clinical medicine and published many non-randomized trials from experience all over the world. He even introduced treatment with intravenous phage for invasive infections, and he summarized all these findings and observations in 1931.⁴ The first published paper on the clinical use of phage, however, was published in Belgium by Bruynoghe and Maisin, who used bacteriophage to treat cutaneous furuncles and carbuncles by injection

of staphylococcal-specific phage near the base of the cutaneous boils. They described clear evidence of clinical improvement within 48 h, with reduction in pain, swelling, and fever in treated patients.¹⁶

At that time, the exact nature of phage had yet to be determined and it remained a matter of active and lively debate. The lack of knowledge of the essential nature of DNA and RNA as the genetic essence of life hampered a fuller understanding about phage biology in the early 20th century. In 1938 John Northrop still concluded from his own work that bacteriophages were produced by living host by the generation of an inert protein which is changed to the active phage by an auto-catalytic reaction.¹⁷ However, several contributions from other investigators did converge to support d’Herelle’s idea that phages were living particles or viruses when replicating in their host cells. In 1928 Wollman assimilated the properties of phages to those of genes,¹⁸ an idea already hypothesized by Muller in 1922.¹⁹ The phenomenon of lysogeny, or the fact that bacteriophages may infect bacteria without the induction of lysis, discovered in 1925 by Bordet²⁰ and Bail, confirmed the idea that the capacity of reproducing phages within bacteria necessitated the insertion of phage-encoded material into the hereditary units of the host microbe. Frank Macfarlane, an Australian scientist awarded the Nobel Prize in 1960 for his work on immunity, also worked on lysogeny and confirmed the viral nature of phages as well as the nature of its interactions with bacterial hosts.²¹ He also demonstrated that different species of phages did exist.^{22,23} Schlesinger confirmed the biochemical nature of phages made of nucleoproteins^{23,24} allowed the existing theories to join together: phages are viral particles that are made of nucleoproteins.

Finally, the invention of the electron microscope (EM) allowed Helmut Ruska, a German doctor, to first describe round particles as well as “sperm-shaped” particles from a phage suspension adhering to a bacterial membrane.²⁵ Two years later, he summarized his principal research into the nature and biology of bacteriophages in his thesis work.²⁶ One year after the first description of phages with EM, Luria and Anderson, in Camden, New Jersey, visualized different types of phages and described their common structure: a non-homogeneous round head with a much thinner tail, giving the peculiar sperm-like appearance.^{27,28} They also described the various stages of bacterial lysis: adsorption which increases with time, extensive bacterial damage and appearance of a large number of newly formed bacteriophages.

While research on phage was never abandoned in the former USSR, with the development of the Eliava Institute in Tbilisi, Georgia, and some other countries such as Poland (and its well-known Hirsfeld Institute in Wroclaw), the English literature re-discovered phage therapy in animals in the 1980s^{29,30} and human experiments started in the 2000s, with the first phase I randomized trial in the US published in 2009.³¹

In August 2004, the so-called Phage Summit was held in Key Biscayne, Florida, and more than 350 conferees attended this first major international gathering in decades devoted to phage biology, demonstrating the explosive resurgence of interest in this field.³² Overall, the phage literature has become one of the most expansive topics, rendering bacteriophages as one of the best

studied microbes known to science. In 1958 and 1967, Raettig published 2 bibliographies, covering about 11 358 references.^{33,34} In 2012, Ackerman analyzed 30 000 phage publications published between 1965 and 2010.³⁴ The names of first authors represent 40 linguistic domains or geographic areas and at least 70 languages, leading to the conclusion that phage particles are studied all over the world (even if English and German languages predominate).

Types of Phages and Phage Biology

More than 6000 different bacteriophages have been discovered and described morphologically, including 6196 bacterial and 88 archeal viruses.³⁵ The vast majority of these viruses are tailed while a small proportion are polyhedral, filamentous or pleomorphic. They may be classified according to their morphology, their genetic content (DNA vs. RNA), their specific host (for instance the staphylococcal phage family,³⁶ the *Pseudomonas* phage family,³⁷ and so on), the place where they live (marine virus vs. other habitats), and their life cycle (see below). Evolving classification formats have been proposed over time^{35,38-41} and abbreviations for these viruses were proposed by Fauquet and Pringle in 2000.⁴²

As obligatory intracellular parasite of a bacterial cell, phages display different life cycles within the bacterial host: lytic, lysogenic, pseudo-lysogenic, and chronic infection.^{2,43} For phage therapy, the main interest has focused upon lytic phages, mainly represented in 3 families of the *Caudovirales* order: the *Myoviridae*, the *Siphoviridae* and the *Podoviridae*. There are also some reports on cubic phages and filamentous phages applications.⁴³ General description of those phages may be summarized as follows: the genetic material is contained in a protein shell or capsid which has a form of an icosahedron; this head is connected through a collar to the tail which may be contractile or not and whose distal extremity is in contact with tail fibers with tips that recognize attachment sites on receptors of the bacterial cell surface.

Whatever the type of cycle of a phage life, the first step is the attachment to receptors of the bacterial cell wall before phages may enter the bacteria. This specific process influences the spectrum of the possible phage-bacteria interactions. For instance, bacteriophage λ interacts only with the LamB receptor of *E. coli*. Spatiotemporal dynamics have demonstrated this event to be of major importance for successful bacterial invasion.⁴⁴ Some phages also are able to synthesize specific enzymes (such as hydrolases or polysaccharidases and polysaccharide lyases) able at degrading exopolysaccharide structure capsules, before they may interact with their specific receptor. This is the case for some phages interacting with strains of *E. coli*, *V. cholerae*, *P. aeruginosa*, *E. agglomerans*, and *P. putida*.⁴³ These enzymes are of potential interest for their therapeutic implications and are in pre-clinical development at present.⁴³

Upon binding to its specific receptor, phages induce a pore in the bacterial cell wall and inject its DNA into the cell, while the viral capsid remains outside of the bacteria. This is followed by the expression of phage early genes, which, in the case of lytic phages, redirects the bacterial synthetic machinery to the reproduction of viral nucleic acids and proteins. Assembly and packing

of phages is then observed before bacterial cell lysis and release of phage progeny occur. Phages' late enzymes such as lysins, holins, and murein synthesis inhibitors are then employed for the virion burst in the extracellular environment. The number of viral particles released, or burst size, greatly varies according to the phage, the state of the bacteria host, and other environmental factors such as nutritive components surrounding the host.²

