

stability of phage preparations during manufacturing, storage and transport. This article describes the past and current status of phage therapy and presents the most recent advances in this domain.

History of Phage Therapy

Origins of phage therapy

Bacteriophage were first described by Frederick Twort in 1915.^[1] However, excitement with the possibilities of bacteriophage can be said to have begun six months before when Félix d'Herelle, a microbiologist at the Pasteur Institute, was sent to Maisons-Laffitte only 50 miles from the Western Front to investigate an outbreak of dysentery among 10 French mounted infantrymen. Returning with samples he described a soon eponymous novel bacillus.^[2] However, in his investigations of this bacteria over the next 18 months, he found that some seemingly sterile Chamberland filtrates of it were capable of effecting the lysis of another dysentery bacillus (likely *Shigella*). In one of the great scientific works of the twentieth century, D'Herelle described in two short pages the experiments that he performed showing that this lytic property could be serially passaged from one culture to the next by transferring 10^{-6} dilutions to new cultures fifty times.^[3] Similarly, he showed that no dilution of these lysed cultures would produce hazy subinhibitory growth when plated over a lawn of bacteria like an antibacterial toxin would, but instead would display a number of clear glassy plaques equal to the concentration that would lyse a liquid culture. From these observations, D'Herelle radically intuited that he had discovered "*un microbe invisible antagoniste des bacilles dysentériques*," described it as "*un bactériophage obligatoire*," suggested that his other bacteria would be found to similarly be infected by these pathogens of pathogens, and (perhaps too radically) posited that these bacteriophage were the true agent of natural immunity.

Phage therapy in the West

Soon after this seminal publication, D'Herelle and others began experimenting with the use of phage as an antimicrobial therapeutic for infections beginning with the treatment of chicken typhoid.^[4] In 1919, two years after phage discovery, Félix d'Hérelle used phages to successfully treat four children with dysentery at the "Hôpital des Enfants-Malades" in Paris (Summers). These were probably the first clinical applications of phages. However, the results of his experiments were not published at that time, and so it was that the use of phages to treat bacterial infections in humans was first published in 1921 by Richard Bruynoghe and Joseph Maisin.^[5] They had used phages to treat staphylococcal infection in surgical lesions and had reported a regression of the infections within 24 to 48 hours. The contemporary paucity of effective antimicrobial treatments and the exciting promise of these early results produced an enthusiastic 'early period' of phage therapy.^[6] However, with the sober hindsight provided by a deeply critical and widely read three-part report by two physicians Eaton and Bayne-Jones in 1934^[7], it became clear that this period was largely characterized by


6. Vésale Bioscience, Vésale Pharma,
Noville-Sur-Mehaigne, Belgium

1. jazeredo@deb.uminho.pt

QID: Q100400597 (<https://wikidata.org/wiki/Q100400597>)

Suggested (provisional) citation format:

Thomas Shafee (ed.), "Phage Therapy", *WikiJournal preprints*, [Wikidata Q100400597](https://wikidata.org/wiki/Q100400597)

License:  This is an open access article distributed under the [Creative Commons Attribution License \(https://creativecommons.org/licenses/by/4.0/\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction, provided the original author and source are credited.

Editors:

Thomas Shafee  (handling editor)

Reviewers: (comments)

Felix Broecker

inconsistent results, unrealistic claims, and unreliable companies. Lacking even a very basic understanding of what bacteriophages were, phage preparations were marketed as effective against implausible ailments such as gallstones, herpes, kidney stones, and various cancers. It is also likely that few commercial preparations available on the market for more plausible indications contained any bacteriophage active against the bacteria they were meant to target. Indeed, it is likely that many rapidly degraded in poor storage conditions, or were isolated against the wrong pathogen species, or against the right species but with the wrong strain. However, while many assume that phage therapy was quietly extinguished in the West in the wake of Eaton and Bayne-Jones's appropriately damning assessment of the phage preparations that were then available as well as the advent of the antibiotic era, there was a great deal of high-quality work that continued in France and the United States and has been reviewed by Abedon et al.^[6] Indeed, phages were widely used in the US and across western Europe well into the 50s and 60s, and only ended in France in the 1990s.

Phage therapy in the Former Soviet Union

Felix D'Herelle was invited to help expand a scientific institute in Tbilisi with Georgian microbiologist Giorgi Eliava to produce vaccine and phage together around 1934. This institute, now called the **George Eliava Institute of Bacteriophage, Microbiology and Virology**, along with others across the Soviet Union, were tasked with providing the Red Army, public health officials, and the general public with preparations that could be used to prevent and treat intestinal and purulent infections. The institute began rapidly isolating and then industrially producing phage preparations for a variety of military and civilian purposes, with bacteriophages used as part of the standard of care for a wide variety of ailments. Thus, while just three years later Eliava and his wife were accused of fantastical crimes against the state and executed after a show trial, the institute thrived and expanded under the leadership of the primarily female scientists who both men trained.

The structure of the Soviet healthcare system and the distinct intellectual framing of infectious disease by Soviet scientists provided a number of particular advantages in exploring phage therapy over the West. Indeed, the significantly centralised control of the Soviet healthcare system allowed for the creation of centralised banks of bacteria that were infecting patients from across the USSR that were more comprehensive than elsewhere. This allowed phage scientists to maintain libraries of phages that would be active against the most current pathogens in a particularly tailored way. At the same time the way that Soviet microbiologists precociously framed bacterial infection as, in part, an ecological problem^[8] made the ecological solution offered by phages particularly natural. Also, importantly, the ideological blinders demanded of Soviet researchers very effectively insulated phage scientists from the criticism that was dominating Western discussions about phage therapy, and antibiotics (particularly specialised antibiotics) were not available at quantities that were considered necessary for a functioning Western medical system.

At its peak in the 80s, Soviet phage production reached 2 tons per week, primarily against intestinal indications and for the Red Army and Central Asian Republics. Notably, the concept was widely considered to be conclusively demonstrated in the 60s after extensive testing^[6]. Indeed, while most of the early phage therapy related scientific work was published in Russian, Georgian or Polish and thus not easily available in the West. Scientists of the Eliava Institute (<http://eliava-institute.org/>) started to publish their phage therapy studies and the results of their clinical experience in English, but these do not yet include phage medicines and RCTs that would pass muster from the experts of the EU and US authorities for medicines for market authorization. In 2012, an English book was published, which reviews the historical publications on phage therapy that were found in the library of the Eliava Institute.^[9] Even though the design and quality of old Soviet clinical trials and scientific publications do not conform with current international standards such as RCTs (e.g. not randomized and/or no controls), they often contain valuable information, which should not be neglected by current phage therapy stakeholders. One of the largest and most imaginative studies was conducted in Tbilisi, Georgia, during 1963 and 1964.^[10] No less than 17,044 children living on one side of the streets were given *Shigella* phages orally (once every 7 days), while 13,725 children living on the other side of the streets did not receive phages.

The incidence of dysentery turned out to be 3.8-fold higher in the placebo group than in the phage-treated group. Based on the culture-confirmed cases, the incidence of dysentery was 2.6-fold higher in the placebo group.

The [Ludwik Hirsfeld Institute of Immunology and Experimental Therapy \(https://www.iitd.pan.wroc.pl/en\)](https://www.iitd.pan.wroc.pl/en) in Wrocław, Poland, continues the tradition of phage therapy in Poland, which originated in the 1920s. A detailed retrospective analysis of the safety and efficacy of phage therapy in 153 patients with a wide range of infections resistant to antibiotic therapy, which were admitted between 2008 and 2010, was published in 2012.^[11] In general, results suggested that phage therapy could provide good clinical results in a significant cohort of patients with otherwise untreatable chronic bacterial infections and this without significant adverse events.

Biological aspects of Phage Therapy

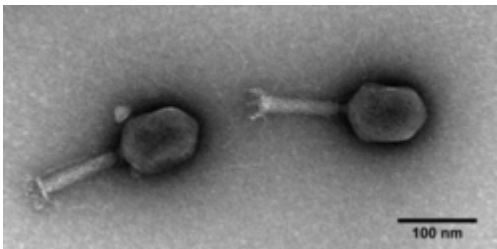


Figure 1 | Bacteriophages; image obtained by Transmission Electron Microscopy

What are bacteriophages?

The word, "phage" is derived from the Greek 'phagos', meaning to eat. Combined with the word "bacteria" it forms the construct bacteriophage, which denotes a type of viruses that "eat" (infect and/or destroy) only bacteria. Phages are viruses and therefore consist of DNA or RNA enclosed within a protein capsid. Bacteriophages (or simply phages) occur naturally and their only known means of replication is inside the specific bacterial strains upon which they prey. When isolated and replicated, phages can form an effective tool to target and destroy bacteria.

Phages are the most abundant organisms on the earth (the number of phage particles on the planet has been speculatively estimated to be approximately 10^{31}). These bacterial viruses are ecologically safe – phages are completely harmless and safe to humans, animals, plants and the environment.

How do phages work?

A specific phage seeks out host bacteria to which it is "active" (potentially lethal), attaching itself to the exterior of the bacterial cell wall, and injecting its DNA into the bacterium. One group of phages (called "temperate" phages) integrate their genetic material into the host bacterial genome; while phages from another group ("virulent" or "lytic" phages) hijack the cell's reproduction mechanism and reprogram it to produce new phage particles. During this active infection process and after the phage has sufficiently assembled and multiplied phage progeny within the cell, specific enzymes are released by the phages, which lyse (rupture) the outer wall of the bacterial host, killing it and releasing new phages into the environment to find and infect other bacterial cells.^[12] Since phages multiply exponentially, a bacterial infection can be decimated in a very short period of time.

Bacteriophage application in Phage Therapy

The ability of phages to replicate in host bacteria, lyse them and form new phage progeny raised the possibility to use these bacterial viruses to combat infectious bacterial diseases. The natural activity of phages harnessed and directed against specific bacterial infections in humans, animals and plants could augment an organism's

own immune system or replace/support antibiotics, particularly those that have become ineffective due to antimicrobial resistance.

Phages can be used for treatment (therapy) of different acute or chronic bacterial infections, including antibiotic resistant infections. The first stage of phage treatment includes the identification of the pathogen – the causative agent of the infection – from clinical samples taken from infected material (mucus, blood, stool, urine, wound, sputum, etc) of a patient. It is possible to give to a patient a multicomponent phage mixture (often called a cocktail) ~~and save time by avoiding a bacteriological analysis~~. In this case, if the bacterial pathogen is not sensitive to the phages in the mixture, it will not cause any adverse reaction to the patient and will be easily cleared from the body. However, preliminary bacteriological analysis is recommended.

After identification of the pathogen (infectious agent), the bacteria should be checked in vitro against a library of phages to select the most effective phage for therapeutic application. The successful use of therapeutic phages in modern clinical settings depends on having a capable diagnostic laboratory. It is essential to have a set of well-characterized phages available covering a broad range of bacterial pathogens to compose the most effective phage preparation.^[13]

In case the library of phages does not include a bacterial virus with the desired activity, an "active" phage (lytic to the specific pathogenic bacterial strain) often can be isolated from different sources. Phages are natural entities ~~on the planet~~ and are also natural enemies of bacteria; however, only a small number of these bacterial viruses have proven to be effective as therapeutic agents. Phages that are used for therapy should exhibit a strictly lytic infection cycle during propagation on the host bacteria^[14] (temperate phages are usually not recommended for therapeutic use, as there is a possibility of transferring undesired genes, coding for virulence or antibiotic resistance, through lysogeny or transduction, which should not be allowed to occur). After the treatment course is completed, the phage, in the absence of host bacterial cells, ~~is released~~ from the body without disturbing any cells or organs.

Phages also can be used as a mono-phage preparation or in combination with other ~~similar or tangentially related, even rather difficult~~ phages in mixture (again, a "cocktail"). Based on the site of the infection, the diagnosed disease, and specific indications, phage preparations can be administered orally, locally, subcutaneously, intravenously, among other. Additionally, phages can be used for prophylactic (prevention) purposes against bacterial diseases, mainly in cases where a high incidence of infection, or an epidemic spread of infections is expected. The best examples of phage usage for prophylaxis include applications against intestinal problems such as diarrheal diseases caused by Salmonella, Shigella, and enteropathogenic E.coli.

