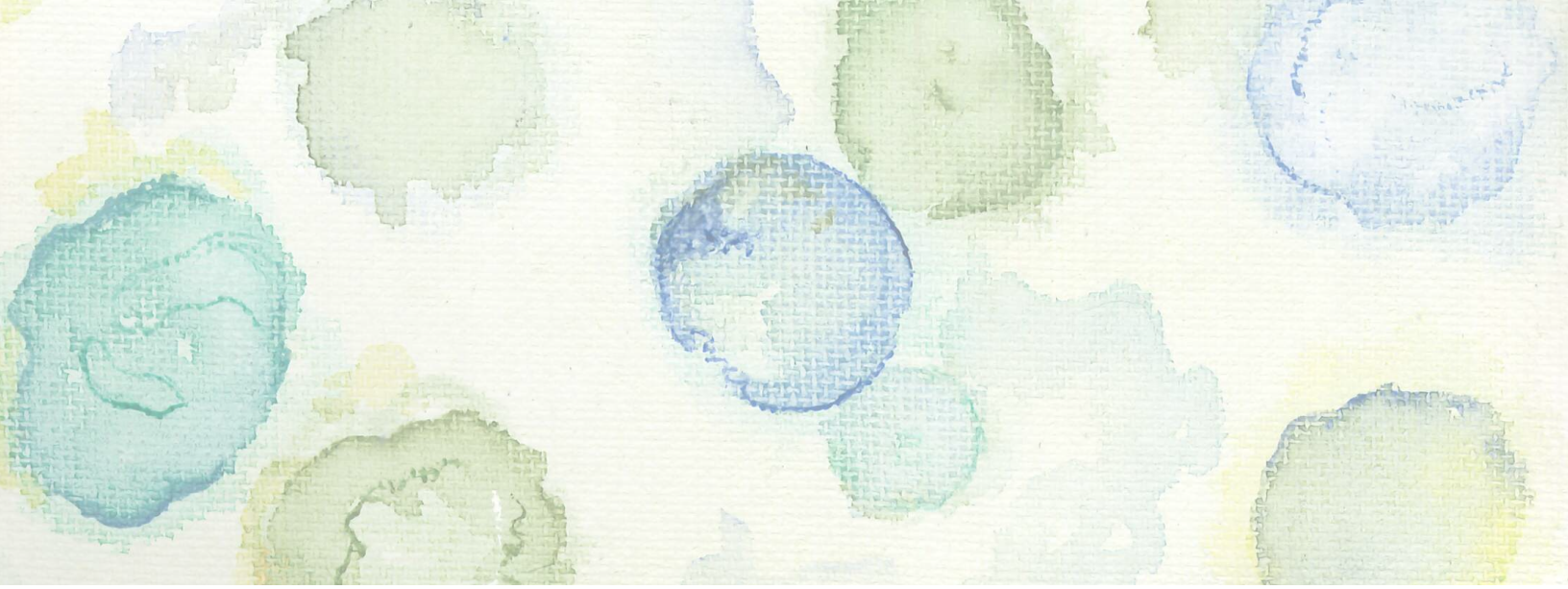

Phage Therapy: Challenges and Opportunities

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Abstract

The development of antibiotic resistance in bacteria is a growing concern. This situation demands a search for antibiotic alternatives. Bacteriophages—natural viral predators of bacteria—are viewed as a possible alternative to treat bacterial infections. Many clinical trials today have not found phages effective as therapeutics. Some of the major challenges regarding usage of bacteriophage as a therapeutic have been: horizontal evolution of bacteria, limited host range of bacteriophage, removal of endotoxins in preparations, the technical feasibility of isolation, mode of administration, rapid clearance and immune rejection. These issues have been addressed in this review. Applications of genetic engineered phages and other remarkable non-human applications are also discussed.

Introduction

After the Golden Age of antibiotics (1950-60), use of antibiotics as the first line of defence has increased dramatically (1). Inappropriate prescription of antibiotics by clinicians is a major problem today (2). Seventy percent of antibiotic use in the USA is attributed to use on cattle (3). Indiscriminate usage and misuse of antibiotics have accelerated the emergence of antimicrobial resistance. A growing list of infections is becoming harder to treat, as antibiotics are becoming less effective (4). Sir Alexander Fleming expected the arrival of the antibiotic resistance era and was worried about the rise in antibiotic resistance by self-medication (5). It is estimated that by 2050, bacterial infections will cause 10 million deaths every year (6, 7). Alternatives are urgently needed to effectively treat these infections and prevent the return of pre-antibiotic era.