In the lysogenic cycle, the so-called temperate phages insert their genetic content (the prophage) in the chromosomes of the bacteria, where it remains silent for extended periods and is replicated as part of the bacterial chromosome. Hence, there is no self-replication. This prophage DNA is vertically transmitted along with the whole bacterial genome to its progeny until the lytic cycle is induced.² During induction lysogenic phage can on occasion transfer host genetic material adjacent to its insertion site on the chromosome from one bacterium to another, a phenomenon called transduction. Actually, the fact that phages are of major importance for bacterial genome evolution is a concept known for years, and Brussow even described bacteriophages as agents for lateral gene transfer.⁴⁵ This process can promote the transfer of genes that are of selective advantage for bacterial host including antibiotic resistance genes; however, the same process could be exploited therapeutically by using phage to transfer genes rendering bacteria more susceptible to some antibiotics. Indeed, by targeting the mechanisms of DNA repair with the injection of a specific gene which led to the overexpression of a protein that inhibits this system, Lu and Collins demonstrated, in vitro, an increased susceptibility of *E. coli* to antibiotics.⁴⁶ Gene insertion was achieved through a specific, and modified, bacteriophage M13. Interestingly, they also used the same technique in mice, intraperitoneally infected with *E. coli*. Survival was increased in mice concomitantly treated with antibiotics and modified phages. This approach was found by other authors to be similar to the general approach of phage therapy that leads to direct killing of bacteria.⁴⁷

Another approach consists in reversing the pathogen drug resistance by injecting specific genes for a sensitizing cassette conferring susceptibility in a dominant fashion. This was recently demonstrated by Edgar and colleagues who were able to render resistant bacteria susceptible to streptomycin and nalidixic acid.⁴⁷ Finally, the chronic infection occurs when the bacteria is infected by lysogenic phage that subsequently mutates and loses the capacity to induce a lytic replication cycle. The phage DNA becomes a new part of the bacterial chromosome and becomes a long-term prophage sequence.

Why Would We Need Phage Therapy?

Over the 2 or 3 last decades, the widespread emergence and spread of antibiotic-resistant bacteria around the world has become a major therapeutic challenge.^{48,49} For instance, MRSA infections in the US were reported with an incidence of about 100 000 serious infections in 2005, contributing to 20 000 deaths.⁵⁰ The limited therapeutic options remaining to treat major multi-drug resistant (MDR) bacteria, known by the acronym as the ESKAPE pathogens (for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*

baumannii, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), has now become a looming healthcare crisis in many ICUs worldwide.⁵¹ Treating patients with MDR pathogens has been demonstrated by Morales et al. to increase the total cost of care and to prolong hospital length of stay.⁵²

An ethical imperative exists throughout the health care professions to do all we can to preserve the efficacy of antibiotics and recognize that this precious resource is being squandered by often unnecessary and inappropriate antibiotic use, promoting the acquisition and dissemination of antibiotic resistance genes.⁵³ Antibiotic drug resistance is now recognized as a health care emergency and appeals for the development of novel means to combat it have been voiced by many; however, antibiotics are developed on the basis of free market criteria, rather than on the basis of direct benefit to the public.⁵⁴ However, despite the call for the development of new antibiotics in the European Union (EU) and in the United States (US),^{55,56} there is dearth of new antibiotics in the developmental pipeline.^{54,57,58} An entirely novel, non-antibiotic approach to treat bacterial pathogens is certainly needed. The re-deployment of phage therapy could become a welcome alternative to antimicrobial chemotherapy in this period of progressive spread of MDR bacterial pathogens with a paucity of new antibiotic to combat these pathogens.

Furthermore, the need for phage applications certainly exceeds its use in human infections. Indeed the use of bacteriophages has been described in various situations including (but not limited to): food safety,⁵⁹ agriculture,⁶⁰ veterinary applications,⁶¹ industry,⁶⁰ and clinical diagnostic application such as detection and typing of bacteria⁶² in human infection.

Potential Advantages of Phage Therapy

Bacteriophages are natural antibacterials able to regulate bacterial populations by the induction of bacterial lysis. They are active against gram-positive,^{63,64} as well as gram-negative bacteria,⁶⁵⁻⁶⁷ including MDR pathogens.⁶³⁻⁶⁷ Indeed, as mechanism of action phage lysis is totally different from antibiotics, retaining activity against bacteria exhibiting multiple mechanisms of antibiotic resistance.³ Because of its specificity, phage therapy has a narrow antibacterial spectrum with an effect limited to one single species or in some cases a single strain within a species. This limits the “pressure” and the heavy collateral damage done to bystander, non-targeted bacteria from antibiotics. The entire microbiome of the patient is altered by antibiotics, not just the intended target pathogen. In contrast, Chibani-Chennoufi et al. demonstrated little impact on the gut microbiota in mice after oral administration of phage therapy directed against *E. coli*.⁶⁸ Preservation of much of the existing microbiome during phage therapy has been confirmed in careful microbial surveys in adult healthy volunteers who ingested a 9-phage cocktail.^{69,70} Phage therapy also avoids the potential overgrowth of secondary pathogens.

Since large, randomized, controlled trials are lacking at the present time, it is difficult to evaluate side effects and their potential impact. Based on the reports gained from Poland and the former Soviet Union, phage therapy seems to be without significant adverse effects; the fact that bacteriophages interact with

bacterial cells only and do not interfere with mammalian cells probably could potentially explain this lack of deleterious side effects. Underreporting could be another explanation. However, the excellent tolerability of phage treatment has been demonstrated in preclinical studies in various animal models and in several observational studies in patients and healthy human volunteers.⁶⁹ There is a wide distribution of phages upon systemic administration, including the ability to penetrate the blood brain barrier, allowing these agents to be used in case of central nervous system infections.⁷¹⁻⁷³ Interestingly, at least some phages also display the capacity to disrupt bacterial biofilms.⁷⁴

Phage therapy may have an impact on the inflammatory response to infection. In 51 patients presenting with various long-term suppurative infection, TNF α release, in vivo and in vitro upon stimulation with LPS, was attenuated based upon the initial pattern of serum TNF α level. Release of IL-6 was only significantly reduced in vivo.⁷⁵ C-reactive protein and white blood cell count were initially not affected in this patient population while it significantly decreased between day 9 and day 32 in 37 patients given oral phage therapy for osteomyelitis, prosthetic joint infection, skin and soft tissue infections, and, in one case, lung infection.⁷⁶ This was an observational study without a control group and therefore should be cautiously interpreted. In a more recent observation, CRP was only affected in patients whose initial CRP serum level was above 10 mg/dl.⁷⁷ White blood cells may also be affected by phage therapy: increased neutrophil precursors and decreased phagocytic index for *Staphylococcus aureus* was observed in patients after 3 weeks and 3 mo of therapy, as compared with healthy donors.⁷⁸ A large review of the alteration of immune responses with phage therapy has recently been published.⁷⁹

Finally, the economic aspects of phage therapy look promising. Despite the fact that the duration of treatment was significantly prolonged, the cost of phage therapy was lower than conventional antibiotic treatment as it was demonstrated in 6 patients presenting with various staphylococcal infections including methicillin-resistant *Staphylococcus aureus*.⁸⁰

Above all, the fact that bacteriophages could have an improved efficacy as compared with antibiotics provides the greatest hope for the future. Smith and colleagues first demonstrated this finding in the early 1980s when they induced a lethal *E. coli* infection in mice using a highly virulent strain expressing a K1 polysaccharide capsule.²⁹ One single intramuscular dose of anti-K1 phage was as effective as multiple streptomycin injections, and was superior to multiple intramuscular doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim in curing the animals. To our knowledge, this observation has never been confirmed in human infection.

Those various potential advantages of phage applications are summarized in Table 1.