Advantages of Phage Therapy

There are numerous advantages of phages over other anti-bacterial agents, namely:

1. *Specificity*: ~~A specific phage~~ can be found to target almost any pathogenic and conditionally-pathogenic bacterial strain, including those that are multidrug resistant. Phages can exhibit extremely high antibacterial activity against target bacteria, making them a strong weapon against infectious bacterial diseases. Phages can be either isolated, selected or adapted to attack antibiotic-resistant bacteria in the case of mostly all disease-caused by bacterial pathogens. Exceptions are for several infections where the bacterial pathogen ~~– the causative agent of infection~~ is intracellular. The range of host bacteria that are targeted by a specific phage is much narrower than that treated by antibiotics or other antibacterial agents: phages recognize and ~~specifically~~ attach to ~~precise receptors of a~~ host bacteria. Most phages can only target a single bacterial species, although some phages reveal non-specific lysis of bacteria outside of the species but still within the same family (e.g. mycobacteriophages (<https://phagesd.b.org/hosts/genera/1/>)). Therapeutic phages can thus be selected to mainly target the bacterial species, or even a relevant subgroup, which is causing the infectious disease, and to spare the patient's beneficial bacteria (e.g. the gut, skin or oral commensal flora). Most routinely employed

antibiotics, in contrast, have a broad spectrum of activity, which can cause "collateral damage" to the patient's microbiome, which in turn can result in adverse effects such as the selection of antibiotic resistant bacteria (e.g. *Clostridium difficile*) or antibiotic-associated diarrhea.^[15] The ~~backside of the coin~~ is that before starting phage therapy, the infecting bacteria ~~need to have been~~ identified. In empiric antibiotic therapy, in contrast, ~~a cocktail of~~ broad-spectrum antibiotics that affect a ~~multitude of~~ Gram-positive and Gram-negative bacteria is typically applied.

2. *Self-replicating*: In contrast to antibiotics, phages replicate at the site of infection, having the ability to multiply ~~in the presence of~~ host bacterial cells and thus making them self-propagating in the ~~face of~~ specific bacteria. ~~But they also are self-regulating, meaning that as~~ bacterial cells are destroyed, phage do not have any new targets to attack and their propagation ~~finishes~~. In contrast, the concentration of an antibiotic introduced into the human organism decreases only with time (through the natural drug clearance mechanism from the body), whereas phages will continue to multiply exponentially, and decrease in numbers only as the target bacterial cells are eliminated.
3. *Local activity*: Phages replicate only at the site of infection, lowering the probability of allergies and **secondary infections**.
4. *Non-toxicity*: Phages are part of the human normal virome and therefore, **they are harmless to human cells**. No significant adverse reactions have been observed in several reported clinical trials (described below).
5. *Anti-biofilm activity*: Phages can effectively penetrate and destroy bacterial biofilms, which make a helpful therapeutic option for the patients with deep wound infections, secondary infections in cystic fibrosis patients and many others (detailed below).
6. *Immune modulation*: Interaction of phages with the cells of the immune system includes two main aspects: phages can induce a specific immune response (immunogenicity) and they also exhibit a non-specific effect on major populations of immune cells involved in both innate and adaptive responses (immunomodulatory activity).^[16] In the first case, a phage-induced immune response is, ~~not necessary~~, an issue for therapy. Formation of phage-neutralizing antibodies can be ~~slower than phage effect on bacteria~~ in vivo, especially when a therapeutic phage is not spread systematically ~~in a large amount, like~~ in local or oral administration. For therapeutic applications, appropriate protocols should be developed for phage preparations in case of treatment of different infectious complications.
7. *Easy production and formulation*: phages present the advantages of rapid isolation, relatively low production costs, and versatility of formulation and application. It is possible to prepare therapeutic phage preparations in different medicinal forms to administer in liquid, tablets (pills), locally (as a cream, tampons, droplets, ointment), rectally or intra-vaginally (suppositories), as aerosols, intrapleural applications, intradermal, subcutaneous and intravenous applications. Phages are compatible with any pharmaceutical or other remedies, such as other antibacterials (antibiotics), vaccines, enzymes, probiotics, etc, and have no known drug interaction complications ~~reported~~.

Current challenges of Phage Therapy

Phage therapy also faces some challenges that should be considered during the preparation of therapeutic phage formulations, as well as during the actual therapy:

1. *High specificity*: The narrow host range of phages can be a complication when the patient is affected by multiple pathogenic bacteria. This challenge is ~~usually~~ addressed by using several phages in a single mixture (cocktails) that are active against different bacterial pathogens; or by using a combined antibiotic-phage approach. At best, ~~they~~ infect a considerable ~~part of~~ one single bacterial species, but generally target only a small number of strains within one species.
2. *Storage conditions*: Bacteriophages ~~should be~~ stored and preserved at 4-5°C temperature, which can create some challenges for long transportation or storage, although for short transfers

it is possible to keep these viruses at room temperature. In general, phages are stable organisms and they keep their viability over several years. Therapeutic phages thus have a significant shelf life (1.5–2 years).

3. *Fast screening methods*: In consequence of the high specificity of phages, finding a phage for a particular strain might require the screening of large phage collections. In a clinical context, the rapid identification of the therapeutic agent(s) is crucial for the success of the therapy and so, high-throughput and fast screening methods are highly needed. Although multiple methods have been developed, most of them are not high-throughput for testing large phage collections or cannot be applied in clinical settings.
4. *Resistance to phages*: ~~Just as with most antimicrobials, bacteria can develop resistance to phages, but, in contrast to static antimicrobials, such as antibiotics, phages have the capacity to overcome emerging bacterial resistance.~~
5. *Activity against biofilms*: The biofilm structure and composition (polysaccharides, lipids, etc.) might impair phage infection in several ways. First, the biofilm matrix can limit phage access and docking to the receptors of bacterial cells by forming a physical barrier for phage diffusion. Second, the presence of dormant persister cells in the deeper layers of the biofilm also constitute an obstacle to phage infection as phages are unable to replicate in metabolically inactive cells.
6. *Standardized protocols*: One challenge associated with the treatment process is the lack of standardized protocols regarding dosing, frequency of dosage, etc., and which vary for phage therapy for different infectious diseases approved by international health community and legal authorities. Current protocols are based on long-term experience of doctors from FSU countries, or ~~currently~~, individual doctors who are selecting the protocols by their individual choice and opinion. ~~As suggested earlier, many emerging practitioners are not aware of, or trained in the many nuances of successful phage therapy~~
7. *Public opinion*: In general, phage application is often connected to some misperception of the approach. First, there is a lack or limited knowledge of phages and phage therapy. Second, is the irrational but understandable fear by the public of being treated by viruses. Third, (conversely) sometimes the public has unrealistically high or uninformed expectations for phage treatment, using phages incorrectly against a non-diagnosed bacterial infection, or even attempting to treat another kind of infection (e.g., a fungal or a viral). ~~This last issue is often the fault of uninformed phage practitioners who may have expertise in isolating phages but limited knowledge on how to therapeutically apply them.~~
8. *Expertise*: the selection and preparation of therapeutic phages and cocktails requires skilled personnel with significant knowledge and experience, and specific laboratories and existing phage collections. Isolation and selection of active phages against specific host bacteria need to follow several preliminary steps, for example, selection of good sources for phage isolation (environmental samples, human or animal samples, soil samples, etc.), and following specific protocols for isolation methodology. Phages in nature are targeted to a specific bacteria, having a narrow range of lytic activity. In order to prepare therapeutic phages for treatment, it is crucial to select virulent phages and to "train" them to enhance their lytic activity, stability and host range. The process requires specific knowledge and experience for scientists.

Phage Therapy approaches

Population medicine vs. precision medicine

When ~~it is intended~~ to use phages for therapeutic purposes, i.e. using them to control bacterial pathogens, then two of their characteristic properties are particularly of interest. First, as already mentioned, phages are highly specific for their target bacteria. Secondly, bacteria and phages are engaged in a host-parasite relationship.

When it comes to phage therapy, two distinct approaches ~~have crystallized to deal with the two above-mentioned aspects of phage-bacterium ecology, i.e. phage specificity and bacterial phage resistance~~.^[17] In what could be called the *one-size-fits-all* phage therapy approach, defined broad spectrum phage cocktails, which are ~~supposed to target the majority of bacteria that are suspected to cause certain infectious diseases, would be industrially produced and widely distributed~~. In **personalized** or **precision** phage therapy concepts, one or more phages are selected from a phage bank, or from the environment, and possibly adapted (i.e. the *in vitro* selection of phage mutants that exhibit an increased infectivity ~~range and time period~~) to infect bacteria that have been isolated from the patient's ~~infection site~~ (as indicated by *in vitro* test, ~~i.e. a so-called phagogram~~), and are produced on a small scale.

The predefined phage cocktails could in principle be developed, produced and marketed within ~~the current pharmaco-economic models, which have been designed for, and together with, 'static' drugs such as aspirin or small molecule-type antibiotics~~. However, truly 'broad spectrum' phage cocktails, active against most Gram-positive and/or Gram-negative bacteria commonly encountered in infectious diseases, would need to contain hundreds of phages and would be very difficult to develop considering the current state of the field. In the future, synthetic biology approaches (e.g. structure-guided design) could generate phages with more predictable and extended host ranges.^[18] Today, it could be feasible to use narrower spectrum phage cocktails that are active against one or a few bacterial species, to be used in certain indications, ~~minding that the infecting bacterial species are known prior to phage therapy~~. For some (clonal) bacterial species, such as *Staphylococcus aureus*, phages have been isolated and characterized that show an exceptionally broad host range, such as phage ISP of the Eliava Institute, which was shown to be active against 86% of *S. aureus* strains.^[19] However, even then one needs to keep in mind that these cocktails will not always be efficient due to the greater biodiversity outside of the laboratory and due to the pre-existing resistance to the chosen phages. In **PhagoBurn** (<http://www.phagoburn.eu/>), a randomized, controlled, double-blind phase I/II trial, ~~assessing the efficacy and tolerability of a cocktail of phages to treat burn wounds infected *Pseudomonas aeruginosa*~~, the success in achieving sustained reduction in bacterial burden was linked to initial (i.e. day 0) *P. aeruginosa* susceptibility to the phage cocktail, highlighting the importance of preliminary phage-susceptibility testing.^[20] The susceptibility of bacterial strains isolated at day 0 was tested in 10 participants in the phage group. Three of the 10 participants (30%) were shown to harbor pre-existing *P. aeruginosa* strains resistant to the phage cocktail, which consisted of no less than 12 natural lytic anti-*P. aeruginosa* phages. It is thus likely that for the time being, most so-called broad-spectrum approaches will greatly depend on rapid pre-treatment identification of the bacterial pathogen(s).

In addition, phage cocktails that are initially suspected to be efficient, based on ~~pathogen isolation and a positive phagogram result~~, will likely need to be regularly updated (e.g. supplemented with new phages) in response to the anticipated emergence of phage resistance or the involvement of newly circulating clinically relevant strains. There are indications, from *in vitro* experiments, that bacterial resistance to phages, even to cocktails containing multiple phages, will inevitably occur, albeit later than if one single phage is used.^[21] Pre-adapting phages to a pathogen was shown to lead, *in vitro*, to increased pathogen clearance and ~~lowered resistance evolution~~.^[22]

Precision phage therapy approaches tend to be more elaborate, but are potentially more sustainable as they are suspected to apply less selection pressure towards bacterial phage resistance. These approaches ~~are best served by~~ phage banks consisting of a large assortment (dozens to hundreds) of characterized phages. From these banks, phages are selected, and – ideally – adapted, to the causative bacterial strain. These precision phage products, which will be used in a limited number of patients, can be rapidly produced on a small scale. Georgian (<http://eliava-institute.org/>) and Polish (<https://www.iitd.pan.wroc.pl/en/OTF/>) phage therapy centers set up and maintain large therapeutic phage banks, which are regularly updated with new phages, widening and adapting the host range of the bank to the ever changing bacterial populations, with regard to the emergence of new clinically relevant bacterial strains and species, as well as phage resistance. However, this concept is not compatible with most (Western) medicinal product (drugs in the US) development and licensing pathways, which require several years and millions of euros (dollars) to complete, even when considering

abbreviated pathways, and this for every phage in the bank. Moreover, it is very difficult to imagine how existing medicinal product pathways will be able to cater for the ad hoc adaptation of banked phages, or the application of newly acquired or isolated (from the environment) phages.

