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Review Article

Phage therapy: an alternative or adjunct to antibiotics?

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Phage therapy is currently discussed as an alternative or adjunct to antibiotics whose activity is increasingly compromised by the emergence of antibiotic-resistant bacterial pathogens. The idea to use lytic bacterial viruses as antimicrobial agents is nearly a century old and is common practice in Eastern Europe. However, safety concerns and lack of controlled clinical trials proving the efficacy of phage therapy have hampered its wider medical use in the West. The present review analyzes safety aspects and compares successful with unsuccessful phage therapy clinical trials to identify potential factors determining success and failure of this approach.

Introduction: a phage renaissance?

The World Health Organization has warned that the antibiotic resistance crisis represents a threat to public health and medical practice [1]. Owing to this specter, phage therapy [2], historically developed as an independent antimicrobial approach to bacterial infections, has regained interest in the scientific community [3]. One speaks with hope, even with some hype, about a renaissance of phage. Phage products are on the market in cosmetics, and phage products for food sanitation have achieved FDA approval. In June 2015, the European Medicine Agency organized a meeting to discuss a framework for the development of medical phage products in the European Union (http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/events/2015/05/event_detail_001155.jsp&mid=WC0b01ac058004d5c3). The European Union has sponsored a multicenter phage therapy trial ('Phagoburn') of infections in burn wound patients (http://www.phagoburn.eu/); clinical results are expected for the end of 2017. At the moment, the scientific proof for the clinical efficacy of phage therapy is thin. What explains this situation? The pharmaceutical industry in Western countries has so far not shown much interest in phage therapy. For small biotech industries, large clinical trials are beyond financial capacities. The prior art argument makes patenting of phage products difficult and investors hesitant. Complaints were formulated in the literature about the lack of a clear, or of a too strict, regulatory framework. However, the EMA has encouraged researchers to discuss regulatory guidelines with them, to assure phage therapy safety without hampering the development of phage products.

Safety aspects of phage preparations

Protocols for phage therapy trials have met substantial safety concerns from clinicians and ethical committees. Indeed, in some bacterial pathogens, virulence genes are encoded by prophages (this is, for example, the case for super-antigens and toxins from *Staphylococcus aureus*) [4]. Temperate phages should therefore be excluded from phage therapy. Transducing phages can transfer genetic material between bacteria, including antibiotic resistance genes, and need likewise to be excluded from therapeutic applications. When phage cocktails are restricted to 'professionally lytic' or 'virulent' phages propagated on a prophage-free bacterial production strain under GMP conditions and when the resulting phage preparations undergo metagenome sequencing and *in silico* safety screening, the genetic safety requirements of EMA for a phage preparation are met.

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Most microbiologists do not consider phage exposure as such a risk since several arguments speak in favor of an intrinsic safety of phage virions [5]. First, there is a long-standing, safe use of phage preparations in Eastern Europe. In addition, generations of students used to mouth-pipette coliphage solutions as part of laboratory practicum. Second, food microbiologists know that we are exposed to phages with fermented food products. Third, microbiome researchers define phages as a natural part of the healthy gut microbiota [6]. Even intravenous coliphage injection in immunocompromised human patients is an FDA-approved process to monitor immunodeficiency since the 1970s and was also used in HIV patients with low CD4 counts without provoking adverse effects [7].

Human safety tests and an unsuccessful phase II trial in *Escherichia coli* diarrhea

Nevertheless, clinicians from the International Center for Diarrheal Diseases Research in Dhaka, Bangladesh (icddr,b), the world's leading diarrhea research hospital, still had safety concerns toward a characterized cocktail of T4-like *E. coli* phages (Figure 1). The phages were isolated from stools of Bangladeshi children [8], and propagated in a sterile fermenter on *E. coli* strain K-12 derivative [9], which is devoid of virulence genes and inducible prophages. The T4-like phages were sequenced and subsequently tested in mice and healthy adult Swiss volunteers [10]. The clinicians asked for three sequential safety tests in healthy subjects from Bangladesh: first in adults, then in 5–10-year- and, finally, in 1–5-year-old children. The subjects were followed clinically and with a large panel of clinical chemistry and hematology tests. Transit of phage and endotoxin, i.e. lipopolysaccharide (LPS) released from phage-lysed *E. coli* cells into the blood stream, was tested. No adverse effects were observed that could be linked to T4 phage or a commercial Russian phage cocktail (Microgen) [11] (Figure 2). As phages are bacterial host species-specific, one major theoretical, yet rarely proven, advantage of phage therapy over antibiotic treatment is the absence of collateral damage to gut commensals. Indeed, oral phage application in healthy Bangladeshi children did not cause shifts in the fecal microbiota composition that went beyond the natural, temporal fluctuation observed in these children [12].