Potential Limitations and Drawbacks of Phage Therapy

Despite all the advantages summarized above, we are far from describing phages as the “magic bullet” to treat any type

Table 1. Summary of potential beneficial effects of phage therapy

1. Activity against all type of bacteria including MDR-pathogens
2. Narrow antibacterial spectrum allowing preservation of the existing microbiome
3. Potential low level of side effects
4. Wide distribution upon systemic administration
5. Possible effect on the inflammatory response
6. Cost effectiveness
7. Improved efficacy as compared with antibiotics

of infection. Actually, the optimal dose, route of administration, frequency, and duration of treatment still need to be defined before widespread clinical trials are contemplated.

The major disadvantage of phage therapy is the need to rapidly determine the precise etiological microorganism causing infection with accuracy. The exquisite specificity of phage therapy against specific pathogens is a major advantage, but also a liability. A clinical sample has to be isolated and cultured, using standard microbiology diagnostic procedures, to identify the pathogen before a specific bacteriophage solution may be defined and later on administered to the patient. Innovations in rapid bacterial diagnosis with genomic methods or the use of mass spectroscopy might help. Nonetheless, this is a time consuming process in most clinical microbiology laboratories and in resource-limited health care settings.

This problem could potentially be solved with the use of ready to use phage “cocktails”. Selection of potent phages from an available collection after phage typing of the isolated bacteria defines the so-called composed phage cocktail treatment. Finally, when no active, existing phage preparation is present against a severe pathogen, it can be isolated directly from the environment before it is prepared for application.⁴³ For instance, in the recent outbreak of *E. coli* O104:H4 in Germany, active lytic phages were found in the collection of the Eliava Institute (Georgia) as well as in the wastewater of the Brussels Military Hospital in Belgium.⁸¹

The choice of bacteriophage for therapy is limited to lytic phages.⁷³ Indeed, lysogenic phages will induce delayed lysis, preventing application of those phages in an acute infection. Although standardized methods to generate phage cocktails do exist,⁸² there are no clear official guidelines.⁸³ Virion stability in terms of their susceptibility to various external and physical factors has recently been reviewed⁸⁴ and could account for some difficulties in preparing stable solutions.

Another concern of phage therapy is the potential ability of bacteriophages to transfer the DNA from a bacterium to another. This transfer of genetic material, or transduction, could be responsible for the transfer of pathogenicity determinants and virulence factors, leading to the development of a new microbe or even more resistant bacteria.⁸⁵⁻⁸⁷ Therefore, the use of phages unable to package extra host DNA or phages that use the host DNA to synthesize its own DNA would be preferred. This technique has already been successfully applied in phage therapy.⁷³

The genome of many phages has been unraveled and each month, there are reports on newly identified gene sequences.

However, we are far from having sequenced the gene of each type of phages⁸⁸ and the function of many of these genes is still unknown. For instance the ORFan genes found in some phages have no similarity to any other gene in the gene database.⁸⁹ The role of those genes in the potential to promote deleterious side effects has still to be elucidated.

At the end of its antibacterial action, lytic phages induce the lysis of bacteria, liberating various bacterial substances such as endotoxin (LPS) from gram-negative bacteria. This may account for several side effects on the host such as the development of an inflammatory cascade leading to multiple organ failure. However, this potential issue applies to currently available rapidly bactericidal antibiotics.⁹⁰

Since they are viruses, bacteriophages may be seen by the immune system of the patient as a potential invader and may therefore rapidly be eliminated from the systemic circulation by reticulo-endothelial system clearance before they are accumulated in the spleen or the liver, or, they may be inactivated by the adaptive immune defense mechanisms.⁹¹ This could lead to a decreased efficacy in case of prolonged or repeated applications.

Finally, the development of resistance mechanisms by the bacterial host, resulting either from mutation and selection or by temperate phage acquisition, could lead to a decreased efficacy of phages. There are at least 4 mechanisms that may be involved in bacterial resistance to a specific phage. Loss or lack of receptor, structural modification and, or masking of the receptor will prevent phage adsorption to the bacteria and prevent further ability to generate new phages. Loss of receptor may occur when cell surface composition is changed, as was demonstrated for *Bordetella* spp.⁹² Structural modification has been noticed for *E. coli* protein TraT which modifies the conformation of the Outer-Membrane Protein A (OmpA), the receptor for T-even-like phages.⁹³ Secretion of various molecules (such as exopolysaccharide by *Pseudomonas* spp. or glycoconjugates by *Enterobacteriaceae*) may mask the receptor, but phages may counteract this by the selection of a new receptor or by secreting exopolysaccharide degrading enzyme.⁴³ The other mechanisms of resistance include the prevention of phage DNA integration by superinfection exclusion system (Sie), degradation of phage DNA by Restriction-Modification defense system or by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and the blocking of phage replication, transcription, translation, or virions assembly by Abortive Infection system.⁴³

Fortunately, thus far the frequency of resistance in vivo during phage therapy is reportedly low,^{43,94} as opposed to the observed in vitro resistance analyses. Furthermore, isolation of novel active phages from the environment or progressive isolation of “adapted” phages could provide a new possibility for treatment.

In most countries, phage therapy is not covered by public health insurance, a potential financial problem for some patients. Some exceptions do exist. Switzerland authorities decided to reimburse complementary medicine for a period of 6 years, while efficacy is evaluated⁹⁵ and the president of the city of Wroclaw (where the Hirsfeld Institute is located), Poland, has established a program covering the costs of phage therapy for the residents of the city; 2 examples to be followed according to Myedzybrodzki.⁷⁷

Table 2. Summary of major experimental studies with phage therapy

Bacteria	Author	Infection model	Animal	Phage therapy
<i>E. coli</i>	Smith ²⁹	Systemic (intramuscular injection) CNS (intracerebral injection)	Mice	Intramuscular injection
<i>E. coli</i>	Smith ³⁰	Diarrhea after oral <i>E. coli</i> administration	Calves Piglets Lambs	Oral administration
<i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	Soothill ⁹⁶	I.P. injection	Mice	I.P. injection
<i>E. coli</i> and <i>S. enterica</i> Typhimurium	Merril ⁹⁷	I.P. injection related systemic infection	Mice	I.P. injection
<i>E. coli</i>	Barrow ⁹⁸	Septicemia and meningitis	Chicken and calves	Intramuscular injection
Vancomycin-resistant <i>E. faecium</i>	Biswas ⁶⁴	I.P. injection related bacteremia	Mice	I.P. injection
<i>Staphylococcus aureus</i>	Matsuzaki ⁶³	I.P. injection related bacteremia	Mice	I.P. injection
<i>E. coli</i>	Chibani-Chennoufi ⁶⁸	Diarrhea after intestinal administration	Mice	Oral administration
MDR <i>Klebsiella pneumoniae</i>	Vinodkumar ⁶⁵	I.P. injection related bacteremia	Mice	I.P. injection
<i>Staphylococcus aureus</i>	Wills ⁹⁹	Wound infection	Rabbit	Subcutaneous injection
Imipenem-resistant <i>Pseudomonas</i> spp.	Wang ⁶⁶	I.P. injection related bacteremia	Mice	I.P. injection
Beta-lactamase producing <i>E. coli</i>	Wang ⁶⁷	I.P. injection related bacteremia	Mice	I.P. injection
<i>Pseudomonas aeruginosa</i>	Watanabe ¹⁰⁰	I.P. injection related bacteremia	Mice	I.P. injection
MDR <i>Pseudomonas aeruginosa</i>	Vinodkumar ¹⁰¹	I.P. injection related bacteremia	Mice	I.P. injection
<i>Pseudomonas aeruginosa</i>	Debarbieux ¹⁰²	Lung infection	Mice	
<i>Staphylococcus aureus</i>	Sunagar ¹⁰³	I.P. injection related bacteremia	Mice	I.P. injection
<i>Klebsiella pneumoniae</i>	Hung ¹⁰⁴	Intragastric administration related liver abscesses and bacteremia	Mice	Intragastric administration I.P. injection
<i>Klebsiella pneumoniae</i>	Kumari ¹⁰⁵	Burn wound infection	Mice	Topical administration
<i>Pseudomonas</i>	Morello ¹⁰⁶	Lung Infection	Mice	Intranasal
<i>Chronobacter turicensis</i>	Thotova ¹⁰⁷	Urinary tract infection	Mice	I.P. injection
<i>Pseudomonas aeruginosa</i>	Alemayehu ¹⁰⁸	Lung infection	Mice	Intranasal
ESBL producing <i>E. coli</i>	Pouillot ⁷¹	I.P. injection Intrathecal injection related meningitis	Rat	I.P. injection Subcutaneous injection
MRSA	Yilmaz ¹⁰⁹	Bone infection	Rat	Medullary injection