It might be wise to develop both options, defined broad spectrum phage medicines, produced at a large scale and globally supplied for first line use, and a local small scale supply of precision phage products for use in personalized therapies or public health or medical emergencies such as the O104:H4 (hybrid EaggEC STEC/VTEC pathotype) *Escherichia coli* outbreak, which caused the death of more than 50 patients in Germany in 2011.^[23] At the Eliava Institute (<http://eliava-institute.org/>) in Tbilisi, scientists verify their commercial phage cocktails at least annually against the pathogen isolates that were recently isolated and then add phages and/or adapt the phage preparations to keep pathogen coverage ranges high. In addition, a personalized "autophage" approach was set up for patients harboring bacterial strains that are not targeted by the "broad spectrum" phage cocktails. These strains are then screened against the phages present in the phage bank of the Eliava Institute, which comprises of hundreds of therapeutically promising phages. If a suitable phage is found, it is produced as quickly as possible for use in that individual patient.

Phage therapy against infectious biofilms

Bacterial biofilms can be defined as microbial communities surrounded by a complex extracellular matrix, also known as EPS (extracellular polymeric substances). These complex structures constitute an important bacterial survival strategy in adverse environments^[24]. The biofilm formation occurs spontaneously on both inert and living systems and are frequently implicated in many chronic infections as consequence of their inherent tolerance to antibiotics and disinfectants.^[24] To deal with this problem, several alternative strategies to antibiotics have been proposed, including phages.

Many researchers have been studying the potential of phages for biofilm control or prevention. In fact, significant reductions of single or dual-species biofilms have been reported when a phage treatment was applied.^{[25][26][27]} However, the majority of these studies have been performed using *in vitro* models of biofilm formation and remains unclear whether such models resemble the biofilms found in real situations.^[25] Although *in vitro* biofilm studies are relevant for an initial screening of the best phages for this application, they cannot replace the *in vivo* studies for an accurate evaluation of the outcome of infection.

In spite of the fact that some *in vivo* models of biofilm infection have been described, there is still a limited knowledge regarding the therapeutic application of phages on real biofilms. ~~The mice model~~ of wound infection is the most commonly used *in vivo* model to study the therapeutic potential of phages against bacterial biofilms. Most of these studies were focused on *P. aeruginosa* and *S. aureus*, two of the most common pathogens present in wound infections, and have demonstrated that phages can indeed be a valuable alternative or complementary approach for biofilm treatment.^[28]

Nonetheless, despite all the promising results achieved, the total eradication of biofilms seems to be a nearly impossible task.^[25] The biofilm structure and composition can indeed pose some limitations to the success phage infection. First, the access of phages to the cell surface receptors can be impaired by the biofilm matrix surrounding the cells. Additionally, the heterogeneity and reduced metabolic activity of the biofilm cells and the fast proliferation of phage-insensitive mutants (BIMs) within the biofilm, constitute some of the major obstacles to the success of phage infection.^[25] To deal with these limitations several strategies have been proposed, namely: i) the use of phage cocktails; ii) combined therapies of phages with antibiotics, antiseptics or enzymes; iii) mechanical debridement of biofilm prior phage application; or iii) genetic manipulation of phage genomes to encode genes that would enhance their anti-biofilm activity.^[25]

Phage-antibiotic combination

To enhance the outcome of the treatment, phages can be combined with other antimicrobial agents such as antibiotics. This type of combined treatment has been widely studied by the scientific community and it has been reported that sublethal concentrations of antibiotics can indeed enhance the activity of virulent phages, a phenomenon that was named as phage-antibiotic synergy (PAS).^[29] This synergy has been shown for several bacterial species grown as planktonic cultures or biofilms.^{[29][30]} For instance, it has been reported that sublethal concentrations of antibiotics can foster the production of virulent phages and increase the size of phage plaques.^{[29][31]} Additionally, some combinations of phages and antibiotics have been shown to almost eradicate bacterial biofilms, depending on the antibiotic concentration and the order of application.^[30] Besides the synergistic effect, it has been further reported that the combination of phages and antibiotics can significantly arrest the emergence of resistant variants, which is a very important feature.^[32]

However, this combination has not always resulted in synergistic effects as, in some cases, additional or antagonistic effects were reported.^{[29][33]} It is important to highlight that the success of the combined therapies is very dependent of key factors such as the dosage levels, order of administration, frequency or time points. Furthermore, potential drawbacks associated with combined therapies can be anticipated such as the development of double-resistant variants or the selection for antibiotic-resistant subpopulations in case that phages preferentially target the antibiotic-sensitive ones.^[33]

In addition to antibiotics, the synergistic effect between phages and antiseptics or other antimicrobial compounds has also been assessed and revealed encouraging results.^[25]

Biotechnological advances in Phage Therapy

The success of phage therapy is highly dependent on the safety of phage preparations, which raises manufacturing, formulation and delivery challenges. Although phage therapy must comply with the strict regulations applied for pharmaceutical products, ~~no clear guidelines were yet developed~~ specifically for phages, which limits their broad application in therapy.^[34] In addition, robust manufacturing processes that will avoid variability as well as production in GMP will probably be required to use phages as a first-line treatment.

~~A group of phage researchers have set~~ some quality and safety requirements for sustainable phage products. One of them is that phages ~~encoding for lysogeny, virulence factors or antibiotic resistance~~ should not be considered suitable for therapy.^[35] However, no strictly virulent phages have been found for some bacterial species such as *C. difficile*,^[36] which might limit the use of phage therapy in some fastidious bacteria. The presence of impurities in phage preparations, such as endotoxins or LPS, should also be avoided, and several purification methods have been developed and optimized to ensure phages' safety.^[37]

Another important feature is the stability of phage preparations since the efficacy of the treatment is highly dependent on the phage concentration delivered to the site of infection.^[38] The reduction of phage titers due to their interaction with host antibodies or other clearance mechanisms might reduce the efficacy of the therapy. Therefore, strategies to enhance phages' stability to pH and increase their circulation time at infective doses in human body are being developed. One of ~~this~~ strategies is the encapsulation of phages on different matrices such as liposomes, alginate, cellulose or other polymers.^[38] Indeed, *in vivo* studies have demonstrated that liposome-encapsulated phages are able to persist for longer periods in the stomach and also adhere to the intestinal membrane.^[39]

The stability of phages can also be enhanced by genetic manipulations of phage genomes. In fact, several phage-engineering tools have been developed and opened a window of opportunities to ~~shape~~ phages with better antibacterial properties.^[40] Using such strategies, it was already possible to expand phages' host ranges, improve their antibacterial and anti-biofilm activities, reduce their immunogenicity or enhance the bactericidal activity of antibiotics.^[40] The tremendous potential of phage engineering was already proved in clinical practice as synthetic phages were successfully used to treat a 15-year-old patient (<https://www.bbc.com/news/h>

ealth-48199915) with a disseminated drug-resistant *Mycobacterium abscessus* infection.^[41] This study clearly indicates that engineered phages may be an important weapon in the future for the treatment of bacterial infections.

Pre-clinical evaluation

A significant number of pre-clinical studies using animal models has been performed to study the efficacy and safety of phage therapy against several types of infections (revised in ^[42]). These studies are the major source of information on the most efficient routes of phage administration for controlling bacterial diseases and the most effective phage dosing. The first obvious conclusion drawn from all animal trials is that phage therapy is safe, as no harmful effects of phage therapy were identified in any of the reported studies. In terms of efficacy, the results are quite variable and very dependent on the type of infection and therapeutic schedule, including moment of the first application, phage dose, and route of administration.

In most cases, stage of infection when a phage was first applied was important, with the tendency for early administrations to be more effective, and more difficult treatments in case of established infections. In general, chronic infections are generally more difficult to tackle than acute infections. Chronic infections are characterized by the presence of biofilms, which are intrinsically tolerant to antimicrobials. Nevertheless, pre-clinical trials demonstrated successful outcomes with topical application of phages usually associated with prior debridement and using high phages titres. Phage concentration seems to be a key factor for the outcome of phage therapy, since *in vivo* trials demonstrated better therapeutic efficacy when high phage titres were applied, usually at an MOI of 10 and concentrations greater than 10^7 phage active particles per treatment (10^7 - 10^9 PFU). In many cases local/topical administration allows for efficient delivery of phage to a site of infection, but in other cases, systemic delivery must be applied. In systemic deliveries, achieving effective concentration of phage depends on the route of administration; the most effective are all types of injections (intramuscular, intravenous, subcutaneous, others) and the least effect is oral delivery with usually very limited spread of phage amount in the body, as revealed by systematic review of phage pharmacokinetics.^[43]

The administration of phage cocktails has usually resulted in a better outcome than single phage applications. Other treatment variables such as the optimal schedule of administration or duration of treatment are difficult to generalize based on pre-clinical studies.

Clinical experience and randomized controlled trials

Today, as a result of changes that occurred in the 1990s, evidence-based medicine defines the standard of care. Randomized controlled trials (RCTs) are recognized as the gold standard for studying causal relationships between medical interventions and outcomes. Even though evidence-based medicine does not necessarily require that patients should receive the treatment option that was (more) effective in RCTs, in practice this is most often the case. Many of today's medical procedures and decisions, however, are not evidence-based, but based on clinical practice experiences. For instance, only 11% of American cardiology guidelines are evidence-based.^[44] For several antibiotics and antiseptics, there is even no RCT evidence that they have any effect on infection rate in certain indications.^[45] In addition, RCTs were shown to be as flawed as other forms of peer-reviewed medical research.^[46]

The competent authorities for medicines in the EU and in the US classified therapeutic phages as "medicinal products (<https://www.ema.europa.eu/en/glossary/medicinal-product>)" and "drugs" respectively, and decided that they cannot be recommended for (marketing) authorization before their efficacy and safety have been

proven in new and appropriately designed controlled trials.^[47] Historical data and clinical experience were not considered.

Several RCTs were launched in Europe, but none of them managed to avowedly prove a sufficient efficacy of phage medicines. Note that, as a result of the medicinal product or drug classification, most of these clinical trials evaluated static phage medicines using conventional clinical trial designs, but did not evaluate the long-established (precision) phage therapy approaches, which use regularly updated phage preparations. Several phase I, I/II and III clinical trials did demonstrate the safety of certain phage medicines, which is consistent with the safety data provided by numerous preclinical animal studies.

To date, two European RCTs showing some phage treatment efficacy have been reported in the scientific literature. In the first one, a small, but well-designed, randomized, double-blind, placebo-controlled phase I/II clinical trial, phage therapy against chronic *P. aeruginosa* otitis was investigated.^[48] A defined cocktail of 6 phages was shown to be successful, as the bacterial burden was found to be significantly lower in 12 phage-treated patients as compared to 12 placebo-treated patients, and no adverse effects were observed. The second one is a small randomized, controlled, double-blind phase I/II clinical trial, designed to evaluate the treatment of *P. aeruginosa* infected burn wounds using a defined phage cocktail.^[20] The study had to deal with many setbacks and delays.^[49] One batch of the investigational phage cocktail, containing 12 phages, took 20 months and a lion's share of the study budget to manufacture in compliance with Good Manufacturing Practices (GMP). Phage specificity issues hampered the recruitment of patients, resulting in only 27 patients enrolled and randomly assigned to receive either phage therapy (n=13) or standard of care (n=14). Standard of care consisted of 1% sulfadiazine silveremulsion cream, an antiseptic that had never been subjected to an RCT before. Due to product stability issues, only a very small number of phages (10-100 PFU/ml instead of the anticipated 10⁶ PFU/ml) were applied. Yet, even at these very low concentrations, the *P. aeruginosa* phage cocktail was shown to decrease the bacterial load in burn wounds, albeit at a slower pace than standard of care.