Also, in the following phase II efficacy trial of oral coliphages in pediatric *E. coli* diarrhea patients, safety aspects of phage therapy were addressed. In diarrhea patients, the pathogen would be lysed by phages (Figure 3) and release LPS/endotoxins, which could enter the circulation through the compromised gut barrier. However, no endotoxin-induced adverse events were observed in the enrolled patients. After the enrolment of 120 patients, an interim analysis was done (Figure 4), which revealed no beneficial effect of the T4 phage or the Microgen phage cocktail over the standard therapy (oral rehydration solution supplemented with zinc) [13]. The phase II clinical trial was therefore stopped for futility.

The successful otitis externa trial

From such experience, one might conclude that oral phage is safe, but not efficient. However, this would be rushing to a premature conclusion. Comparing this unsuccessful phage therapy trial with a successful example, conducted by Biocontrol Ltd in a London ear clinic, might reveal factors deciding on success and failure of phage trials (Table 1). The British researchers targeted antibiotic-resistant chronic otitis externa with a *Pseudomonas aeruginosa* phage cocktail [14]. Otitis externa ranges from mild swimmer's ear to a chronic, persistent, draining infection of the external ear canal. To optimize chances for success, the researchers only enrolled patients with a microbiologically proven *P. aeruginosa* infection. In addition, they only enrolled patients whose isolate was sensitive to at least one of the six phages in the cocktail. A single phage dose (6×10^5 pfu) in a 0.2 ml volume was applied once into the external ear canal. Phage remained detectable for 3 weeks and experienced a 200-fold titer increase, which was accompanied by a variable *P. aeruginosa* titer decrease not seen in placebo recipients. The microbiological effects were accompanied by significant symptom amelioration in patients obtained by self-report and physician diagnosis, which was not seen in placebo recipients. Owing to this encouraging result, the test was interrupted after a blinded interim analysis and a follow-up in a phase III trial was recommended. A drawback of the study was its small size: only a total of 12 phage-treated and 12 placebo patients were investigated. A phase III trial was never started, possibly because Biocontrol was bought by AmpliPhi Biosciences.

Learning lessons

Chronic versus acute infections

What are the critical differences between the two trials? First, otitis externa is a chronic condition, while patients enrolled into the icddr,b trial suffered from an acute infection. The children experienced on average

1–2 days of diarrhea, at home before hospitalization, leaving at most only 3–4 days of diarrhea in the hospital to achieve an effect. While this looks like an uphill battle, it is not impossible to succeed. Treatment effects of an *Lactobacillus paracasei* probiotic were seen with the same protocol in a subgroup of non-viral diarrhea patients at icddr,b [15].

Animal pretesting and pathogen targeting

Second, the otitis trial was preceded by a small veterinary trial where the Biocontrol phage cocktail produced positive effects in pet dogs with *P. aeruginosa* ear infections [16]. No veterinary trial was done with the T4 phage cocktails, and the preclinical model of mice colonized with *E. coli* and treated with phages does not produce diarrheal symptoms [17]. Therefore, only limited efficacy indications in animals were obtained for the phage cocktails of the diarrhea trial [18]. Third, for practical purposes, otitis externa can be considered to be specific to *P. aeruginosa*, as it is rarely caused by fungi and *S. aureus*. In contrast, acute childhood diarrhea in Bangladesh is caused by a plethora of pathogens. In the trial, rotavirus infections were excluded by a quick ELISA test, *Vibrio cholerae* infections by dark field microscopy and shigellosis by the detection of blood and mucus in the stool. However, the detection of *E. coli* pathogens was done by culture, combined with PCR, which took days for a diagnosis. Therefore, only 60% of enrolled patients had microbiologically proven *E. coli* infection [13].