IP, intraperitoneal; MDR, multidrug-resistant; ESBL, extended spectrum β -lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*

Since bacterial viruses are currently not recognized as medicinal products, current European pharmacological regulations, definitions and standards are not adequately adapted to phage preparations.⁷⁷ Therefore, a Belgian Research group and some members of the Pasteur Institute in Paris, developed the P.H.A.G.E. (for Phages for Human Application Group Europe; <http://www.p-h-a-g-e.org>), an international non-profit organization, with the aim to develop a specific framework for the use of bacteriophages.

Regulatory clearance remains another hurdle. In addition to the inherent safety concern, neither the US Food and Drug Administration nor the European Medicines Agency has an approval process in place that can easily accommodate the ever-changing combinations of phages that companies need to develop to stay one step ahead of evolving MDR bacteria.⁷⁷

Experimental Data with Phage Therapy

Many experimental data were conducted since the 2 landmark studies by Smith and Huggins who demonstrated, in the early 80s, the potential role of bacteriophages in controlling systemic infections, and enteritis in mice, calves, piglets and lambs.^{29,30} Some of those studies^{29,30,64-68,71,96-109} are summarized in **Table 2**.

Mice have been widely studied as experimental animals but there are also reports on phage therapy in laboratory models of infections in rat, chicken, rabbits, calves, and lambs. Various models of infections were evaluated such as intraperitoneal injection of live bacteria leading to systemic infection with bacteremia, intramuscular injection of bacteria, central nervous system infection, lung infection, liver abscesses, enteritis, urinary tract infection, bone infection, skin, and wound

infections. Bacteria used in these models included *E. coli*, MDR bacteria (*Pseudomonas aeruginosa*, ESBL-producing *E. coli* and *K. pneumoniae*, vancomycin-resistant *Enterococcus faecium*), *Staphylococcus aureus*, and *Chronobacter turicensis*. Some strains were directly isolated from patients.^{64,104} The method of administration of phage therapy tested includes intraperitoneal injection, oral or intragastric administration, topical, sub-cutaneous, and intramuscular injections and intranasal administration. While in some studies, phage administration was considered as a prophylactic measure,^{102,106} treatment was usually administered as a single dose after the bacterial challenge and in some studies was delayed until the animals displayed infectious symptoms such as diarrhea³⁰ or clear signs of severe infection.¹⁰¹

Overall those studies demonstrated positive effects on mortality with phage therapy and in 3 studies where it was assessed, outcomes were significantly better than antibiotics used as comparators.^{29,103,105} In one study of infected bone model in rats, the combined antibiotic-bacteriophage treatment significantly decreased the quantitative culture from the infected site at the end of the study as compared with either treatment modality given alone.¹⁰⁹

Already Described Human Applications

The first report on the use of bacteriophage in humans described its efficacy in staphylococcal skin furuncles¹⁶ and d'Herelle summarized all his clinical work in 1931.⁴ There were a large amount of publications in the 1930s and a full monograph of the journal *La Médecine* covered phage applications in human disease.¹¹⁰ It described the treatment of typhoid fever, *Shigella* and *Salmonella* spp.-related colitis, peritonitis, skin infections, surgical infections (mainly abscesses of various locations), septicemia, urinary tract infections, and otolaryngology infections (external otitis and nasal furuncles).

However, as already described, the enthusiasm for phage therapy declined in the western countries in the 1930s because of the questions regarding scientific rigor in testing phage therapy in the reports by Eaton and colleagues⁷⁻⁹ and also as a consequence of the discovery and the ease of use of antibiotics. The use of bacteriophages continued in the eastern countries and large number of reports were published over time, mainly in Poland and Georgia (former USSR). The use of non-English literature (mainly Russian and Polish) probably explain the fact those reports were confined to the country of origin of the authors. A summary of this literature have been published by various authors more recently,^{3,77,94,110-115} showing extensive experience for some authors with several hundred treated patients.^{77,111} We, however, have to note that most of the published data are from non-randomized, uncontrolled trials.

Indeed, the first phase I randomized controlled trial conducted in the United States was published in 2009.³¹ It evaluated the safety of a cocktail of phages directed against *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* in 42 patients with chronic venous leg ulcers. The study was not powered to detect any positive outcome such as rate or frequency of healing but the authors did not find any adverse event related to the treatment. Another randomized trial was conducted in the UK and studied the efficacy of one application of a solution containing 6 bacteriophages in the ears of patients suffering chronic *Pseudomonas aeruginosa*-related otitis.¹¹⁶ The colony counts of *P. aeruginosa* significantly decreased in the treated group in this well done, double-blind, placebo-controlled study while various subjective clinical indicators improved in those patients. Indeed, patients reported lower intensity of symptoms such as discomfort, itching, wetness, and unpleasant odor. Likewise, physicians in charge of the patients (and blinded to the assigned treatment) reported decreased clinical observations such as erythema/inflammation, ulceration/granulation/polyps, and odor. There were no reported adverse reactions.

A small phase I study of 9 patients treated at the Burn Wound Centre of the Queen Astrid Military Hospital, Brussels, Belgium, was recently performed.¹¹⁰ Patients were locally treated with the BFC-1 phage cocktail containing 3 lytic phages: a *Myovirus*, a *Podovirus* against *Pseudomonas aeruginosa*, and a *Myovirus* directed against *Staphylococcus aureus*.¹¹⁷ A large burned section was exposed to a single spray application while a distant portion of the wound served as control. While complete results are yet to be published, there was no safety issue reported.¹¹⁰

Finally, a randomized controlled trial confirmed the safety of an orally administered phage solution in healthy non-infected patients.⁶⁹

Conclusions

Bacteriophages are a possible alternative tool for the treatment of bacterial infections, including those caused by MDR pathogens. Indeed, phage therapy displays several advantages and few adverse events are reported but underreporting cannot be ruled out. However, further well-conducted studies are required to define the role and safety of phage therapy in daily clinical practice to treat patients with various infections.