In Bangladesh, an RCT of phage therapy with two different coliphage cocktails was performed in children with *E. coli* diarrhea, between June 2009 and September 2011.^[50] Oral coliphage were shown to reach the children's intestines, but they did not achieve treatment effects over placebo, consisting of reduced osmolarity oral rehydration solution (ORS) supplemented with zinc, the standard treatment for uncomplicated watery diarrhea at the clinical trial center. As a possible reason for this disappointing result, the authors stated that microbiota analysis had revealed a marked outgrowth of fecal *streptococci* during the acute phase of diarrhea with *E. coli* titers close to the replication threshold of the coliphages.

In Australia, a phase I, first-in-humans, open-label clinical trial was conducted to assess the safety, tolerability, and preliminary efficacy of ascending multiple intranasal doses of an investigational phage cocktail, containing three *S. aureus* phages, in patients with recalcitrant chronic rhinosinusitis due to *S. aureus*.^[51] Intranasal irrigation with phage cocktail doses to 3 × 10⁹ PFU for 14 days were shown to be safe and well tolerated. Preliminary efficacy observations were promising with patients showing clinical and microbiological evidence of eradication of infection.

In Georgia, the feasibility, tolerability, safety, and clinical/microbiological outcomes of adapted phages in the treatment of urinary tract infections were evaluated in a case series as a pilot for a double-blind RCT.^[52] Nine patients that had undergone transurethral resection of the prostate (TURP) and had positive urinary cultures with uropathogens sensitive to the adapted Pyo bacteriophage, one of the commercial phage preparations produced by the Eliava Biopreparations (company associated with Eliava Institute), were selected for phage therapy. Bacterial burden decreased in six of the nine patients (67%) and no phage-associated adverse events were detected. Meanwhile, the RCT has been completed and the results are being published. The safety of oral exposure to the Eliava Pyophage and to a *S. aureus* monophage, as compared to a placebo, was assessed in healthy human carriers of *S. aureus*.^[53] No adverse effects were observed in any of the treatment groups, which supports the clinical safety of *S. aureus* phages administered either as a single phage or as part of a phage cocktail.

Another commercial preparation of the Eliava Institute, the anti-staphylococcal phage Sb-1, was used in the treatment of a series of diabetic toe ulcers **in view of** future full-scale controlled clinical trials.^[54] Nine diabetes patients with poorly perfused toe ulcers infected (culture-proven) with *S. aureus* and who had responded poorly to recommended antibiotic therapy, were treated with Sb-1 in combination with infected bone and soft tissue debridement. The ulcers healed in an average of seven weeks and one ulcer with very poor vascularity required 18 weeks of treatment.

The effect of a commercial *E. coli* phage cocktail on gut microbiota and markers of intestinal and systemic inflammation in a healthy human population was examined in a double-blinded, placebo-controlled crossover trial.^[55] Reductions in fecal *E. coli* loads were observed, without global disruption of the gut microbiota. Specific populations were altered in response to the phage treatment, including increases in members of the butyrate-producing genera *Eubacterium* and a decreased proportion of taxa most closely related to *Clostridium perfringens*, which demonstrated the potential of phages to selectively reduce target organisms without global disruption of the gut community.

In addition to the above-mentioned clinical trials, an increasing number of (small series of) case studies, involving different clinical indications and infecting bacterial species, have been reported to show promising results.^{[41][56][57][58][59][60][61][62][63][64][65][66][67]} **The pharmaceutical industry has so far demonstrated little to no interest in phage therapy,** and RCTs to evaluate and compare safety and efficacy might be too costly for smaller biotechnology companies. Hence, there is a relatively low number of completed ~~(a handful of phage RCTs are currently in the pipeline)~~ phage-related RCTs so far. If medicinal product or drug classification is ~~retained~~, a coordinated private-public effort might be necessary to forward the phage therapy field.^[68]

Development of resistance to phages

One of the major concerns usually associated to phage therapy is the emergence of phage-insensitive mutants (BIMs) that could hinder the success of this therapy. In fact, several *in vitro* studies have reported a fast emergence of BIMs within a short period of time after phage treatment.^{[69][70][71]} The emergence of BIMs has also been observed *in vivo* using different animal models, although usually occurs later than *in vitro* (reviewed in ^[72]). This fast adaptation of bacteria to phage attack is usually caused by mutations on genes encoding phage receptors,^{[70][73]} which include lipopolysaccharides (LPS), outer membrane proteins, capsules, flagella, pili, among others.^[74] However, some studies suggested that when phage resistance is caused by bacterial mutations on phage receptors, this might result in fitness costs to the resistance bacterium, which will ultimately become less virulent.^{[72][75]}

Besides the prevention of phage adsorption by loss or modification of bacterial receptors, other described mechanisms used by bacteria to avoid phage predation are: (i) prevention of phage DNA integration by superinfection exclusion systems; (ii) degradation of phage DNA by restriction-modification systems or by CRISPR-Cas systems; and (iii) use of abortive infection systems that block phage replication, transcription or translation.^[76] Altogether, these mechanisms promote a quick adaptation of bacteria to phage attack and therefore, the emergence of phage-resistance mutants is frequent and unavoidable.

In case of phages being widely used in the future as antibiotics had been in the past, it is still unclear whether it could lead to a widespread of phage resistance. In theory this is not very likely to occur, since phages are very specific and therefore their selective pressure would affect a very narrow group of bacteria. Currently, in contrast to antibiotics, phage preparations for therapeutic applications are expected to be developed in a personalized way because of phages specificity. In addition, strategies have been proposed to counterattack the problem of phage resistance. One of the strategies is use of phage cocktails with complementary host ranges

and targeting different bacterial receptors. Another strategy is the combination of phages with other antimicrobials such as antibiotics, disinfectants or enzymes that could enhance their antibacterial activity. The genetic manipulation of phage genomes can also be a strategy to circumvent phage resistance.

Safety aspects

Bacteriophages are bacterial viruses, evolved to infect bacterial cells; to do that, phages must use characteristic structures at cell surfaces (receptors), and to propagate they need appropriate molecular tools inside the cells. Bacteria are Prokaryotes and their cells differ substantially to Eukaryotes including humans or animals. For this reason phages meet the major safety requirement: they do not infect treated individuals. Even engineered phages and induced artificial internalization of phage into mammalian cells did not result in phage propagation. Internalization can be induced e.g. by adding adenovirus penton base protein on the phage surface, it allows for the attachment of engineered phages to integrin receptors and for endocytosis. These mimic adenoviral infection, but no resulting propagation of phage nor any cell damage were observed.^[77] Natural transcytosis of unmodified phages, that is: uptake and internal transport to the other side of a cell, which was observed in human epithelial cells, did not result in phage propagation or cell damage.^[78]

Due to many experimental treatments in human patients conducted in past decades, and to already existing RTCs (see section: Clinical experience and randomized controlled trials), phage safety can be assessed directly. The very first safety trial in healthy human volunteers for a phage was conducted by Bruttin and Brüssow in 2005;^[79] they investigated *Escherichia coli* phage T4 and they found no adverse effects of the treatment. Historical record shows that phages are safe, with maybe mild side effects if any. The most frequent (though still rare) adverse reactions to phage preparations found in patients were symptoms from the digestive tract, local reactions at the site of administration of a phage preparation, superinfections, and a rise in body temperature.^{[11][80][81]} Notably, these reactions may have been (i) due to the liberation of endotoxins from bacteria lysed *in vivo* by the phages, since such effects also can be observed when antibiotics are used,^[82] or (ii) caused by bacterial debris that accompanied phage in cases where unpurified lysates were used.

Bacteriophages must be produced in bacteria that are lysed (i.e. fragmented) during phage propagation. As such, phage lysates contain bacterial debris that may affect human organism even when phage is harmless. For this reason, purification of bacteriophages is considered important and phage preparations need to be assessed for their safety as the whole. This is consistent to general procedure for other drug candidates. In the year 2015, a group of phage therapy experts summarized Quality and Safety Requirements for Sustainable Phage Therapy.^[35]

Phage effects on human microbiome also contribute to the safety issues in phage therapy. First, some bacterial viruses may acquire and contain bacterial genes related to bacterial pathogenicity (virulence factors). Capability to acquire bacterial genes is linked mainly to the type of phage life cycle, and it is increased in those capable to enter lysogenic life cycle (temperate, lysogenic phages). For this reason, temperate bacteriophages are disrecommended as therapeutics. Even obligatory lytic bacteriophages should be analyzed to exclude potential presence of any undesired genes, preferentially by whole genome sequencing.

As antibacterials, phages may also affect composition of microbiomes, by infecting and killing phage-sensitive strains of bacteria. However, a major advantage of bacteriophages over antibiotics is the high specificity of bacteriophages; this specificity limits phage antibacterial activity even more narrow than to bacterial species, typically a phage kills only selected bacterial strains. For this reason phages are much less likely (than antibiotics) to disturb composition of natural microbiome or to induce dysbiosis. This was demonstrated in experimental studies where microbiome composition was assessed by next-generation sequencing (NGS) that revealed no important changes correlated with phage treatment.^{[50][53][83][84][85][86]}

Regulatory aspects

Since its inception in 1923, and until the 1980s, the Eliava Institute in Georgia has produced tons of therapeutic phage preparations, for the Red Army as well as for the civil sector, which led to the registration of phages for oral and topical applications as an over-the-counter product in pharmacies in several Member States of the former Soviet Union. Today, the phage production center and the pharmacy of the Eliava Institute manufacture and deliver several commercial phage preparations under a license from the Georgian government.

The Ludwik Hirsfeld Institute of Immunology and Experimental Therapy in Wrocław has produced numerous phage formulations for phage therapy in different hospitals in Poland, and this for many decades.

In France, phage therapy disappeared officially with the withdrawal of the Vidal dictionary (<https://www.vidal.fr/>) (France's official drug directory) in 1978. The last phage preparation, produced by l'Institut du Bactériophage, was an ointment against skin infections. Phage therapy research ceased at about the same time in France, with the closure of the bacteriophage department the Pasteur Institute. However, Professor J.-F. Vieu had collected several hundreds of phages potentially usable against staphylococci and digestive infections and the Pasteur Institutes of Paris and Lyon continued to provide these phages for medical use. As such, some hospital physicians continued to practice compassionate phage therapy until the 1990s when production eventually died out.^[87]

Upon their rediscovery, at the end the 1990s, phage preparations were logically classified as medicines, i.e. "medicinal products" in the EU or "drugs" in the US ^[88]. However, the pharmaceutical legislation that had been implemented since their disappearance from Western medicine was mainly designed to cater for industrially-made pharmaceuticals, devoid of any customization and intended for large-scale distribution ^[89], and it was not deemed necessary to provide phage-specific requirements or concessions. Today's phage therapy products need to comply with the entire battery of medicinal product licensing requirements: manufacturing according to GMP, preclinical studies, phase I, II and III clinical trials, and marketing authorization. Technically speaking, industrially produced predefined phage preparations, could make it through the conventional pharmaceutical processes, minding some adaptations. However, phage specificity and resistance issues are likely to cause that these defined preparations will have a relatively short useful lifespan.^[90] In addition, it appeared that the pharmaceutical industry, the stakeholder which is foreseen to develop and market industrially-made medicines, is currently not considering phage therapy products. Yet, a handful of small and medium-sized enterprises (SMEs) picked up the gauntlet, with the help of risk capital and/or public funding. The reality today is that decades after the renewed interest in the Western world, not one defined therapeutic phage product has made it to the EU or US markets, and clinicians are under increasing pressure to use phages in the treatment of multidrug-resistant bacterial infections.