There is active research currently undertaken for the development of novel classes of antibiotics (8, 9). Bacteriophages (also known as phages) might provide us with a promising alternative for antibiotics. These are the viruses that infect bacteria and are the most abundant living entities in the world. It is predicted that every millimeter of a natural sample has 10^7 phage particles (10). The application of phage as therapeutics against bacteria is called phage therapy (Bacteriophage Therapy). Phage therapy is drawing global attention due to the rise in antimicrobial resistance. Early studies on phage therapy were conducted in Georgia (11). To date, only a few clinical trials have been conducted to modern standards (randomized, placebo-controlled, double-blinded) by the United States (US) Food and Drug Administration (FDA) as well as the European Medical Agency (EMA) jurisdictions.

It is necessary to re-evaluate the challenges involved in phage therapy. Here, a review of the challenges, possible solutions, safety and concerns for therapeutic phage applications is presented. Other potential applications of phages and their studies in humans are also discussed.

Why the Forgotten Magic?

Bacteriophages were first discovered independently by Frederick Twort in 1915 (12) and Félix d'Herelle in 1917 (13). d'Herelle realized the potential of these devourers of bacteria as a therapeutic and conducted further research on phages. One of the early investigations by d'Herelle was in India in 1927 (14). The mortality rates of cholera-infected study subjects decreased from 66.66% in control groups to 5.8% in phage treated groups. Phages offered a great scope of enquiry, but the simplicity in the production of antibiotics gave antibiotic therapy a lead over phage therapy.

The global spread of antibiotic-resistant bacteria and the comeback of the pre-antibiotic era alarmed the scientific community and warranted a search for antibiotic alternatives. Phage therapy is a superior alternative to antibiotics with many theoretical advantages. While antibiotics kill bacteria broadly, phages bind and infect the bacteria specifically. High specificity is an important advantage, as it might minimally impact beneficial microflora, making phages safer than antibiotics. The specificity also limits the number of bacterial types gaining specific phage-resistance mechanisms (15).

Bacteriophages follow lytic and lysogenic pathways of infection. Phages that follow the lysogenic pathway, integrate their genome into the bacterial genome, eventually lysing the cell. Phages following the lytic pathway enter bacteria, reproduce within and lyse the cell (Figure 1). These released phages infect other bacteria. In this process, the number of phages increases exponentially. This exponential growth of phage is advantageous, as theoretically, a smaller dose is needed. The exponential increase is seen specifically where hosts are present, making phages themselves contribute to the dosage at the required site (16).

Biofilm forming bacteria cause infections such as bacterial vaginosis, urinary tract infections, and middle-ear infections. Biofilms are polymeric matrices produced by bacteria as a defence mechanism that allows them to adhere to surfaces (17). Even when a bacterium is sensitive to an antibacterial agent, the antibiotic fails to penetrate through the biofilm matrix, increasing the resistance of the bacteria by 1000-fold (18). A phage has a distinctive capability of tackling biofilms efficiently (5) by encoding depolymerases that allow their direct penetration into the biofilm (19). Phages are also

known for stimulating an immune response. Receptor-binding proteins of a few phages display collagen motifs (20), which can co-stimulate the number and longevity of T cells (21). Furthermore, Van Belleghem *et al*. (22) demonstrated that phages can induce reproducible immune responses from monocytes. Recent studies have also shown that phages have antiviral properties and that phage therapy may also hold promise as a treatment for SARS-82 CoV-2 (23, 24).