Moreover, direct use of phage encoded proteins such as endolysins, exopolysaccharidases and holins have proved their ability as a promising alternative to antibacterial products. This topic is, however, beyond the scope of this review.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Sulakvelidze A. Bacteriophage: A new journal for the most ubiquitous organisms on Earth. *Bacteriophage* 2011; 1:1-2; PMID:21687529; <http://dx.doi.org/10.4161/bact.1.1.15030>
- Weinbauer MG. Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 2004; 28:127-81; PMID:15109783; <http://dx.doi.org/10.1016/j.femsre.2003.08.001>
- Hanlon GW. Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *Int J Antimicrob Agents* 2007; 30:118-28; PMID:17566713; <http://dx.doi.org/10.1016/j.ijantimicag.2007.04.006>
- d'Herelle F. Bacteriophage as a treatment in acute medical and surgical infections. *Bull N Y Acad Med* 1931; 7:329-48; PMID:19311785
- Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. *Bacteriophage* 2011; 1:66-85; PMID:22334863; <http://dx.doi.org/10.4161/bact.1.2.15845>
- Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches. *Adv Virus Res* 2012; 83:3-40; PMID:22748807; <http://dx.doi.org/10.1016/B978-0-12-394438-2.00001-3>
- Eaton MD, Stanhope BJ. Bacteriophage therapy: review of the principle and results of the use of bacteriophage in the treatment of infection (I). *J Am Med Assoc* 1934; 103:1769-76; <http://dx.doi.org/10.1001/jama.1934.72750490003007>
- Eaton MD, Stanhope BJ. Bacteriophage therapy: review of the principle and results of the use of bacteriophage in the treatment of infection (II). *J Am Med Assoc* 1934; 103:1847-53; <http://dx.doi.org/10.1001/jama.1934.72750500003009>
- Eaton MD, Stanhope BJ. Bacteriophage therapy: review of the principle and results of the use of bacteriophage in the treatment of infection (III). *J Am Med Assoc* 1934; 103:1934-9; <http://dx.doi.org/10.1001/jama.1934.72750510005009>
- Hankin EH. L'action bactericide des eaux de la Jumna et du Gange sur le vibron du cholera. *Ann Inst Pasteur (Paris)* 1896; 10:511-23
- Twort FW. An investigation on the nature of ultramicroscopic viruses. *Lancet* 1915; 186:1241-3; [http://dx.doi.org/10.1016/S0140-6736\(01\)20383-3](http://dx.doi.org/10.1016/S0140-6736(01)20383-3)
- d'Herelle F. Sur un microbe invisible antagoniste des bacilles dysentériques. *C R Acad Sci Paris* 1917; 165:173-5
- Bordet J, Ciuca M. Remarques sur l'historique des recherches concernant la lyse microbienne transmissible. *C R Soc Biol* 1921; 84:745-7
- Duckworth DH. "Who discovered bacteriophage?" *Bacteriol Rev* 1976; 40:793-802; PMID:795414
- Summers WC. In the beginning... *Bacteriophage* 2011; 1:50-1; PMID:21687535; <http://dx.doi.org/10.4161/bact.1.1.14070>
- Brunynoghe R, Maisin J. Essais de thérapeutique au moyen du bacteriophage du Staphylocoque. *Compt Rend Soc Biol* 1921; 85:1120-1
- Northrop JH. Concentration and purification of bacteriophage. *J Gen Physiol* 1938; 21:335-66; PMID:19873054; <http://dx.doi.org/10.1085/jgp.21.3.335>
- Wollman E. Bacteriophage et processus similaire: Héritéité ou Infection? *Bull Inst Pasteur Paris* 1928; 26:1-14
- Muller HJ. Variation due to change in the individual gene. *Am Nat* 1922; 56:32-50; <http://dx.doi.org/10.1086/279846>
- Bordet J. Le problème de l'autolyse microbienne transmissible ou du bacteriophage. *Ann Inst Pasteur (Paris)* 1925; 39:711-63
- Sankaran N. The bacteriophage, its role in immunology: how Macfarlane Burnet's phage research shaped his scientific life. *Studies in History and Philosophy of Biological and Biomedical Sciences* 2010; 41:367-75; <http://dx.doi.org/10.1016/j.shpsc.2010.10.012>
- Burnet FM. The serological classification of Coli-Dysentery phages. *J Pathol Bacteriol* 1933; 36:307-18; <http://dx.doi.org/10.1002/path.1700360212>
- Pennazio S. The origin of phage virology. *Riv Biol* 2006; 99:103-29; PMID:16791793
- Schlesinger M. Zur frage der chemischen zusammensetzung des bakteriohagen. *Biochem Z* 1934; 273:306-11
- Ruska H. Die Sichtbarmachung der bakteriohagen Lyse im Übermikroskop. *Naturwissenschaften* 1940; 28:45-6; <http://dx.doi.org/10.1007/BF01486931>
- Kruger DH, Schneck P, Gelderblom HR, Helmut Ruska and the visualisation of viruses. *Lancet* 2000; 355:1713-7; PMID:10905259; [http://dx.doi.org/10.1016/S0140-6736\(00\)02250-9](http://dx.doi.org/10.1016/S0140-6736(00)02250-9)
- Luria SE, Anderson TF. The identification and characterization of bacteriophages with the electron microscope. *Proc Natl Acad Sci U S A* 1942; 28:127-130.1; PMID:16588529; <http://dx.doi.org/10.1073/pnas.28.4.127>
- Luria SE, Delbrück M, Anderson TF. Electron Microscope Studies of Bacterial Viruses. *J Bacteriol* 1943; 46:57-77; PMID:16560678
- Smith HW, Huggins MB. Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotics. *J Gen Microbiol* 1982; 128:307-18; PMID:7042903
- Smith HW, Huggins MB. Effectiveness of phages in treating experimental Escherichia coli diarrhoea in calves, piglets and lambs. *J Gen Microbiol* 1983; 129:2659-75; PMID:6355391
- Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *J Wound Care* 2009; 18:237-8, 240-3; PMID:19661847
- Adhya S, Black L, Friedman D, Hatfull G, Kreuzer K, Merrill C, Oppenheim A, Rohwer F, Young R. 2004 ASM Conference on the New Phage Biology: the "Phage Summit". *Mol Microbiol* 2005; 55:1300-14; PMID:15720541; <http://dx.doi.org/10.1111/j.1365-2958.2005.04509.x>
- Ackermann HW. Obituary – Hansjürgen Raettig – Collector of bacteriophages references. *BEG news* 2000; 3:5 (<http://phage.org/2000>)
- Ackermann HW. Who went into phage research? *Bacteriophage* 2012; 2:55-9; PMID:22666657; <http://dx.doi.org/10.4161/bact.18680>
- Ackermann HW, Prangishvili D. Prokaryote viruses studied by electron microscopy. *Arch Virol* 2012; 157:1843-9; PMID:22752841; <http://dx.doi.org/10.1007/s00705-012-1383-y>
- Deghorain M, Van Melderen L. The Staphylococci phages family: an overview. *Viruses* 2012; 4:3316-35; PMID:23342361; <http://dx.doi.org/10.3390/v4123316>
- Ceysens PJ, Lavigne R. Bacteriophages of Pseudomonas. *Future Microbiol* 2010; 5:1041-55; PMID:20632804; <http://dx.doi.org/10.2217/fmb.10.66>
- Ackermann HW. Bacteriophages: characteristics and 1st stages of a classification. *Pathol Biol (Paris)* 1969; 17:1003-24; PMID:4907644
- Ackermann HW. Bacteriophage taxonomy in 1987. *Microbiol Sci* 1987; 4:214-8; PMID:3153614
- Ackermann HW. Frequency of morphological phage descriptions in 1995. *Arch Virol* 1996; 141:209-18; PMID:8634015; <http://dx.doi.org/10.1007/BF01718394>
- Rohwer F, Edwards R. The Phage Proteomic Tree: a genome-based taxonomy for phage. *J Bacteriol* 2002; 184:4529-35; PMID:12142423; <http://dx.doi.org/10.1128/JB.184.16.4529-4535.2002>
- Fauquet CM, Pringle CR. Abbreviations for bacterial and fungal virus species names. *Arch Virol* 2000; 145:197-203; PMID:10664418; <http://dx.doi.org/10.1007/s007050050017>
- Drulis-Kawa Z, Majkowska-Skropek G, Maciejewska B, Delattre AS, Lavigne R. Learning from bacteriophages - advantages and limitations of phage and phage-encoded protein applications. *Curr Protein Pept Sci* 2012; 13:699-722; PMID:23305359; <http://dx.doi.org/10.2174/138920312804871193>
- Chatterjee S, Rothenberg E. Interaction of bacteriophage I with its E. coli receptor, Lamb. *Viruses* 2012; 4:3162-78; PMID:23202520; <http://dx.doi.org/10.3390/v4113162>
- Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüßow H. Phage as agents of lateral gene transfer. *Curr Opin Microbiol* 2003; 6:417-24; PMID:12941415; [http://dx.doi.org/10.1016/S1369-5274\(03\)00086-9](http://dx.doi.org/10.1016/S1369-5274(03)00086-9)
- Lu TK, Collins JJ. Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc Natl Acad Sci U S A* 2009; 106:4629-34; PMID:19255432; <http://dx.doi.org/10.1073/pnas.0800442106>
- Edgar R, Friedman N, Molshanski-Mor S, Qimron U. Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. *Appl Environ Microbiol* 2012; 78:744-51; PMID:22113912; <http://dx.doi.org/10.1128/AEM.05741-11>
- Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. *Int J Antimicrob Agents* 2010; 36(Suppl 2):S50-4; PMID:21129924; <http://dx.doi.org/10.1016/j.ijantimicag.2010.11.014>
- Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century--a clinical super-challenge. *N Engl J Med* 2009; 360:439-43; PMID:19179312; <http://dx.doi.org/10.1056/NEJMp0804651>
- Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, et al.; Active Bacterial Core surveillance (ABCs) MRSA Investigators. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007; 298:1763-71; PMID:17940231; <http://dx.doi.org/10.1001/jama.298.15.1763>
- Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther* 2013; 11:297-308; PMID:23458769; <http://dx.doi.org/10.1586/eri.13.12>
- Morales E, Cots F, Sala M, Comas M, Belvis F, Riu M, Salvadó M, Grau S, Horcajada JP, Montero MM, et al. Hospital costs of nosocomial multi-drug resistant *Pseudomonas aeruginosa* acquisition. *BMC Health Serv Res* 2012; 12:122; PMID:22621745; <http://dx.doi.org/10.1186/1472-6963-12-122>
- Selgelid MJ. Ethics and drug resistance. *Bioethics* 2007; 21:218-29; PMID:17845480; <http://dx.doi.org/10.1111/j.1467-8519.2006.00542.x>
- Aiello AE, King NB, Foxman B. Ethical conflicts in public health research and practice: antimicrobial resistance and the ethics of drug development. *Am J Public Health* 2006; 96:1910-4; PMID:17018817; <http://dx.doi.org/10.2105/AJPH.2005.077214>
- Gravitz L. Turning a new phage. *Nat Med* 2012; 18:1318-20; PMID:22961150; <http://dx.doi.org/10.1038/nm0912-1318>
- World Medical Association Statement on Resistance to antimicrobial agents www.wma.net/e/policy/a19.htm
- Freire-Moran L, Aronsson B, Manz C, Gyssens IC, So AD, Monnet DL, Cars O; ECDC-EMA Working Group. Critical shortage of new antibiotics in development against multidrug-resistant bacteria--Time to react is now. *Drug Resist Updat* 2011; 14:118-24; PMID:21435939; <http://dx.doi.org/10.1016/j.drug.2011.02.003>
- Jabes D. The antibiotic R&D pipeline: an update. *Curr Opin Microbiol* 2011; 14:564-9; PMID:21873107; <http://dx.doi.org/10.1016/j.mib.2011.08.002>
- Mahony J, McAuliffe O, Ross RP, van Sinderen D. Bacteriophages as biocontrol agents of food pathogens. *Curr Opin Biotechnol* 2011; 22:157-63; PMID:21115341; <http://dx.doi.org/10.1016/j.copbio.2010.10.008>