According to some, therapeutic phages should be prepared individually and kept in large phage banks, ready to be used, upon testing for effectiveness against the patient's bacterial pathogen(s). Intermediary or combined (industrially-made as well as precision phage preparations) approaches could be appropriate.^[90] However, it turns out to be difficult to reconcile the classical phage therapy concepts, which are based on the timely adaptation of phage preparations, with the current Western pharmaceutical R&D and marketing models. The repeated calls for a specific regulatory framework have not been heard by the European policymakers, who appear to be resilient to change in this regard.^[89] A phage therapy

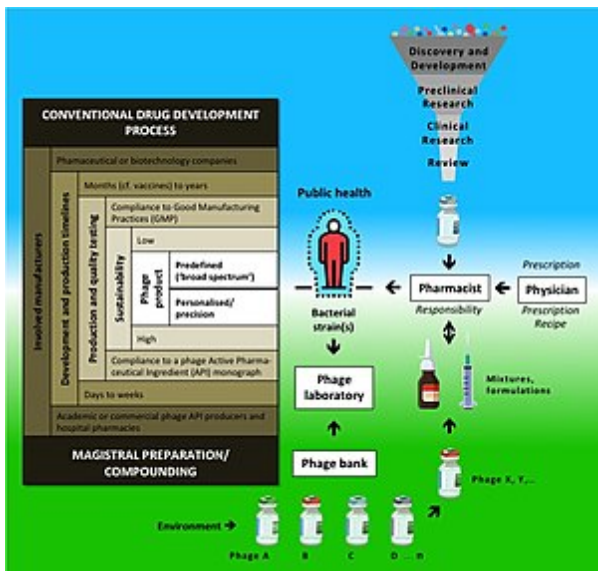


Figure 2 | Conventional drug development process vs. Magistral preparation

framework based on the Biological Master File (BMF) concept has been proposed as a (European) solution to the regulatory issues, but the European regulation did not allow for an extension of this concept to biologically active substances such as phages.^[91]

Meanwhile, individuals, responsible representatives from the medical, academic and regulatory communities have found each other provide some (temporary) national solutions. For instance, phage applications have been performed in Europe under the umbrella of Article 37 (Unproven Interventions in Clinical Practice) of the Helsinki Declaration. To enable the application of phage therapy after Poland had joined the EU in 2004, the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław opened its own Phage Therapy Unit (PTU). Phage therapy performed at the PTU is considered an "Experimental Treatment", covered by the adapted Act of 5 December 1996 on the Medical Profession (Polish Law Gazette, 2011, No. 277 item 1634) and Article 37 of the Declaration of Helsinki.^[68]

Some patients have been treated with phages under the umbrella of "compassionate use", which is a treatment option that allows a physician to use a not yet authorized medicine in desperate cases. Under strict conditions, medicines under development can be made available for use in patients for whom no satisfactory authorized therapies are available, and who cannot participate in clinical trials. In principle, this approach can only be applied to products for which earlier study results have demonstrated efficacy and safety, but have not yet been approved. Much like Article 37 of the Helsinki Declaration, the compassionate use treatment option can only be applied when the phages are expected to help in life-threatening or chronic and/or seriously debilitating diseases that are not treatable with formally approved products.

In France, l'Agence Nationale de Sécurité du Médicament et des produits de santé (<https://ansm.sante.fr/>) (ANSM), the French medicine agency, has organized a specific committee "Comité Scientifique Spécialisé Temporaire (CSST)" for phage therapy, which consists of experts in various fields. Their task is to evaluate and guide each phage therapy requests that ends up at the ANSM. Phage therapy requests are discussed in concentration with the treating physicians and a consensus advice is sent to the ANSM (<https://ansm.sante.fr/>), which will grant permission or not. Between 2006 and 2018, 15 patients have been treated in France (11 healed) using this pathway.^[61]

In Belgium, in 2016 and in response to a number of parliamentary questions, the Minister of Social Affairs and Health acknowledged that it is indeed not evident to treat phages as industrially-made drugs and therefore she proposed to investigate if the magistral preparation pathway could offer a solution.^[90] Magistral preparations (compounding pharmacies in the US) are not subjected to certain constraints such as GMP compliance and marketing authorization. As the "magistral preparation framework" was created to allow for adapted patient treatments and/or to use medicines for which there is no commercial interest, it seemed a suitable framework for precision phage therapy concepts. Magistral preparations are medicines prepared in a pharmacy in accordance with a medical prescription for an individual patient. They are made by a pharmacist (or under his/her supervision) from their constituent ingredients, according to the technical and scientific standards of pharmaceutical technology. Phage Active Pharmaceutical Ingredients (APIs) to be included in magistral preparations must meet the requirements of a monograph, which describes their production and quality control testing. They must be accompanied by a certificate of analysis, issued by a "Belgian Approved Laboratory (BAL)", which has been granted an accreditation to perform batch release testing of medicinal products. Since 2019, phages are delivered in the form of magistral preparations to nominal patients in Belgium.

Dozens of patients have been treated thanks to the above-mentioned national solutions. No safety issues were reported and most targeted infections seemed to have been resolved, but the diversity of these "desperate" phage therapy cases, in terms of clinical indications, involved bacterial pathogens, phage products, and treatment and sampling protocols, make it impossible to unambiguously demonstrate that the positive clinical outcomes were due to phages.

It is time to find a broader solution to the phage therapy regulatory issues. Supranational medicine agencies, such as the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) are urged to build on the initiatives that were developed by some national regulatory authorities.^[89] Policymakers need to be convinced to open the door for a broad and fast (interim) solution with reduced stringency until the present-day pharmaceutical requirements can be fulfilled, which may require many years.^[92] Phage banks containing large amounts of well-characterized (e.g. host range, genomic passport) and safe phages need to be set up. Physicians must be aware of the existence and content of these banks.

Additional information

Acknowledgements

Any people, organisations, or funding sources that you would like to thank.

Competing interests

The Authors declare no competing interests.

Ethics statement

An ethics statement, if appropriate, on any animal or human research performed should be included here or in the methods section.

References

1. Twort, F. W. (1915-12-04). "AN INVESTIGATION ON THE NATURE OF ULTRA-MICROSCOPIC VIRUSES.". *The Lancet*. Originally published as Volume 2, Issue 4814 **186** (4814): 1241–1243. doi:10.1016/S0140-6736(01)20383-3. ISSN 0140-6736. <http://www.sciencedirect.com/science/article/pii/S0140673601203833>.
2. d'Herelle, F. (1916). Sur un bacille dysentérique atypique. *Ann. de l'Inst.Pasteur*,30, 145.
3. D'Herelle, M. F. (1961-02). *Sur un microbe invisible antagoniste des bacilles dysenteriques* (in fr). ISSN 0515-2909. <http://sgc.anlis.gob.ar/handle/123456789/753>.
4. d'HERELLE, F. (1919). Sur le role du microbe bacteriophage dans la typhose aviare. *CR Acad. Sci*, 169, 932-934.
5. Lavigne, Rob; Robben, Johan (2012-01-01). "Professor Dr. Richard Bruynoghe: A 1951 overview of his bacteriophage research spanning three decades". *Bacteriophage* **2** (1): 1–4. doi:10.4161/bact.20024. ISSN 2159-7073. PMID 22666651. PMC 3357380. <https://pubmed.ncbi.nlm.nih.gov/22666651/>.

6. Abedon, Stephen T.; Kuhl, Sarah J.; Blasdel, Bob G.; Kutter, Elizabeth Martin (2011-03-01). "Phage treatment of human infections". *Bacteriophage* **1** (2): 66–85. doi:10.4161/bact.1.2.15845. PMID 22334863. PMC PMC3278644. <https://doi.org/10.4161/bact.1.2.15845>.
7. Eaton, Monroe D.; Bayne-Jones, Stanhope (1934-12-08). "BACTERIOPHAGE THERAPY: REVIEW OF THE PRINCIPLES AND RESULTS OF THE USE OF BACTERIOPHAGE IN THE TREATMENT OF INFECTIONS". *Journal of the American Medical Association* **103** (23): 1769–1776. doi:10.1001/jama.1934.72750490003007. ISSN 0002-9955. <https://jamanetwork.com/journals/jama/fullarticle/255543>.
8. Myelnikov, Dmitriy (2018-10-01). "An Alternative Cure: The Adoption and Survival of Bacteriophage Therapy in the USSR, 1922–1955". *Journal of the History of Medicine and Allied Sciences* **73** (4): 385–411. doi:10.1093/jhmas/jry024. ISSN 0022-5045. <https://academic.oup.com/jhmas/article/73/4/385/5128730>.
9. Chanishvili, Nina (2012). *A Literature Review of the Practical Application of Bacteriophage Research*. Nova Biomedical Books. ISBN 978-1-62100-851-4.
10. Sulakvelidze, A.; Alavidze, Z.; Morris, J. G. (2001-03). "Bacteriophage therapy". *Antimicrobial Agents and Chemotherapy* **45** (3): 649–659. doi:10.1128/AAC.45.3.649-659.2001. ISSN 0066-4804. PMID 11181338. <https://pubmed.ncbi.nlm.nih.gov/11181338/>.
11. Międzybrodzki, Ryszard; Borysowski, Jan; Weber-Dąbrowska, Beata; Fortuna, Wojciech; Letkiewicz, Sławomir; Szufnarowski, Krzysztof; Pawełczyk, Zdzisław; Rogóż, Paweł *et al.* (2012). "Clinical aspects of phage therapy". *Advances in Virus Research* **83**: 73–121. doi:10.1016/B978-0-12-394438-2.00003-7. ISSN 1557-8399. PMID 22748809. <https://pubmed.ncbi.nlm.nih.gov/22748809/>.
12. Ellis, E. L.; Delbrück, M. (1939-01-20). "THE GROWTH OF BACTERIOPHAGE". *The Journal of General Physiology* **22** (3): 365–384. doi:10.1085/jgp.22.3.365. ISSN 0022-1295. PMID 19873108. PMC 2141994. <https://pubmed.ncbi.nlm.nih.gov/19873108/>.
13. Gill, Jason J.; Hyman, Paul (2010-01). "Phage choice, isolation, and preparation for phage therapy". *Current Pharmaceutical Biotechnology* **11** (1): 2–14. doi:10.2174/138920110790725311. ISSN 1873-4316. PMID 20214604. <https://pubmed.ncbi.nlm.nih.gov/20214604/>.
14. Goodridge, Lawrence D. (2010-01). "Designing phage therapeutics". *Current Pharmaceutical Biotechnology* **11** (1): 15–27. doi:10.2174/138920110790725348. ISSN 1873-4316. PMID 20214605. <https://pubmed.ncbi.nlm.nih.gov/20214605/>.
15. Jernberg, Cecilia; Löfmark, Sonja; Edlund, Charlotta; Jansson, Janet K. (2010-11). "Long-term impacts of antibiotic exposure on the human intestinal microbiota". *Microbiology (Reading, England)* **156** (Pt 11): 3216–3223. doi:10.1099/mic.0.040618-0. ISSN 1465-2080. PMID 20705661. <https://pubmed.ncbi.nlm.nih.gov/20705661/>.
16. Górski, Andrzej; Międzybrodzki, Ryszard; Borysowski, Jan; Dąbrowska, Krystyna; Wierzbicki, Piotr; Ohams, Monika; Korczak-Kowalska, Grażyna; Olszowska-Zaremba, Natasza *et al.* (2012). "Phage as a modulator of immune responses: practical implications for phage therapy". *Advances in Virus Research* **83**: 41–71. doi:10.1016/B978-0-12-394438-2.00002-5. ISSN 1557-8399. PMID 22748808. <https://pubmed.ncbi.nlm.nih.gov/22748808/>.
17. Pirnay, Jean-Paul; De Vos, Daniel; Verbeken, Gilbert; Merabishvili, Maia; Chanishvili, Nina; Vaneechoutte, Mario; Zizi, Martin; Laire, Geert *et al.* (2011-04). "The phage therapy paradigm: prêt-à-porter or sur-mesure?". *Pharmaceutical Research* **28** (4): 934–937. doi:10.1007/s11095-010-0313-5. ISSN 1573-904X. PMID 21063753. <https://pubmed.ncbi.nlm.nih.gov/21063753/>.
18. Dunne, Matthew; Rupf, Beatrice; Tala, Marc; Qabrati, Xhem; Ernst, Patrick; Shen, Yang; Sumrall, Eric; Heeb, Laura *et al.* (2019-10-29). "Reprogramming Bacteriophage Host Range through Structure-Guided Design of Chimeric Receptor Binding Proteins". *Cell Reports* **29** (5): 1336–1350.e4. doi:10.1016/j.celrep.2019.09.062. ISSN 2211-1247. PMID 31665644. <https://pubmed.ncbi.nlm.nih.gov/31665644/>.
19. Vandersteegen, Katrien; Mattheus, Wesley; Ceyssens, Pieter-Jan; Bilocq, Florence; De Vos, Daniel; Pirnay, Jean-Paul; Noben, Jean-Paul; Merabishvili, Maia *et al.* (2011). "Microbiological