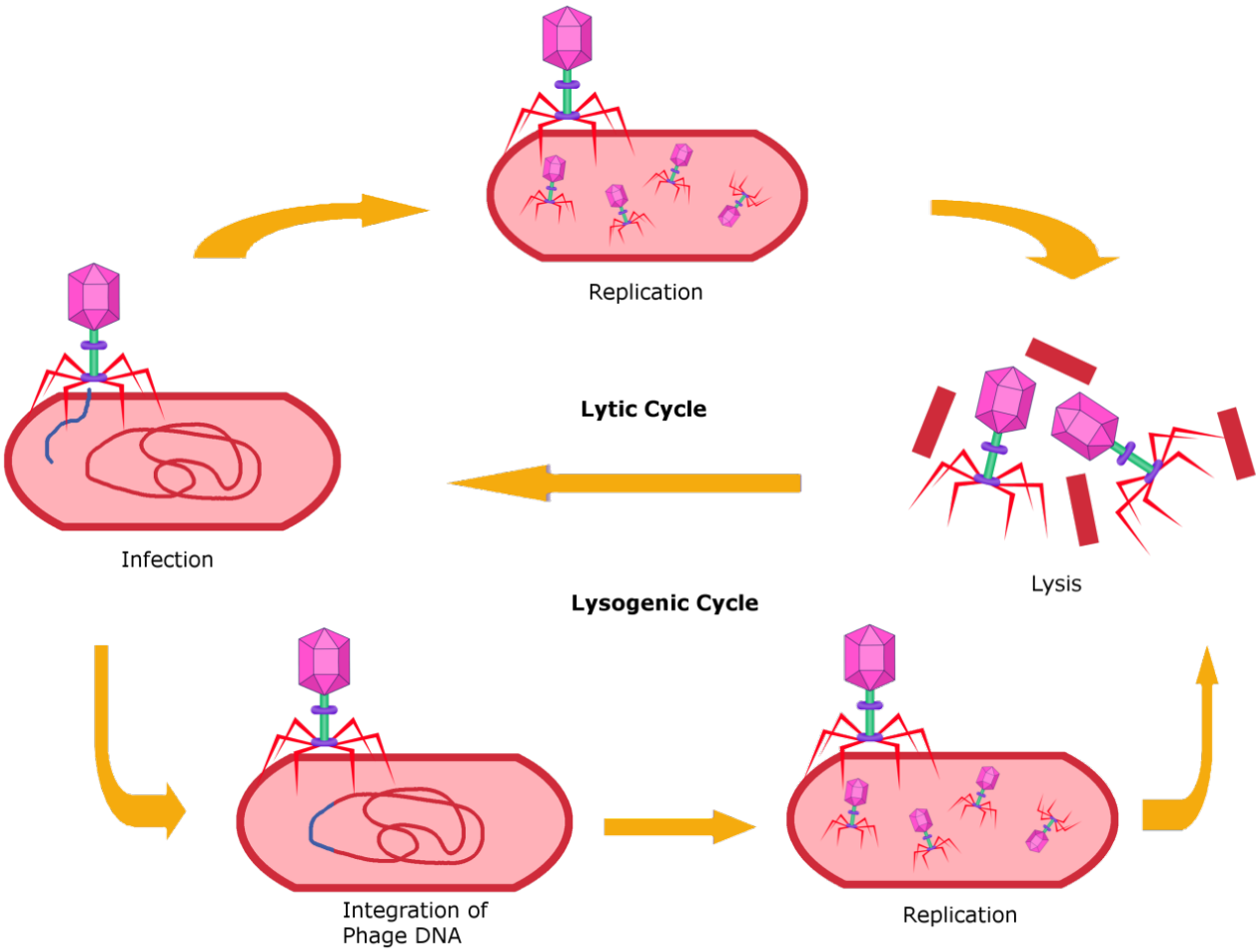

***Figure 1*: Phage attaches to the host cell and injects DNA to initiate the infection. In lytic cycle, phage DNA and proteins are synthesized and assembled into virions. These replicated phages lyse the cell wall and infect another host. Lysogenic cycle involves an additional step of integrating their genetic material to form an endogenous prophage, which compromises with the safety of the therapy.**

Challenges and Potential Solutions in Phage Therapy

1. Host Range

Challenge:

Bacteriophages selectively bind to specific receptors of bacteria, which confer a relatively narrow range of infectivity (25). This would narrow the infectivity range challenging the choice of phage for therapeutic use. As a minimum requirement, phages used in phage therapy should follow only the lytic pathway to ensure the safety of the patient (Figure 1). Bacterial infections, where the currently isolated phages lack a lytic cycle, are therefore not treatable with phages, and the bacteria include *Rickettsia, Coxiella africanum, Mycobacterium leprae, Proteuspenneri, Citrobacterkoseri, Salmonella arizonae, Porphyromonas* spp, and *Hafniaalvei* spp (26).

Potential Solutions:

Antibiotics: At present, phage therapy is generally considered as a last resort when a single bacterial strain dominates. In such situations, the synergistic use of antibiotics like ciprofloxacin in combination with phages can reduce the bacterial load by 10,000 times (27, 28). This combined therapy can boost bactericidal activity with their different mechanisms of attack. However, the choice of the combination is crucial. Antibiotics must not interfere with phage replication (29).

Phage Cocktails: Another possible solution is by employing a combination of phages (so-called phage cocktails), which cover a large spectrum of bacterial strains. Bacterial isolates of a patient are screened against a library of lytic phages for infection susceptibility. The infectious phages are administered together as multivalent phages (30). D'Herelle's pyophage and intestiphage (11) are a few well known accessible commercial phage cocktails.