Difficulty with polymicrobial infections

Childhood diarrhea in Bangladesh is frequently a polymicrobial infection with three to five different pathogens isolated from the stool [19]. In addition, large epidemiological surveys have seeded doubts about the role of *E. coli* pathogens in acute diarrhea since they are frequently also found with a comparable rate in asymptomatic local control children [20,21]. The pathogenicity index of many *E. coli* pathogens is thus rather low, and this applies not only to newer pathotypes, but also to well-investigated types like heat labile toxin (LT)-producing enterotoxigenic *E. coli* (ETEC). One therefore does not know whether *E. coli* was the driver of the diarrhea or a bystander, and it becomes unclear whether its elimination will lead to a clinical amelioration of the patient. The species specificity of phages necessitates either an accurate pathogen diagnosis or the use of complex phage cocktails directed against many pathogens, as found in some commercial Russian phage products, but not the Microgen cocktail used in the trial, which only contained phages against *E. coli* and *Proteus*.

Pretesting for phage sensitivity

A fourth difference is the selection of patients harboring a pathogen that is sensitive towards the phage cocktail in the otitis, but not in the diarrhea phage trial. This is probably a critical parameter with a complex target like *E. coli*, which presents with many pathotypes and many O-serotypes (determined by the LPS side chain, which is also one receptor for T4 phages). Ten different T4-like phages were needed to achieve a 60% coverage of *E. coli* pathogens from strain collections with variations according to geographical origin [22]. The Microgen cocktail experienced the same coverage problems. From stool analysis, it was indeed estimated that only 50% of the isolated *E. coli* colonies were susceptible to the phage cocktails [13].

Pathogen below threshold concentration

The microbiological analysis of stool samples from the icddr,b trial raised further doubts about a phage approach against acute *E. coli* diarrhea. *E. coli* represented only a small fraction of the stool microbiota, while a marked stool microbiota dysbiosis with intestinal streptococci was observed [13]. When enumerating ETEC strains by qPCR to test for either heat LT or heat stable toxin (ST)-encoding genes, only a low median peak titer of 10^5 cfu/g stool was detected, and the titers only peaked at the second day of hospitalization. Titers of 10^5 cfu/g stool titers were also described for enteropathogenic *E. coli* (EPEC) pathogens in Peruvian children with diarrhea [23]. Pathogen titers play a major role for phage therapy. *In vitro* experiments have revealed that thresholds of target cell concentrations exist for phages, below which no phage replication is observed. This threshold was determined to be 10^3 cfu/ml for T4 phage and *E. coli* in broth culture [24]. This number can also be rationalized with a simple model. Phage will not be able to replicate if the product of target bacterium density N, phage adsorption rate d and its burst size B is less than the phage inactivation rate w. With $d \times N \times B > w$ and realistic estimates of $d = 10^{-8}$, $B = 100$ and $w = 0.01$ per hour, the minimum 'threshold' density of bacteria for phage replication is $N \geq 10^{-2}/(10^{-8} \times 10^2) = 10^4$ cells per ml, assuming a physiological state of the bacteria supporting phage replication [25]. The stool titers of ETEC and EPEC in diarrhea patients are thus

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close to the *in vitro* threshold, considering that many factors reduce the effective concentration of *E. coli* in the gut (biofilms, accessibility in mucus and low replication rate of *E. coli in vivo*) [26]. Indeed, no amplification of phage was seen when comparing the fecal with the oral phage titers; phage experienced probably a passive transit through the gut without much *in situ* phage replication on the intestinal *E. coli* cells [13].

Difficulty with pharmacokinetics

Finally, the location of the infection site, its accessibility to the applied phages and the effective phage concentration reached are also likely factors that have a major impact on the efficacy of phage therapy. Clearly, when applying phage into the outer ear canal, one might expect that therapeutically relevant phage concentrations are reached at the infection site, provided that phage penetrates biofilms, which has been shown by *in vitro* and *ex vivo* experiments. In contrast, applying phages orally without any further pharmaceutical formulation (e.g. an acid-resistant microencapsulation) has major drawbacks. Even when given at higher doses (3×10^8 and 10^9 pfu as in the diarrhea trial), substantial dilution can be expected in secretions of the alimentary tract. In addition, the physiological task of gut secretions is to digest food, and the stomach represents a particularly potent barrier for gut passage of phages, as shown in simulated stomach juice exposure [27]. Oral phages are given in Eastern Europe after neutralizing gastric acidity with oral bicarbonate. This procedure was, however, not accepted by the icddr,b clinicians so as not to compromise the gastric barrier protection against cross-infection in a diarrhea hospital with a high pathogen load. One thus has to anticipate a substantial decrease in effective phage dose in the small intestine. The increased peristalsis during diarrhea also means shorter contact times with the *E. coli* pathogen.