60. Haq IU, Chaudhry WN, Akhtar MN, Andleeb S, Qadri I. Bacteriophages and their implications on future biotechnology: a review. *Virol J* 2012; 9:9; PMID:22234269; <http://dx.doi.org/10.1186/1743-422X-9-9>
61. Atterbury RJ. Bacteriophage biocontrol in animals and meat products. *Microb Biotechnol* 2009; 2:601-12; PMID:21255295; <http://dx.doi.org/10.1111/j.1751-7915.2009.00089.x>
62. Jain P, Hartman TE, Eisenberg N, O'Donnell MR, Kriakov J, Govender K, Makume M, Thaler DS, Hatfull GF, Sturm AW, et al. $\phi(2)$ GFP10, a high-intensity fluorophore, enables detection and rapid drug susceptibility testing of *Mycobacterium tuberculosis* directly from sputum samples. *J Clin Microbiol* 2012; 50:1362-9; PMID:22278833; <http://dx.doi.org/10.1128/JCM.06192-11>
63. Matsuzaki S, Yasuda M, Nishikawa H, Kuroda M, Ujihara T, Shuin T, Shen Y, Jin Z, Fujimoto S, Nasimuzzaman MD, et al. Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage phi MR11. *J Infect Dis* 2003; 187:613-24; PMID:12599078; <http://dx.doi.org/10.1086/374001>
64. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, Carlton R, Merrill CR. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect Immun* 2002; 70:204-10; PMID:11748184; <http://dx.doi.org/10.1128/IAI.70.1.204-210.2002>
65. Vinodkumar CS, Neelagund YF, Kalsurmath S. Bacteriophage in the treatment of experimental septicemic mice from a clinical isolate of multidrug resistant *Klebsiella pneumoniae*. *J Commun Dis* 2005; 37:18-29; PMID:16637396
66. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X, Sun Z, Reed E, Ding L, Gong J, et al. Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa*. *Int J Mol Med* 2006; 17:309-17; PMID:16391831
67. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X, Sun Z, Tao D, Ding L, Reed E, et al. Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum β -lactamase-producing *Escherichia coli* bacteremia. *Int J Mol Med* 2006; 17:347-55; PMID:16391836
68. Chibani-Chennoufi S, Sidoti J, Bruttin A, Kutter E, Sarker S, Brüssow H. In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob Agents Chemother* 2004; 48:2558-69; PMID:15215109; <http://dx.doi.org/10.1128/AAC.48.7.2558-2569.2004>
69. Bruttin A, Brüssow H. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob Agents Chemother* 2005; 49:2874-8; PMID:15980363; <http://dx.doi.org/10.1128/AAC.49.7.2874-2878.2005>
70. Sarker SA, McCallin S, Barretto C, Berger B, Pittet AC, Sultana S, Krause L, Huq S, Bibiloni R, Bruttin A, et al. Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. *Virology* 2012; 434:222-32; PMID:23102968; <http://dx.doi.org/10.1016/j.virol.2012.09.002>
71. Pouillot F, Chomton M, Blois H, Courroux C, Noelig J, Bidet P, Bingen E, Bonacorsi S. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b:H4-ST131 *Escherichia coli* strain producing CTX-M-15. *Antimicrob Agents Chemother* 2012; 56:3568-75; PMID:22491690; <http://dx.doi.org/10.1128/AAC.06330-11>
72. Alisky J, Iczkowski K, Rapoport A, Troitsky N. Bacteriophages show promise as antimicrobial agents. *J Infect* 1998; 36:5-15; PMID:9515662; [http://dx.doi.org/10.1016/S0163-4453\(98\)92874-2](http://dx.doi.org/10.1016/S0163-4453(98)92874-2)
73. Górski A, Miedzybrodzki R, Borysowski J, Weber-Dabrowska B, Lobočka M, Fortuna W, Letkiewicz S, Zimecki M, Filby G. Bacteriophage therapy for the treatment of infections. *Curr Opin Investig Drugs* 2009; 10:766-74; PMID:19649921
74. Azeredo J, Sutherland IW. The use of phages for the removal of infectious biofilms. *Curr Pharm Biotechnol* 2008; 9:261-6; PMID:18691087; <http://dx.doi.org/10.2174/138920108785161604>
75. Weber-Dabrowska B, Zimecki M, Mulczyk M. Effective phage therapy is associated with normalization of cytokine production by blood cell cultures. *Arch Immunol Ther Exp (Warsz)* 2000; 48:31-7; PMID:10722229
76. Miedzybrodzki R, Fortuna W, Weber-Dabrowska B, Górski A. A retrospective analysis of changes in inflammatory markers in patients treated with bacterial viruses. *Clin Exp Med* 2009; 9:303-12; PMID:19350363; <http://dx.doi.org/10.1007/s10238-009-0044-2>
77. Miedzybrodzki R, Borysowski J, Weber-Dabrowska B, Fortuna W, Letkiewicz S, Szufnarowski K, Pawelczyk Z, Rogóź P, Klak M, Wójcisiak E, et al. Clinical aspects of phage therapy. *Adv Virus Res* 2012; 83:73-121; PMID:22748809; <http://dx.doi.org/10.1016/B978-0-12-394438-2.00003-7>
78. Weber-Dabrowska B, Zimecki M, Mulczyk M, Górski A. Effect of phage therapy on the turnover and function of peripheral neutrophils. *FEMS Immunol Med Microbiol* 2002; 34:135-8; PMID:12381464; <http://dx.doi.org/10.1111/j.1574-695X.2002.tb00614.x>
79. Górski A, Miedzybrodzki R, Borysowski J, Dąbrowska K, Wierzbicki P, Oham S, Korczak-Kowalska G, Olszowska-Zaremba N, Lusiak-Szelachowska M, Klak M, et al. Phage as a modulator of immune responses: practical implications for phage therapy. *Adv Virus Res* 2012; 83:41-71; PMID:22748808; <http://dx.doi.org/10.1016/B978-0-12-394438-2.00002-5>
80. Miedzybrodzki R, Fortuna W, Weber-Dabrowska B, Górski A. Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. *Postepy Hig Med Dosw (Online)* 2007; 61:461-5; PMID:17679835