Genetic Engineering: Genetic engineering can be used to improve the host range by modifying tail fibres (31). It can also improve the efficacy of phage therapy by converting a temperate phage to a lytic phage by removing its repressor genes (32).

2. Endotoxins

Challenge:

In the recent PhagoBurn clinical trial, a team of doctors had to reduce the dosage of phage administration from expected 10^6 PFU/ml to 10-100 PFU/ml due to high endotoxin concentrations in the phage preparations (33). Bacterial debris may remain in the phage preparations even after filtration. In the historic era (around 100 years ago) of phage therapy, not all the debris was removed, and the authors reported a few chemical contaminants which brought about death and illness (30, 34–36). The typical phage purification process (Ultracentrifugation in CsCl gradient) requires intensive labour, high expense and is time consuming (37).

Potential Solutions:

The bacterial debris, having pyrogens and toxins (38), can be cleared and high purity levels can be achieved with nanofibrillated filters (39). Endotoxin removal proteins are now available commercially (40). For a large scale production, usage of surrogate hosts could offer a superior solution(37).

Challenge:

In recent clinical trials, patients suffered many side effects due to increased concentrations of endotoxins among which abdominal pain, sudden fever and chills were common. Many biologists attribute endotoxins as the prime cause for these side effects (41). Expression of endotoxin genes in phages or rapid lysis of bacteria in patients can release toxins (38, 42).

Potential Solutions:

A therapeutic phage having a Lys^- gene (endolysin-deficient phage) cannot lyse the peptidoglycan layer of bacteria after infecting the bacteria. Phages attack the bacteria and do not lyse the host membrane, which does not lead to the release of endotoxins. The macrophages then eliminates these incapacitated bacteria (43).

3. Immune Rejection and Rapid Clearance

Challenge:

Large phage titers trigger the release of neutralizing antibodies in high amounts (44), which would hinder the action of phages. Being in continuous exposure with phages, 81% of healthy individuals show antibodies to T4 phage, prior to the treatment itself (45). Though the phage kinetics are much faster than the release of neutralizing antibodies (30, 46), the presence of such anti-phage antibodies before phage administration, and the release of anti-phage antibodies during the treatment brings concerns (47).

Potential Solutions:

Liposomal delivery of phages can decrease the clearance rate of phages by guarding the phages against anti-phage antibodies (48). Frequent administration of phages can reduce the rate of neutralizing antibodies (49). Cell-mediated immunity can be combated by making the phage protein coat express polyethylene glycols, which increase the phage circulation time in the blood (50).

Challenge:

Geier *et al.* (51) first observed rapid clearance of phages when Lambda phages were injected in high titers into transgenic mice lacking immune response. The administered phages are rapidly cleared by the reticuloendothelial system (RES) and are not available for therapeutic use in the body (52, 53).

Potential Solutions:

Longer circulating phages can be obtained by "serial passage" into the bloodstream of a mouse, and selecting phages with higher circulation time than the original wild-type phage (54).

According to Levin and Bull (55), phage treatment should only decrease the pathogen to an extent where the immune system can successfully clear the bacterial load. Phage engineering can help us generate phages that don't replicate or proliferate (56, 57). These can make an immune safe therapeutic phage.

4. Horizontal Gene Transfer

Once a phage infects a bacterium, the phage genome is replicated inside the host and eventually, phages assemble and lyse the bacterium (Figure 1). While the phage genome gets packed in the phage capsids, accidentally 1 in 10^7 phages receive the bacterial genome (58). This phage is now called a transducing particle. Infection of transducing particles in bacteria causes Horizontal Gene Transfer (HGT) or transduction (59). Transduction enhances HGT of virulent, resistant, metabolic and other fitness genes (60, 61), which enable the bacteria to rapidly adapt and evolve to changing environmental conditions.

4.1 Development of Phage Resistance

A complication of phage therapy is that the bacteria can gain resistance to the phage during the treatment (15, 62–64). Among 12 phage therapy clinical trials, seven studies reported phage resistance (65). Bacteria show phage resistance generally by modifying phage receptors (64). Often, such changes would affect bacterial fitness and could reduce its virulence (66, 67) as seen in Tom's case (see Human Trials- below).

The development of phage resistance is partially advantageous, but phages interfere with many cellular pathways, including translation, transcription, replication, and so, it is harder for bacteria to gain resistance against phages compared to antibiotics (68). While Khawaldeh *et al*. (69) did not find any development of phage resistance after administering a phage cocktail to a patient detected with *Pseudomonas aeruginosa* UTI (Urinary Tract Infection), Zhvania *et al*. (70) did report phage resistance in *Staphylococcus aureus* after phage administration to a patient with Netherton Syndrome.