- and molecular assessment of bacteriophage ISP for the control of *Staphylococcus aureus*". *PloS One* **6** (9): e24418. doi:10.1371/journal.pone.0024418. ISSN 1932-6203. PMID 21931710. PMC 3170307. <https://pubmed.ncbi.nlm.nih.gov/21931710/>.
20. Jault, Patrick; Leclerc, Thomas; Jennes, Serge; Pirnay, Jean Paul; Que, Yok-Ai; Resch, Gregory; Rousseau, Anne Françoise; Ravat, François *et al.* (01 2019). "Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial". *The Lancet. Infectious Diseases* **19** (1): 35–45. doi:10.1016/S1473-3099(18)30482-1. ISSN 1474-4457. PMID 30292481. <https://pubmed.ncbi.nlm.nih.gov/30292481/>.
21. Hall, Alex R.; De Vos, Daniel; Friman, Ville-Petri; Pirnay, Jean-Paul; Buckling, Angus (2012-08). "Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa* in vitro and in wax moth larvae". *Applied and Environmental Microbiology* **78** (16): 5646–5652. doi:10.1128/AEM.00757-12. ISSN 1098-5336. PMID 22660719. PMC 3406105. <https://pubmed.ncbi.nlm.nih.gov/22660719/>.
22. Friman, V.-P.; Soanes-Brown, D.; Sierocinski, P.; Molin, S.; Johansen, H. K.; Merabishvili, M.; Pirnay, J.-P.; De Vos, D. *et al.* (01 2016). "Pre-adapting parasitic phages to a pathogen leads to increased pathogen clearance and lowered resistance evolution with *Pseudomonas aeruginosa* cystic fibrosis bacterial isolates". *Journal of Evolutionary Biology* **29** (1): 188–198. doi:10.1111/jeb.12774. ISSN 1420-9101. PMID 26476097. <https://pubmed.ncbi.nlm.nih.gov/26476097/>.
23. Muniesa, Maite; Hammerl, Jens A.; Hertwig, Stefan; Appel, Bernd; Brüßow, Harald (2012-06). "Shiga toxin-producing *Escherichia coli* O104:H4: a new challenge for microbiology". *Applied and Environmental Microbiology* **78** (12): 4065–4073. doi:10.1128/AEM.00217-12. ISSN 1098-5336. PMID 22504816. PMC 3370534. <https://pubmed.ncbi.nlm.nih.gov/22504816/>.
24. Azeredo, J.; Sutherland, I. W. (2008-08). "The use of phages for the removal of infectious biofilms". *Current Pharmaceutical Biotechnology* **9** (4): 261–266. doi:10.2174/138920108785161604. ISSN 1873-4316. PMID 18691087. <https://pubmed.ncbi.nlm.nih.gov/18691087/>.
25. Pires, D. P.; Melo, Ldr; Vilas Boas, D.; Sillankorva, S.; Azeredo, J. (2017-10). "Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections". *Current Opinion in Microbiology* **39**: 48–56. doi:10.1016/j.mib.2017.09.004. ISSN 1879-0364. PMID 28964986. <https://pubmed.ncbi.nlm.nih.gov/28964986/>.
26. Chan, Benjamin K.; Abedon, Stephen T. (2015). "Bacteriophages and their enzymes in biofilm control". *Current Pharmaceutical Design* **21** (1): 85–99. doi:10.2174/1381612820666140905112311. ISSN 1873-4286. PMID 25189866. <https://pubmed.ncbi.nlm.nih.gov/25189866/>.
27. Gutiérrez, Diana; Rodríguez-Rubio, Lorena; Martínez, Beatriz; Rodríguez, Ana; García, Pilar (2016). "Bacteriophages as Weapons Against Bacterial Biofilms in the Food Industry". *Frontiers in Microbiology* **7**: 825. doi:10.3389/fmicb.2016.00825. ISSN 1664-302X. PMID 27375566. PMC 4897796. <https://pubmed.ncbi.nlm.nih.gov/27375566/>.
28. Melo, Luís D. R.; Pires, Diana Priscila; Monteiro, Rodrigo; Azeredo, Joana (2019). Górski, Andrzej; Międzybrodzki, Ryszard; Borysowski, Jan (eds.). *Phage Therapy: A Practical Approach*. Cham: Springer International Publishing. pp. 295–313. doi:10.1007/978-3-030-26736-0_11. ISBN 978-3-030-26736-0.
29. Tagliaferri, Thaysa Leite; Jansen, Mathias; Horz, Hans-Peter (2019). "Fighting Pathogenic Bacteria on Two Fronts: Phages and Antibiotics as Combined Strategy". *Frontiers in Cellular and Infection Microbiology* **9**: 22. doi:10.3389/fcimb.2019.00022. ISSN 2235-2988. PMID 30834237. PMC 6387922. <https://pubmed.ncbi.nlm.nih.gov/30834237/>.
30. Akturk, Ergun; Oliveira, Hugo; Santos, Sílvio B.; Costa, Susana; Kuyumcu, Suleyman; Melo, Luís D. R.; Azeredo, Joana (2019-07-25). "Synergistic Action of Phage and Antibiotics: Parameters to Enhance the Killing Efficacy Against Mono and Dual-Species Biofilms". *Antibiotics (Basel, Switzerland)* **8** (3). doi:10.3390/antibiotics8030103. ISSN 2079-6382. PMID 31349628. PMC 6783858. <https://pubmed.ncbi.nlm.nih.gov/31349628/>.

31. Comeau, André M.; Tétart, Françoise; Trojet, Sabrina N.; Prère, Marie-Françoise; Krisch, H. M. (2007-08-29). "Phage-Antibiotic Synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth". *PLoS One* **2** (8): e799. doi:10.1371/journal.pone.0000799. ISSN 1932-6203. PMID 17726529. PMC 1949050. <https://pubmed.ncbi.nlm.nih.gov/17726529/>.
32. Verma, Vivek; Harjai, Kusum; Chhibber, Sanjay (2009-12). "Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment". *The Journal of Antimicrobial Chemotherapy* **64** (6): 1212–1218. doi:10.1093/jac/dkp360. ISSN 1460-2091. PMID 19808232. <https://pubmed.ncbi.nlm.nih.gov/19808232/>.
33. Torres-Barceló, Clara; Hochberg, Michael E. (2016-04). "Evolutionary Rationale for Phages as Complements of Antibiotics". *Trends in Microbiology* **24** (4): 249–256. doi:10.1016/j.tim.2015.12.011. ISSN 1878-4380. PMID 26786863. <https://pubmed.ncbi.nlm.nih.gov/26786863/>.
34. Mutti, Michele; Corsini, Lorenzo (2019). "Robust Approaches for the Production of Active Ingredient and Drug Product for Human Phage Therapy". *Frontiers in Microbiology* **10**: 2289. doi:10.3389/fmicb.2019.02289. ISSN 1664-302X. PMID 31649636. PMC 6791927. <https://pubmed.ncbi.nlm.nih.gov/31649636/>.
35. Pirnay, Jean-Paul; Blasdel, Bob G.; Bretaudeau, Laurent; Buckling, Angus; Chanishvili, Nina; Clark, Jason R.; Corte-Real, Sofia; Debarbieux, Laurent *et al.* (2015-07). "Quality and safety requirements for sustainable phage therapy products". *Pharmaceutical Research* **32** (7): 2173–2179. doi:10.1007/s11095-014-1617-7. ISSN 1573-904X. PMID 25585954. PMC 4452253. <https://pubmed.ncbi.nlm.nih.gov/25585954/>.
36. Hargreaves, Katherine R.; Clokie, Martha R. J. (2014). "Clostridium difficile phages: still difficult?". *Frontiers in Microbiology* **5**: 184. doi:10.3389/fmicb.2014.00184. ISSN 1664-302X. PMID 24808893. PMC 4009436. <https://pubmed.ncbi.nlm.nih.gov/24808893/>.
37. Hietala, Ville; Horsma-Heikkinen, Jenni; Carron, Annelie; Skurnik, Mikael; Kiljunen, Saija (2019). "The Removal of Endo- and Enterotoxins From Bacteriophage Preparations". *Frontiers in Microbiology* **10**: 1674. doi:10.3389/fmicb.2019.01674. ISSN 1664-302X. PMID 31396188. PMC 6664067. <https://pubmed.ncbi.nlm.nih.gov/31396188/>.
38. Malik, Danish J.; Sokolov, Ilya J.; Vinner, Gurinder K.; Mancuso, Francesco; Cinquerrui, Salvatore; Vladisavljevic, Goran T.; Clokie, Martha R. J.; Garton, Natalie J. *et al.* (2017-11). "Formulation, stabilisation and encapsulation of bacteriophage for phage therapy". *Advances in Colloid and Interface Science* **249**: 100–133. doi:10.1016/j.cis.2017.05.014. ISSN 1873-3727. PMID 28688779. <https://pubmed.ncbi.nlm.nih.gov/28688779/>.
39. Otero, Jennifer; García-Rodríguez, Alba; Cano-Sarabia, Mary; MasPOCH, Daniel; Marcos, Ricard; Cortés, Pilar; Llagostera, Montserrat (2019). "Biodistribution of Liposome-Encapsulated Bacteriophages and Their Transcytosis During Oral Phage Therapy". *Frontiers in Microbiology* **10**: 689. doi:10.3389/fmicb.2019.00689. ISSN 1664-302X. PMID 31019499. PMC 6458305. <https://pubmed.ncbi.nlm.nih.gov/31019499/>.
40. Pires, Diana P.; Cleto, Sara; Sillankorva, Sanna; Azeredo, Joana; Lu, Timothy K. (09 2016). "Genetically Engineered Phages: a Review of Advances over the Last Decade". *Microbiology and molecular biology reviews: MMBR* **80** (3): 523–543. doi:10.1128/MMBR.00069-15. ISSN 1098-5557. PMID 27250768. PMC 4981678. <https://pubmed.ncbi.nlm.nih.gov/27250768/>.
41. Dedrick, Rebekah M.; Guerrero-Bustamante, Carlos A.; Garland, Rebecca A.; Russell, Daniel A.; Ford, Katrina; Harris, Kathryn; Gilmour, Kimberly C.; Sothill, James *et al.* (05 2019). "Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*". *Nature Medicine* **25** (5): 730–733. doi:10.1038/s41591-019-0437-z. ISSN 1546-170X. PMID 31068712. PMC 6557439. <https://pubmed.ncbi.nlm.nih.gov/31068712/>.
42. Melo, Luís D. R.; Oliveira, Hugo; Pires, Diana P.; Dabrowska, Krystyna; Azeredo, Joana (2020-02). "Phage therapy efficacy: a review of the last 10 years of preclinical studies". *Critical Reviews in Microbiology* **46** (1): 78–99. doi:10.1080/1040841X.2020.1729695. ISSN 1549-7828. PMID 32091280. <https://pubmed.ncbi.nlm.nih.gov/32091280/>.