81. Merabishvili M, De Vos D, Verbeken G, Kropinski AM, Vandenhoevel D, Lavigne R, Watiau P, Mast J, Ragimbeau C, Mossong J, et al. Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. *PLoS One* 2012; 7:e52709; PMID:23285164; <http://dx.doi.org/10.1371/journal.pone.0052709>
82. Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, et al. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS One* 2009; 4:e4944; PMID:19300511; <http://dx.doi.org/10.1371/journal.pone.0004944>
83. Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, Zizi M, Laire G, Lavigne R, Huys I, et al. The phage therapy paradigm: prêt-à-porter or sur-mesure? *Pharm Res* 2011; 28:934-7; PMID:21063753; <http://dx.doi.org/10.1007/s11095-010-0313-5>
84. Jończyk E, Klak M, Miedzybrodzki R, Górski A. The influence of external factors on bacteriophages—review. *Folia Microbiol (Praha)* 2011; 56:191-200; PMID:21625877; <http://dx.doi.org/10.1007/s12223-011-0039-8>
85. Brabban AD, Hite E, Callaway TR. Evolution of food-borne pathogens via temperate bacteriophage-mediated gene transfer. *Foodborne Pathog Dis* 2005; 2:287-303; PMID:16366852; <http://dx.doi.org/10.1089/fpd.2005.2.287>
86. O'Shea YA, Boyd EF. Mobilization of the *Vibrio* pathogenicity island between *Vibrio cholerae* isolates mediated by CP-T1 generalized transduction. *FEMS Microbiol Lett* 2002; 214:153-7; PMID:12351223; <http://dx.doi.org/10.1111/j.1574-6968.2002.tb11339.x>
87. Maigues E, Ubeda C, Tormo MA, Ferrer MD, Lasa I, Novick RP, Penadés JR. Role of staphylococcal phage and SaPI integrase in intra- and interspecies SaPI transfer. *J Bacteriol* 2007; 189:5608-16; PMID:17545290; <http://dx.doi.org/10.1128/JB.00619-07>
88. Hatfull GF. Bacteriophage genomics. *Curr Opin Microbiol* 2008; 11:447-53; PMID:18824125; <http://dx.doi.org/10.1016/j.mib.2008.09.004>
89. Ackermann HW, Kropinski AM. Curated list of prokaryote viruses with fully sequenced genomes. *Res Microbiol* 2007; 158:555-66; PMID:17889511; <http://dx.doi.org/10.1016/j.resmic.2007.07.006>
90. Goodridge LD. Designing phage therapeutics. *Curr Pharm Biotechnol* 2010; 11:15-27; PMID:20214605; <http://dx.doi.org/10.2174/138920110790725348>
91. Dabrowska K, Swiata-Jelen K, Opolski A, Weber-Dabrowska B, Gorski A. Bacteriophage penetration in vertebrates. *J Appl Microbiol* 2005; 98:7-13; PMID:15610412; <http://dx.doi.org/10.1111/j.1365-2672.2004.02422.x>
92. Liu M, Deora R, Doulatov SR, Gingery M, Eiserling FA, Preston A, Maskell DJ, Simons RW, Cotter PA, Parkhill J, et al. Reverse transcriptase-mediated tropism switching in *Bordetella* bacteriophage. *Science* 2002; 295:2091-4; PMID:11896279; <http://dx.doi.org/10.1126/science.1067467>
93. Riede I, Eschbach ML. Evidence that TraT interacts with OmpA of *Escherichia coli*. *FEBS Lett* 1986; 205:241-5; PMID:3527751; [http://dx.doi.org/10.1016/0014-5793\(86\)80905-X](http://dx.doi.org/10.1016/0014-5793(86)80905-X)
94. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. Phage therapy in clinical practice: treatment of human infections. *Curr Pharm Biotechnol* 2010; 11:69-86; PMID:20214609; <http://dx.doi.org/10.2174/138920110790725401>
95. Stafford N. Switzerland is to fund complementary therapies for six years while effectiveness is evaluated. *BMJ* 2011; 342:d819; PMID:21300707; <http://dx.doi.org/10.1136/bmj.d819>
96. Soothill JS. Treatment of experimental infections of mice with bacteriophages. *J Med Microbiol* 1992; 37:258-61; PMID:1404324; <http://dx.doi.org/10.1099/00222615-37-4-258>
97. Merrill CR, Biswas B, Carlton R, Jensen NC, Creed GJ, Zullo S, Adhya S. Long-circulating bacteriophage as antibacterial agents. *Proc Natl Acad Sci U S A* 1996; 93:3188-92; PMID:8622911; <http://dx.doi.org/10.1073/pnas.93.8.3188>
98. Barrow P, Lovell M, Berchieri A Jr. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clin Diagn Lab Immunol* 1998; 5:294-8; PMID:9605979
99. Wills QF, Kerrigan C, Soothill JS. Experimental bacteriophage protection against *Staphylococcus aureus* abscesses in a rabbit model. *Antimicrob Agents Chemother* 2005; 49:1220-1; PMID:15728933; <http://dx.doi.org/10.1128/AAC.49.3.1220-1221.2005>
100. Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, Sumiyama Y, Uchiyama J, Sakurai S, Matsuzaki S, Imai S, et al. Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. *Antimicrob Agents Chemother* 2007; 51:446-52; PMID:17116686; <http://dx.doi.org/10.1128/AAC.00635-06>
101. Vinodkumar CS, Kalsurmath S, Neelagund YF. Utility of lytic bacteriophage in the treatment of multi-drug-resistant *Pseudomonas aeruginosa* septicemia in mice. *Indian J Pathol Microbiol* 2008; 51:360-6; PMID:18723958; <http://dx.doi.org/10.4103/0377-4929.42511>
102. Debarbieux L, Leduc D, Maura D, Morello E, Criscuolo A, Grossi O, Balloy V, Touqui L. Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. *J Infect Dis* 2010; 201:1096-104; PMID:20196657; <http://dx.doi.org/10.1086/651135>
103. Sunagar R, Patil SA, Chandrakanth RK. Bacteriophage therapy for *Staphylococcus aureus* bacteremia in streptozotocin-induced diabetic mice. *Res Microbiol* 2010; 161:854-60; PMID:20868746; <http://dx.doi.org/10.1016/j.resmic.2010.09.011>