5. Intracellular Treatment

Challenge:

Antibiotics can treat intracellular bacterial pathogens (like *M. tuberculosis*) as they have the capability of entering the

cell (71). A phage requires bacterial receptors to bind and kill a pathogen. The inability of phages to enter macrophages brings concern in tackling intracellular pathogens using phage therapy. Internalization of phages into the infected cells is a crucial step to treat intracellular pathogens.

Potential Solutions:

Targeting extracellular stage: Phage therapy was found to efficiently decrease pathology and prevent ulceration in *Mycobacterium ulcerans* infection, where phages targeted a temporary extracellular stage of the bacterium (72).

Using cell penetrating peptides: The model phage M13 decorated with cell penetrating peptides was localized in the ER, Endosomes, and Golgi within 6 hours of internalization in Hela cells (73). However, phages displaying such peptides might circulate for a lesser time (74).

Liposomal internalization: Liposomes with a positive charge fuses with the negatively charged cell membrane to deliver phages inside the cell membrane (75). Using non-pathogenic host: TM4 phage was utilized for intracellular drug delivery into infected macrophages. Non-pathogenic *M. smegmatis* was loaded with phages, and these were phagocytosed by macrophages. The phage lytic cycle then reduced intra-phagosomal bacterial counts (76).

6. Which Route Works Best?

6.1 Oral

Oral administration of a therapeutic is the most convenient and often desired mode of administration. Oral delivery of phage poses two major challenges regarding viability and gut transit.

The lower survival rates of phages (7%) meeting the hostile acidic environment of the stomach (77) presents a major challenge. Even, administering phages with alkali could increase the risk of opportunistic infections (78, 79). Administration of phages with yoghurt, or encapsulation of phages, are a possible solution to enhance the survival rate of phages (80, 81). Genetically modified phages could offer a simplified and cheaper methodology than encapsulation (82).

The gut transit of phages is a question without a clear understanding. There are examples of phages entering the bloodstream (83–85) and not entering the bloodstream (54, 86, 87) after oral administration. Furthermore, Majewska *et al*. (88) showed that phage-induced IgG and IgA hinder the gut transit of phages. Experiments by Międzybrodzki *et al*. (80) conclude that the phage entry into the bloodstream depends on the type of phage and the host. Therefore, more studies need to be conducted to find specific phage strains which can easily enter the human gut.

6.2 Other Routes

The most effective mode of phage administration is unclear yet. For pulmonary infections, phage delivery through inhalation seems a more convenient and effective administration (89, 90). Contradicting this view, few studies show greater effectiveness through intraperitoneal and intravenous routes of administration for pulmonary infections (91, 92).

Although intravenous administration of phages strongly elicits an immune response, Czaplewski *et al*. (93) believe that phage administration intravenously is also a promising alternative for antibiotics. The two famous success stories in phage therapy (see Human Trials) utilized systemic phage delivery. Considering simplicity and effectiveness, topical administration is highly advisable for eye, ear, nose and skin infections; oral administration for gut infections; intrarectal for prostate infections; and intravenous for systemic infections (94).

7. Side Effects in Phage Therapy

Until recently, phages were considered safe for human use as they selectively bind to only bacteria. Also, the abundance of phages in the human body indicates that phages are inherently safe since we are continuously exposed to them enterically and topically (34, 95). However, recent studies question this concept of phages "Generally Regarded as Safe" (96). A systematic review conducted by Steele *et al*. (97) concludes that there is limited evidence supporting the safety of phages. Tetz *et al*. (98) goes on to call phages "Potential Mammalian Pathogens."

The complex interactions of phages with the human body can cause serious side effects in phage therapy. Few such side effects could be chronic glomerulonephritis by the accumulation of antiphage-antibody complexes in the glomerular region (23, 99); increased concentration of endotoxins and inflammatory cytokines in the blood (84, 98); increased gut permeability, weight loss, messy hair (100); sudden fever and chills (101). Another potential side effect could be the unpredictable consequences of the human microbiome by the introduction of phages (102).

The most feared phenomenon by many phage biologists is the integration of virulence genes—like Cholera toxin, Staphylococcal enterotoxin and Shiga toxin—from the phage that can enhance the virulence armoury of bacteria. This could lead to the "evolution of new human pathogens" (103). It is therefore necessary that the therapeutic phages must be fully sequenced to confirm the absence of undesirable genes such as toxins.