43. Dąbrowska, Krystyna (09 2019). "Phage therapy: What factors shape phage pharmacokinetics and bioavailability? Systematic and critical review". *Medicinal Research Reviews* **39** (5): 2000–2025. doi:10.1002/med.21572. ISSN 1098-1128. PMID 30887551. PMC 6767042. <https://pubmed.ncbi.nlm.nih.gov/30887551/>.
44. Tricoci, Pierluigi; Allen, Joseph M.; Kramer, Judith M.; Califf, Robert M.; Smith, Sidney C. (2009-02-25). "Scientific evidence underlying the ACC/AHA clinical practice guidelines". *JAMA* **301** (8): 831–841. doi:10.1001/jama.2009.205. ISSN 1538-3598. PMID 19244190. <https://pubmed.ncbi.nlm.nih.gov/19244190/>.
45. Voigt, Jeffrey; Mosier, Michael; Darouiche, Rabih (2015-11). "Systematic review and meta-analysis of randomized controlled trials of antibiotics and antiseptics for preventing infection in people receiving primary total hip and knee prostheses". *Antimicrobial Agents and Chemotherapy* **59** (11): 6696–6707. doi:10.1128/AAC.01331-15. ISSN 1098-6596. PMID 26259793. PMC 4604400. <https://pubmed.ncbi.nlm.nih.gov/26259793/>.
46. Steen, R. Grant; Dager, Stephen R. (2013-09). "Evaluating the evidence for evidence-based medicine: are randomized clinical trials less flawed than other forms of peer-reviewed medical research?". *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* **27** (9): 3430–3436. doi:10.1096/fj.13-230714. ISSN 1530-6860. PMID 23695156. <https://pubmed.ncbi.nlm.nih.gov/23695156/>.
47. Debarbieux, Laurent; Pirnay, Jean-Paul; Verbeken, Gilbert; De Vos, Daniel; Merabishvili, Maia; Huys, Isabelle; Patey, Olivier; Schoonjans, Dirk *et al.* (2016-01). "A bacteriophage journey at the European Medicines Agency". *FEMS microbiology letters* **363** (2): fnv225. doi:10.1093/femsle/fnv225. ISSN 1574-6968. PMID 26656541. PMC 5812529. <https://pubmed.ncbi.nlm.nih.gov/26656541/>.
48. Wright, A.; Hawkins, C. H.; Anggård, E. E.; Harper, D. R. (2009-08). "A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy". *Clinical otolaryngology: official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery* **34** (4): 349–357. doi:10.1111/j.1749-4486.2009.01973.x. ISSN 1749-4486. PMID 19673983. <https://pubmed.ncbi.nlm.nih.gov/19673983/>.
49. Servick, Kelly (2016-06-24). "DRUG DEVELOPMENT. Beleaguered phage therapy trial presses on". *Science (New York, N.Y.)* **352** (6293): 1506. doi:10.1126/science.352.6293.1506. ISSN 1095-9203. PMID 27339963. <https://pubmed.ncbi.nlm.nih.gov/27339963/>.
50. Sarker, Shafiqul Alam; Berger, Bernard; Deng, Ying; Kieser, Silas; Foata, Francis; Moine, Deborah; Descombes, Patrick; Sultana, Shamima *et al.* (01 2017). "Oral application of *Escherichia coli* bacteriophage: safety tests in healthy and diarrheal children from Bangladesh". *Environmental Microbiology* **19** (1): 237–250. doi:10.1111/1462-2920.13574. ISSN 1462-2920. PMID 27750388. <https://pubmed.ncbi.nlm.nih.gov/27750388/>.
51. Ooi, Mian Li; Drilling, Amanda Jane; Morales, Sandra; Fong, Stephanie; Moraitis, Sophia; Macias-Valle, Luis; Vreugde, Sarah; Psaltis, Alkis James *et al.* (2019-06-20). "Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to *Staphylococcus aureus*". *JAMA otolaryngology-- head & neck surgery*. doi:10.1001/jamaoto.2019.1191. ISSN 2168-619X. PMID 31219531. PMC 6587246. <https://pubmed.ncbi.nlm.nih.gov/31219531/>.
52. Ujmajuridze, Aleksandre; Chanishvili, Nina; Goderdzishvili, Marina; Leitner, Lorenz; Mehnert, Ulrich; Chkhotua, Archil; Kessler, Thomas M.; Sybesma, Wilbert (2018). "Adapted Bacteriophages for Treating Urinary Tract Infections". *Frontiers in Microbiology* **9**: 1832. doi:10.3389/fmicb.2018.01832. ISSN 1664-302X. PMID 30131795. PMC 6090023. <https://pubmed.ncbi.nlm.nih.gov/30131795/>.
53. McCallin, Shawna; Sarker, Shafiqul A.; Sultana, Shamima; Oechslin, Frank; Brüssow, Harald (09 2018). "Metagenome analysis of Russian and Georgian Pyophage cocktails and a placebo-controlled safety trial of single phage versus phage cocktail in healthy *Staphylococcus aureus* carriers". *Environmental Microbiology* **20** (9): 3278–3293. doi:10.1111/1462-2920.14310. ISSN 1462-2920. PMID 30051571. <https://pubmed.ncbi.nlm.nih.gov/30051571/>.

54. Fish, Randolph; Kutter, Elizabeth; Wheat, Gordon; Blasdel, Bob; Kutateladze, Mzia; Kuhl, Sarah (2018). "Compassionate Use of Bacteriophage Therapy for Foot Ulcer Treatment as an Effective Step for Moving Toward Clinical Trials". *Methods in Molecular Biology (Clifton, N.J.)* **1693**: 159–170. doi:10.1007/978-1-4939-7395-8_14. ISSN 1940-6029. PMID 29119440. <https://pubmed.ncbi.nlm.nih.gov/29119440/>.
55. Febvre, Hallie P.; Rao, Sangeeta; Gindin, Melinda; Goodwin, Natalie D. M.; Finer, Elijah; Vivanco, Jorge S.; Lu, Shen; Manter, Daniel K. *et al.* (2019-03-20). "PHAGE Study: Effects of Supplemental Bacteriophage Intake on Inflammation and Gut Microbiota in Healthy Adults". *Nutrients* **11** (3). doi:10.3390/nu11030666. ISSN 2072-6643. PMID 30897686. PMC 6471193. <https://pubmed.ncbi.nlm.nih.gov/30897686/>.
56. Letkiewicz, S.; Miedzybrodzki, R.; Fortuna, W.; Weber-Dabrowska, B.; Górski, A. (2009-09). "Eradication of *Enterococcus faecalis* by phage therapy in chronic bacterial prostatitis--case report". *Folia Microbiologica* **54** (5): 457–461. doi:10.1007/s12223-009-0064-z. ISSN 1874-9356. PMID 19937220. <https://pubmed.ncbi.nlm.nih.gov/19937220/>.
57. Jennes, Serge; Merabishvili, Maia; Soentjens, Patrick; Pang, Kim Win; Rose, Thomas; Keersebilck, Elkana; Soete, Olivier; François, Pierre-Michel *et al.* (2017-06-04). "Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury-a case report". *Critical Care (London, England)* **21** (1): 129. doi:10.1186/s13054-017-1709-y. ISSN 1466-609X. PMID 28583189. PMC 5460490. <https://pubmed.ncbi.nlm.nih.gov/28583189/>.
58. Schooley, Robert T.; Biswas, Biswajit; Gill, Jason J.; Hernandez-Morales, Adriana; Lancaster, Jacob; Lessor, Lauren; Barr, Jeremy J.; Reed, Sharon L. *et al.* (10 2017). "Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection". *Antimicrobial Agents and Chemotherapy* **61** (10). doi:10.1128/AAC.00954-17. ISSN 1098-6596. PMID 28807909. PMC 5610518. <https://pubmed.ncbi.nlm.nih.gov/28807909/>.
59. Zhvania, Pikria; Hoyle, Naomi Sulinger; Nadareishvili, Lia; Nizharadze, Dea; Kutateladze, Mzia (2017). "Phage Therapy in a 16-Year-Old Boy with Netherton Syndrome". *Frontiers in Medicine* **4**: 94. doi:10.3389/fmed.2017.00094. ISSN 2296-858X. PMID 28717637. PMC 5494523. <https://pubmed.ncbi.nlm.nih.gov/28717637/>.
60. Hoyle, N.; Zhvaniya, P.; Balarjishvili, N.; Bolkvadze, D.; Nadareishvili, L.; Nizharadze, D.; Wittmann, J.; Rohde, C. *et al.* (2018-11). "Phage therapy against *Achromobacter xylosoxidans* lung infection in a patient with cystic fibrosis: a case report". *Research in Microbiology* **169** (9): 540–542. doi:10.1016/j.resmic.2018.05.001. ISSN 1769-7123. PMID 29777836. <https://pubmed.ncbi.nlm.nih.gov/29777836/>.
61. Patey, Olivier; McCallin, Shawna; Mazure, Hubert; Liddle, Max; Smithyman, Anthony; Dublanchet, Alain (12 28, 2018). "Clinical Indications and Compassionate Use of Phage Therapy: Personal Experience and Literature Review with a Focus on Osteoarticular Infections". *Viruses* **11** (1). doi:10.3390/v11010018. ISSN 1999-4915. PMID 30597868. PMC 6356659. <https://pubmed.ncbi.nlm.nih.gov/30597868/>.
62. Chan, Benjamin K.; Turner, Paul E.; Kim, Samuel; Mojibian, Hamid R.; Elefteriades, John A.; Narayan, Deepak (2018). "Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*". *Evolution, Medicine, and Public Health* **2018** (1): 60–66. doi:10.1093/emph/eoy005. ISSN 2050-6201. PMID 29588855. PMC 5842392. <https://pubmed.ncbi.nlm.nih.gov/29588855/>.
63. Onsea, Jolien; Soentjens, Patrick; Djebara, Sarah; Merabishvili, Maia; Depypere, Melissa; Spriet, Isabel; De Munter, Paul; Debaveye, Yves *et al.* (09 23, 2019). "Bacteriophage Application for Difficult-to-treat Musculoskeletal Infections: Development of a Standardized Multidisciplinary Treatment Protocol". *Viruses* **11** (10). doi:10.3390/v11100891. ISSN 1999-4915. PMID 31548497. PMC 6832313. <https://pubmed.ncbi.nlm.nih.gov/31548497/>.
64. Corbellino, Mario; Kieffer, Nicolas; Kutateladze, Mzia; Balarjishvili, Nana; Leshkasheli, Lika; Askilashvili, Lia; Tsertsvadze, George; Rimoldi, Sara Giordana *et al.* (2020-04-15). "Eradication of a Multidrug-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Isolate Following

Oral and Intra-rectal Therapy With a Custom Made, Lytic Bacteriophage Preparation". *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **70** (9): 1998–2001. doi:10.1093/cid/ciz782. ISSN 1537-6591. PMID 31414123. <https://pubmed.ncbi.nlm.nih.gov/31414123/>.