104. Hung CH, Kuo CF, Wang CH, Wu CM, Tsao N. Experimental phage therapy in treating *Klebsiella pneumoniae*-mediated liver abscesses and bacteremia in mice. *Antimicrob Agents Chemother* 2011; 55:1358-65; PMID:21245450; <http://dx.doi.org/10.1128/AAC.01123-10>
105. 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; 60:205-10; PMID:20965914; <http://dx.doi.org/10.1099/jmm.0.018580-0>
106. Morello E, Saussereau E, Maura D, Huerre M, Touqui L, Debarbieux L. Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. *PLoS One* 2011; 6:e16963; PMID:21347240; <http://dx.doi.org/10.1371/journal.pone.0016963>
107. Tóthová L, Celec P, Bábíčková J, Gajdošová J, Al-Alami H, Kamodyova N, Drahovská H, Liptáková A, Turňa J, Hodosy J. Phage therapy of *Cronobacter*-induced urinary tract infection in mice. *Med Sci Monit* 2011; 17:BR173-8; PMID:21709627; <http://dx.doi.org/10.12659/MSM.881844>
108. Alemayehu D, Casey PG, McAuliffe O, Guinane CM, Martin JG, Shanahan F, Coffey A, Ross RP, Hill C. Bacteriophages ϕ MR299-2 and ϕ NH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. *MBio* 2012; 3:e00029-12; PMID:22396480; <http://dx.doi.org/10.1128/mBio.00029-12>
109. Yilmaz C, Colak M, Yilmaz BC, Ersoz G, Kutateladze M, Gozlugol M. Bacteriophage therapy in implant-related infections: an experimental study. *J Bone Joint Surg Am* 2013; 95:117-25; PMID:23324958; <http://dx.doi.org/10.2106/JBJS.K.01135>
110. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. *Bacteriophage* 2011; 1:66-85; PMID:22334863; <http://dx.doi.org/10.4161/bact.1.2.15845>
111. Slopek S, Weber-Dabrowska B, Dabrowski M, Kucharewicz-Krukowska A. Results of bacteriophage treatment of suppurative bacterial infections in the years 1981-1986. *Arch Immunol Ther Exp (Warsz)* 1987; 35:569-83; PMID:3455647
112. Chanishvili N, Chanishvili T, Tediashvili M, Parrow PA. Phages and their applications against drug resistant bacteria. *J Chem Technol Biotechnol* 2001; 76:689-99; <http://dx.doi.org/10.1002/jctb.438>
113. Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. *Antimicrob Agents Chemother* 2001; 45:649-59; PMID:11181338; <http://dx.doi.org/10.1128/AAC.45.3.649-659.2001>
114. Summers WC. Bacteriophage therapy. *Annu Rev Microbiol* 2001; 55:437-51; PMID:11544363; <http://dx.doi.org/10.1146/annurev.micro.55.1.437>
115. Sulakvelidze A, Kutter E. Bacteriophage therapy. In Kutter E, Sulakvelidze editors. *Bacteriophage biology and applications*. Boca Raton, FL: CRC Press; 2005 p381-436.
116. Wright A, Hawkins CH, Anggård EE, Harper DR. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin Otolaryngol* 2009; 34:349-57; PMID:19673983; <http://dx.doi.org/10.1111/j.1749-4486.2009.01973.x>
117. Merabishvili M, Pirnay JP, Verbeke G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, et al. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS One* 2009; 4:e4944; PMID:19300511; <http://dx.doi.org/10.1371/journal.pone.0004944>