8. Technical Feasibility

In a review by Czaplewski *et al*. (93) about alternatives for antibiotics, phage enzymes were thought to have the highest potential to replace antibacterials, while phages were scored relatively lower for their technical feasibility. Unlike antibiotics, phage (virus) preparation and storage is costlier. The shelf-life of phages must be long enough for laboratory study or commercial application (104). A therapeutic bacteriophage should not lose its activity before treating patients. Attainment of stability is a crucial part of development (105). The phage should grow well under industrial and laboratory cultures and must be easy to store and maintain (37). Since every phage strain has different optimal conditions (temperature, pH, buffer) for preservation, not all the diverse number of phages can presently be stored efficiently. Lyophilization and spray-drying (107) are current methods available to obtain phage powders, and the optimal conditions of storage for different therapeutic phages are yet to be explored. Zhang *et al*. (106) have successfully produced a freeze-dried phage powder of the model phage M13.

9. Other Challenges

To date, bioethical theories regarding phages have not been published; Intellectual Property protection is limited in the case of natural phages (108); Statistically evident double-blind clinical studies were not reported in adequate numbers. These factors create uncertainty in the development of a dedicated regulatory framework for phage therapy. The peculiar characteristics of phages have made their clinical assessment more complex, demanding further clinical research. Regardless of whether there is abundant research performed, commercial implementation of phage therapy would pose a significant challenge. Investment factors as well as profitability are exceedingly unknown (95), which hinders pharmaceutical investments (109).

Potential Solutions: In-depth research and large-scale phage production is possible through industrial investments. Phage producing centres and hospitals should have good collaboration (110) and be able to work together to provide positive evidence for the regulatory bodies which would grab the attention of pharmaceutical industries.

Human Trials

In vitro studies of phage therapy do not consider complex biological interactions, which influence the treatment. Our knowledge of phages *in vitro* is exceptional, but in vivo behaviour of phages is less well-known (111). This demands a need to study phages in a biological system (preferably in humans) to evaluate the efficacy and side effects of the treatment. Two major institutes conducting such investigations since the historical era (1917-1995) are Eliava Institute of Bacteriophages, Microbiology and Virology (IBMV, Georgia) and Hirfeld Institute of Immunology and Experimental Therapy (ITET, Poland). These institutes, along with Felix d'Herelle Reference Center for bacterial viruses (Canada), The Leibniz Institute DSMZ (Germany), and Queen Astrid Military Hospital (Belgium) are empowered with huge phage banks to store therapeutic phage cocktails (112–114). Eliava Institute has treated around a hundred foreign patients since 2012, and the numbers are expanding every year (115). The US Navy has also developed

a proprietary capacity to purify phage strains for specific infections (116). Phage therapy is now being studied globally due to the development of antibacterial resistance. One of the earliest and well-designed controlled trials in Georgia was during the 1960s (112). A total of 30,769 children less than 7 years were included. During the annual dysentery period, an anti-Shigella phage cocktail targeting Shigella boydi, S. newcastle, S. sonnei, S. flexenerei was administered to the children present on one side of a street. The children on the other side of the street received a placebo. A nurse reviewed the subjects once a week for 109 days. Dysentery was encountered by 1.76 in 1000 children receiving phage treatment, while 6.7 in 1000 from the controlled group were affected. But, many such studies conducted in the historic era (1917-1995) needed methodological evidence, and the results are not reliable and reproducible (30). Many phage biologists, therefore, believe these results to be spurious. A need for clinical trials set to modern standards—placebo-controlled, randomized, double-blind studies—are required to settle the debate on the efficiency of phage therapy. The first US-FDA approved phase I clinical trial was executed in 2009 (117), and numerous other studies followed. Most of the studies concluded phages as not a significant therapeutic (Table 1). Many of these studies failed to recruit statistically significant numbers, having small patient groups, which drastically limited the conclusions drawn (10). A recent Phase II clinical trial (Phagoburn) approved by France, Belgium, and Switzerland national health regulators was terminated

Table 1: **Data from clinical trials evaluating the efficacy of phages as therapeutic agents**

Year of Study	Problem and Etiologic agent	Route of Administration	Success Rate	Reference
1981 to 1986	Suppurative infections by *Staphylococcus, Pseudomonas, E. coli,, Klebsiella,* and *Salmonella*	Various	92% (n=550)	(41)
1987 to 1999	Suppurative infections by *Escherichia, Klebsiella, Proteus, Enterobacter, Pseudomonas* and *Staphylococcus*	Oral, Local, Intraperitoneal, Topical	86% (n=1307)	(84)
1987	Skin infections by *Pseudomonas, Staphylococcus, Klebsiella, Proteus* and *E. coli*	Oral and Local	74% (n=31)	(118)
1989	Post operative wound infections by *Pseudomonas* and *Staphylococcus*	Local	82% (n=65)	(119)