35. Law, Nancy; Logan, Cathy; Yung, Gordon; Furr, Carrie-Lynn Langlais; Lehman, Susan M.; Morales, Sandra; Rosas, Francisco; Gaidamaka, Alexander *et al.* (2019-08). "Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* infection in a cystic fibrosis patient". *Infection* **47** (4): 665–668. doi:10.1007/s15010-019-01319-0. ISSN 1439-0973. PMID 31102236. <https://pubmed.ncbi.nlm.nih.gov/31102236/>.
36. Aslam, Saima; Courtwright, Andrew M.; Koval, Christine; Lehman, Susan M.; Morales, Sandra; Furr, Carrie-Lynn Langlais; Rosas, Francisco; Brownstein, Michael J. *et al.* (09 2019). "Early clinical experience of bacteriophage therapy in 3 lung transplant recipients". *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons* **19** (9): 2631–2639. doi:10.1111/ajt.15503. ISSN 1600-6143. PMID 31207123. PMC 6711787. <https://pubmed.ncbi.nlm.nih.gov/31207123/>.
37. Maddocks, Susan; Fabijan, Aleksandra Petrovic; Ho, Josephine; Lin, Ruby C. Y.; Ben Zakour, Nouri L.; Dugan, Chris; Kliman, Ivana; Branston, Steven *et al.* (11 01, 2019). "Bacteriophage Therapy of Ventilator-associated Pneumonia and Empyema Caused by *Pseudomonas aeruginosa*". *American Journal of Respiratory and Critical Care Medicine* **200** (9): 1179–1181. doi:10.1164/rccm.201904-0839LE. ISSN 1535-4970. PMID 31437402. <https://pubmed.ncbi.nlm.nih.gov/31437402/>.
38. Henein, Alexandra (2013-04-01). "What are the limitations on the wider therapeutic use of phage?". *Bacteriophage* **3** (2): e24872. doi:10.4161/bact.24872. ISSN 2159-7073. PMID 24228220. PMC 3821673. <https://pubmed.ncbi.nlm.nih.gov/24228220/>.
39. Fu, Weiling; Forster, Terri; Mayer, Oren; Curtin, John J.; Lehman, Susan M.; Donlan, Rodney M. (2010-01). "Bacteriophage cocktail for the prevention of biofilm formation by *Pseudomonas aeruginosa* on catheters in an in vitro model system". *Antimicrobial Agents and Chemotherapy* **54** (1): 397–404. doi:10.1128/AAC.00669-09. ISSN 1098-6596. PMID 19822702. PMC 2798481. <https://pubmed.ncbi.nlm.nih.gov/19822702/>.
70. Pires, Diana P.; Dötsch, Andreas; Anderson, Erin M.; Hao, Youai; Khursigara, Cezar M.; Lam, Joseph S.; Sillankorva, Sanna; Azeredo, Joana (2017). "A Genotypic Analysis of Five *P. aeruginosa* Strains after Biofilm Infection by Phages Targeting Different Cell Surface Receptors". *Frontiers in Microbiology* **8**: 1229. doi:10.3389/fmicb.2017.01229. ISSN 1664-302X. PMID 28713356. PMC 5492357. <https://pubmed.ncbi.nlm.nih.gov/28713356/>.
71. Le, Shuai; Yao, Xinyue; Lu, Shuguang; Tan, Yinling; Rao, Xiancai; Li, Ming; Jin, Xiaolin; Wang, Jing *et al.* (2014-04-28). "Chromosomal DNA deletion confers phage resistance to *Pseudomonas aeruginosa*". *Scientific Reports* **4**: 4738. doi:10.1038/srep04738. ISSN 2045-2322. PMID 24770387. PMC 4001099. <https://pubmed.ncbi.nlm.nih.gov/24770387/>.
72. Oechslin, Frank (06 30, 2018). "Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy". *Viruses* **10** (7). doi:10.3390/v10070351. ISSN 1999-4915. PMID 29966329. PMC 6070868. <https://pubmed.ncbi.nlm.nih.gov/29966329/>.
73. Oechslin, Frank; Piccardi, Philippe; Mancini, Stefano; Gabard, Jérôme; Moreillon, Philippe; Entenza, José M.; Resch, Gregory; Que, Yok-Ai (03 01, 2017). "Synergistic Interaction Between Phage Therapy and Antibiotics Clears *Pseudomonas Aeruginosa* Infection in Endocarditis and Reduces Virulence". *The Journal of Infectious Diseases* **215** (5): 703–712. doi:10.1093/infdis/jiw632. ISSN 1537-6613. PMID 28007922. PMC 5388299. <https://pubmed.ncbi.nlm.nih.gov/28007922/>.
74. Bertozzi Silva, Juliano; Storms, Zachary; Sauvageau, Dominic (2016-02). "Host receptors for bacteriophage adsorption". *FEMS microbiology letters* **363** (4). doi:10.1093/femsle/fnw002. ISSN 1574-6968. PMID 26755501. <https://pubmed.ncbi.nlm.nih.gov/26755501/>.
75. León, Marcela; Bastías, Roberto (2015). "Virulence reduction in bacteriophage resistant bacteria". *Frontiers in Microbiology* **6**. doi:10.3389/fmicb.2015.00343. ISSN 1664-302X. <https://www.frontiersin.org/articles/10.3389/fmicb.2015.00343/full>.

76. Labrie, Simon J.; Samson, Julie E.; Moineau, Sylvain (2010-05). "Bacteriophage resistance mechanisms". *Nature Reviews. Microbiology* **8** (5): 317–327. doi:10.1038/nrmicro2315. ISSN 1740-1534. PMID 20348932. <https://pubmed.ncbi.nlm.nih.gov/20348932/>.
77. Di Giovine, M.; Salone, B.; Martina, Y.; Amati, V.; Zambruno, G.; Cundari, E.; Failla, C. M.; Saggio, I. (2001-03-30). "Binding properties, cell delivery, and gene transfer of adenoviral penton base displaying bacteriophage". *Virology* **282** (1): 102–112. doi:10.1006/viro.2000.0809. ISSN 0042-6822. PMID 11259194. <https://pubmed.ncbi.nlm.nih.gov/11259194/>.
78. Nguyen, Sophie; Baker, Kristi; Padman, Benjamin S.; Patwa, Ruzeen; Dunstan, Rhys A.; Weston, Thomas A.; Schlosser, Kyle; Bailey, Barbara *et al.* (11 21, 2017). "Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers". *mBio* **8** (6). doi:10.1128/mBio.01874-17. ISSN 2150-7511. PMID 29162715. PMC 5698557. <https://pubmed.ncbi.nlm.nih.gov/29162715/>.
79. Bruttin, Anne; Brüssow, Harald (2005-07). "Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy". *Antimicrobial Agents and Chemotherapy* **49** (7): 2874–2878. doi:10.1128/AAC.49.7.2874-2878.2005. ISSN 0066-4804. PMID 15980363. PMC 1168693. <https://pubmed.ncbi.nlm.nih.gov/15980363/>.
30. Kutter, Elizabeth; De Vos, Daniel; Gvasalia, Guram; Alavidze, Zemphira; Gogokhia, Lasha; Kuhl, Sarah; Abedon, Stephen T. (2010-01). "Phage therapy in clinical practice: treatment of human infections". *Current Pharmaceutical Biotechnology* **11** (1): 69–86. doi:10.2174/138920110790725401. ISSN 1873-4316. PMID 20214609. <https://pubmed.ncbi.nlm.nih.gov/20214609/>.
31. Speck, Peter; Smithyman, Anthony (2016-02). "Safety and efficacy of phage therapy via the intravenous route". *FEMS microbiology letters* **363** (3). doi:10.1093/femsle/fnv242. ISSN 1574-6968. PMID 26691737. <https://pubmed.ncbi.nlm.nih.gov/26691737/>.
32. Prins, J. M.; van Deventer, S. J.; Kuijper, E. J.; Speelman, P. (1994-06). "Clinical relevance of antibiotic-induced endotoxin release". *Antimicrobial Agents and Chemotherapy* **38** (6): 1211–1218. doi:10.1128/aac.38.6.1211. ISSN 0066-4804. PMID 8092816. <https://pubmed.ncbi.nlm.nih.gov/8092816/>.
33. Sarker, Shafiqul Alam; McCallin, Shawna; Barretto, Caroline; Berger, Bernard; Pittet, Anne-Cécile; Sultana, Shamima; Krause, Lutz; Huq, Sayeda *et al.* (2012-12-20). "Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh". *Virology* **434** (2): 222–232. doi:10.1016/j.virol.2012.09.002. ISSN 1096-0341. PMID 23102968. <https://pubmed.ncbi.nlm.nih.gov/23102968/>.
34. Sarker, Shafiqul Alam; Sultana, Shamima; Reuteler, Gloria; Moine, Deborah; Descombes, Patrick; Charton, Florence; Bourdin, Gilles; McCallin, Shawna *et al.* (2016-02). "Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh". *EBioMedicine* **4**: 124–137. doi:10.1016/j.ebiom.2015.12.023. ISSN 2352-3964. PMID 26981577. PMC 4776075. <https://pubmed.ncbi.nlm.nih.gov/26981577/>.
35. Galtier, Matthieu; De Sordi, Luisa; Maura, Damien; Arachchi, Harindra; Volant, Stevann; Dillies, Marie-Agnès; Debarbieux, Laurent (07 2016). "Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition". *Environmental Microbiology* **18** (7): 2237–2245. doi:10.1111/1462-2920.13284. ISSN 1462-2920. PMID 26971586. <https://pubmed.ncbi.nlm.nih.gov/26971586/>.
36. Majewska, Joanna; Kaźmierczak, Zuzanna; Lahutta, Karolina; Lecion, Dorota; Szymczak, Aleksander; Miernikiewicz, Paulina; Drapała, Jarosław; Harhala, Marek *et al.* (2019). "Induction of Phage-Specific Antibodies by Two Therapeutic Staphylococcal Bacteriophages Administered per os". *Frontiers in Immunology* **10**: 2607. doi:10.3389/fimmu.2019.02607. ISSN 1664-3224. PMID 31803179. PMC 6871536. <https://pubmed.ncbi.nlm.nih.gov/31803179/>.
37. Dublanchet, A.; Fruciano, E. (2008-08). "[A short history of phage therapy]". *Medecine Et Maladies Infectieuses* **38** (8): 415–420. doi:10.1016/j.medmal.2008.06.016. ISSN 0399-077X. PMID 18692974. <https://pubmed.ncbi.nlm.nih.gov/18692974/>.
38. Verbeken, Gilbert; Pirnay, Jean-Paul; Lavigne, Rob; Jennes, Serge; De Vos, Daniel; Casteels, Minne; Huys, Isabelle (2014-04). "Call for a dedicated European legal framework for

bacteriophage therapy". *Archivum Immunologiae Et Therapiae Experimentalis* **62** (2): 117–129. doi:10.1007/s00005-014-0269-y. ISSN 1661-4917. PMID 24500660. PMC 3950567. <https://pubmed.ncbi.nlm.nih.gov/24500660/>.

39. Fauconnier, Alan (04 17, 2019). "Phage Therapy Regulation: From Night to Dawn". *Viruses* **11** (4). doi:10.3390/v11040352. ISSN 1999-4915. PMID 30999559. PMC 6521264. <https://pubmed.ncbi.nlm.nih.gov/30999559/>.
30. Pirnay, Jean-Paul; Verbeken, Gilbert; Ceysens, Pieter-Jan; Huys, Isabelle; De Vos, Daniel; Ameloot, Charlotte; Fauconnier, Alan (02 06, 2018). "The Magistral Phage". *Viruses* **10** (2). doi:10.3390/v10020064. ISSN 1999-4915. PMID 29415431. PMC 5850371. <https://pubmed.ncbi.nlm.nih.gov/29415431/>.
31. Fauconnier, Alan (02 2017). "Regulating phage therapy: The biological master file concept could help to overcome regulatory challenge of personalized medicines". *EMBO reports* **18** (2): 198–200. doi:10.15252/embr.201643250. ISSN 1469-3178. PMID 28082313. PMC 5286392. <https://pubmed.ncbi.nlm.nih.gov/28082313/>.
32. Moelling, Karin; Broecker, Felix; Willy, Christian (12 05, 2018). "A Wake-Up Call: We Need Phage Therapy Now". *Viruses* **10** (12). doi:10.3390/v10120688. ISSN 1999-4915. PMID 30563034. PMC 6316858. <https://pubmed.ncbi.nlm.nih.gov/30563034/>.

Retrieved from "https://en.wikiversity.org/w/index.php?title=WikiJournal_Preprints/Phage_Therapy&oldid=2235182"

This page was last edited on 2 November 2020, at 15:37.

Text is available under the Creative Commons Attribution-ShareAlike License; additional terms may apply. By using this site, you agree to the Terms of Use and Privacy Policy.