Year of Study	Problem and Etiologic agent	Route of Administration	Success Rate	Reference
1992	Skin and nasal mucosal infections by *K. ozaenae, K. rhinoscleromatis* and *K. pneumoniae*	Intraperitoneal	100% (n=109)	(120)
1995	Urinogenital inflamation by various agents	Oral and Local	92% (n=46) (8% more than antibiotic treated group)	(121)
2009 (Double Blind)	Otitis by *P. aeruginosa*	Local	76% decrease in bacterial count (n=12)	(122)
2009 (Double Blind)	Chronic venus leg ulcers by *P. aerginosa, S. aureus,* and *E. coli*	Local	No significant difference from control. (n=39)	(117)
2016 (Double Blind)	Diarrhea by *E. coli*	Oral	No significant decrease in diarrhea in different groups	(83)
2018 (Double Blind)	Urinary Tract Infection by *Staphylococcus aureus, E. coli, Streptococcus spp., Pseudomonas aaeruginosa, Enterococcus spp.*	Intravesical	Decrease of bacterial titers is 67% (n=9)	(101)
2019	Burn wounds by *P. aeruginosa*	Topical	69% Cured, 23%-adverse, 1 person died (n=13)	(33)
2020	Systemic infection with *Staphylococcus aureus*	Intravenous	61.5% cured within 7 days (n=13)	(123)

prematurely (several times before the trial had started), as the eligible patient recruitment was inadequate (33).

In light of the WMA-Declaration of Helsinki, which states "In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, reestablishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published." (124), physicians offer phage therapy as the last resort. Often this includes combined therapy with antibiotics and in many cases, the patients recovered.One such case is that of Tom Patterson who is a 68-year-old professor in the Psychiatry Department, University of California Medical School. During his vacation to Egypt, he contracted a systemic infection (initially thought to be Food poisoning) by MDR (Multi Drug Resistant) A. baumannii. All standard antibiotic treatments failed. His wife Steffanie Strathdee—Associate Dean of Global Health Sciences, University of California—obtained an emergency authorization for treating her husband with phages. Tom was administered phage cocktails intravenously. There was a change in antibiotic resistance profiles. Bacteria had developed phage resistance. Tom finally got treated. The team then received a $1.2 million grant over three years and became the directors of IPATH (Innovative Phage Applications and Therapeutics) (79, 125, 126).

Another case study is that of Isabelle Holdaway, a 15-year-old girl who suffered from P. aeruginosa and *Mycobacterium* abscessus infection. Doctors performed a lung transplant, but the infection was still not cleared. One month post the transplant, *Mycobacterium* abscessus was isolated and the patient was diagnosed with a Mycobacterial infection. Though her survival chance was predicted to be less than 1%, doctors gave a try for phage therapy. Holdaway's Mycobacterial isolates were screened against more than 10,000 phages. Two of the 3 selected phages were following temperate life cycle. Bacteriophage Recombineering of Electroporated DNA (BRED) was used to prepare the lytic derivative of these phages. The cocktail of these phages was used for phage therapy. Holdaway received the intravenous phage treatment without any significant side effects. She was discharged after 9 days of her treatment. After 11 months of her treatment, virtually all her lesions disappeared (32, 127). Evaluation of clinical trials is necessary to evaluate the effectiveness of phage therapy (see Table-1). Phages are found to be safe in all the trials, but only a single double-blind clinical trial claims the efficacy of phage therapy. Thus, well-designed clinical trials are highly warranted to further evaluate the efficacy of phage therapy.

Other Applications

Bacteriophage has its application in varied arenas, from targeting MDR infections to targeting rot in harvested potatoes. Phages also may be used in decontaminating the hospital environment, which would decrease the incidence of nosocomial infections.

1. Agriculture and Food Safety

Bacteriophage application is becoming advanced in animal husbandry, food safety, and agriculture (128). It was in 2005 when for the first time, a bacteriophage product—Agriphage™—was formally approved by the regulatory agency of the US government to treat crop diseases (129). Since then, many phage products have hit the commercial markets (Table 2). The use of phages in agriculture was also exploited by OmniLytics Inc. When a customer sends in infected plant material, customized phage products are prepared and given to the customer (37).

Phages are also gaining popularity in the food industry to attack foodborne bacterial pathogens. In 2006, the FDA approved ListShield™, a phage cocktail targeting Listeria monocytogenes (which contaminates ready-made food products) as safe for consumption. Few other products include AgriPhage, BioTector, Ecoshield, Finalise, ListShield (130). Other than phages, phage-derived enzymes are also attractive investments. The usage of phage lytic enzymes in food conservation (as an antibacterial) was first reviewed in 2005 (131). Few commercially available enzymes are LISTEX™ & LMP-102 (from phages targeting Listeria) (132), ECP-100 (from *Escherichia coli* O157:H7 phage)

Table 2: **Examples of commercially available phage products**

Product Name	Company	Targeted Bacteria	Reference
LMP 102	Intralytix	*Listeria monocytogenes*	(135)
ListShield	Intralytix	*Listeria monocytogenes*	(136)
EcoShield	Intralytix	*E. coli O157:H7*	(137)
SalmoFresh	Intralytix	*Salmonella spp.*	(138)
Shiga Shield	Intralytix	*Shigella flexneri, S. sonnei, S. dysenteriae*	(139)
ListeX™ P100	Micreos Ltd	*Listeria monocytogenes*	(132)
SalmoPro	Phagelux Inc	*Salmonella enterica*	(140)
AgriPhage	Certis USA LLC	*Xanthomanas campestris*	(141)
PhageGuard	Micreos Food Safety	*L- Listeria monocytogene S- Salmonella E- E. coli O157*	(142)

(133), and SalmoFresh™ (from phage targeting Salmonella enterica) (134) are used in the food industry.

2. Promising Applications

Usage of phages is highly promising in eye drops and antiseptics, which follow topical administration. Such commercial phage products are highly effective and can attract high investments from pharmaceutical companies. Eye drops, for example, were found to be a statistically significant treatment for *P. aeruginosa* infection (143). Regarding antiseptics, Eliava Institute in Georgia has developed a commercial biopolymer bandage, with phage cocktails called "PhagoBioderm". For the development of other such commercial phage therapy products, projects like PhagoFlow (144) and PhagoMed (145) are implemented.

3. Genetic Engineered Phage

The lack of efficiency and other challenges met by phages in the therapeutic domain in the modern synthetic biology era can now be met by genetic engineering of phages (113, 146, 147). Genetically engineered phages have found their application in many other areas. Phage engineering is used for the targeted delivery of phages (148) and is also being exploited for protein and gene delivery. Tao P. *et al.* delivered proteins and genes in *vitro* and in vivo by using the T4 phage (149). Przystal *et al*. used phages as vectors to target orthotopic glioblastoma and suppressed the growth of glioblastoma by a systemic combination of temozolomide and suicide gene therapy (150). Folate-conjugated M13 coated by Poly(caprolactone-b-2-vinylpyridine) which encapsulated hydrophobic antitumor drug doxorubicin acted as a nanosized drug delivery vehicle (151). Phage coat protein can be modified by expressing immunogenic peptides that could deliver vaccines (152). Phages are also exploited to edit the microbiota by artificially synthesizing phages and modifying their tail fibers (153).

Summary

Though phage therapy is a promising and attractive source of treatment for emerging bacterial infections, further understanding of phage biology is essential before reimplementation of phage therapy. *In vivo* studies are required on liposomal phage delivery or delivery of genetically modified phages. There is no consensus view on the most effective route of phage administration, dosage and pharmacokinetics. Also, phage interaction with the immune system is not well known when compared to the knowledge we possess in regarding antibiotics.

The efficacy of genetically modified phages is to be evaluated *in vivo*. There is a need to be careful with genetically modified phages used in therapeutics. If phage resistance arises, switching to new phages with different technology, achieving the same efficacy would be difficult.

Programs like the "Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science" (SEA-PHAGES) trains students to isolate phages characterize genomes against a particular pathogen. Such programs need to be conducted with increased rigour across the globe. The establishment of more phage banks to store such newly found isolates can lessen the challenges faced by the host range.

Since phage therapy deals with viruses, the high cost involved in phage isolation is an obvious hurdle for commercial production. For industrial-scale production of phages, a search for surrogate hosts is necessary, which might marginally reduce the production cost. Despite these challenges, the author believes that bacteriophages can lead us into a progressive future with varied applications in agriculture, aquaculture, poultry, sewage treatment, and therapeutic use in humans aquaculture, poultry, sewage treatment, and therapeutic use in humans. aquaculture, poultry, sewage treatment, and therapeutic use in humans.

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