Solutions: Ex oriente lux?

The unsuccessful icddr,b trial is thus not an argument against phage therapy in general, but an argument against targeting *E. coli* diarrhea with phage therapy. Do literature data suggest a more suitable target for phage therapy? One way is to look into the Eastern European experience. A lead is provided here by a clinical trial conducted in Tbilisi during the 1960s [28].

The *Shigella* prevention trial

This is a remarkable example since it would fulfill the criteria of a large modern clinical trial [2]. The trial was controlled: half of the children received a *Shigella* phage in a pressed tablet and the other half a similar tablet without phage as placebo. The trial was randomized: children living one side of the street received the active pill and those on the other side received placebo. The trial was blinded: neither the physicians diagnosing the disease nor the microbiologists detecting the pathogen knew the group attribution of the children. The trial was statistically relevant: more than 30 000 children were enrolled and children were followed for >3 months. Notably, the trial was successful: phage treatment resulted in a 3.8-fold decrease in dysentery incidence detected clinically and in a 2.3-fold decrease in culture-confirmed shigellosis. Finally, the trial was credible: the authors described a decrease in *E. coli* diarrhea, which was not understood at the time, while it is known today that *Shigella* qualifies not even as a subspecies of *E. coli* [29]. The drawback of this fine study is that it was only documented in a 75-line Russian publication containing a single table [28] (an English translation of this publication is provided in Supplementary Text), and neither the archive in the Eliava Institute nor the central medical archive of clinical trials in Moscow conserved more documents on the present study.

Shigella–E. coli comparison

What factors set this successful *Shigella* trial apart from the unsuccessful *E. coli* diarrhea trial and what can we learn from such a comparison for future phage therapy trials against gut infections (Table 2)? First, dysentery is closely associated with *Shigella* and the pathogenicity index of *Shigella* is higher than that of *E. coli* pathogens [20,21]. In addition, dysentery is not a polymicrobial infection. Second, *Shigella* is genetically much more homogeneous than *E. coli* [29], and a 90% coverage can easily be achieved with a mixture of a few phages, obviating coverage problems [22]. Third, *Shigella* infections occur in the colon, whereas ETEC and EPEC infections are localized in the small intestine. Fourth, the *Shigella* trial was a prevention trial in healthy children in contrast with the *E. coli* trial, which was a treatment trial of acute diarrhea. The gut transit of phage in the colon of healthy children is much slower than that in the small intestine during diarrhea, therefore allowing for longer interaction times between oral phage and intestinal pathogen. As in the comparison of the otitis and *E. coli* trials, many factors distinguish the two trials — one cannot easily single out a factor predicting success or

Table 1 Comparison of distinguishing factors between the successful *P. aeruginosa* otitis externa [14] and the unsuccessful *E. coli* diarrhea [13] phage therapy trials

Successful *Pseudomonas* otitis trial	Unsuccessful *E. coli* diarrhea trial
Chronic infection	Acute infection
Single cause (*P. aeruginosa* in >95%)	Many pathogens involved (*E. coli* <30%)
Prescreening for phage cocktail sensitivity	No prescreening
Monoinfection	Polymicrobial infection
Pathogen above phage replication threshold	Pathogen below replication threshold
Pathogen clearly involved in disease	Doubts about pathogen–disease relationship (streptococcal dysbiosis?)
Application: easy (ear canal)	Dilution and digestion in gut, no antacid

failure. However, the phage therapy research community is well advised to repeat a *Shigella* prevention trial to give the phage therapy field a more solid foundation and to work against the reputation of phage therapy as merely 'Stalin's cure'.

Evaluation of case reports

Likewise, one should analyze the published reports of the Polish and Georgian phage therapy centers summarizing their experience with phage therapy from the past in large series of case reports [30,31]. This analysis can help to identify clinical conditions where phage is likely to have, or not to have, an effect. The combined microbiology and clinical approach in ambulatory patients of the joint Georgian-US-EU phage therapy center on the campus of the Eliava Institute could potentially provide further valuable data associating microbiological readouts with clinical outcomes. This could lead to the identification of infection types that empirically respond to phage interventions, which could then become the target of controlled clinical trials.

Phage resistance and antibiotic resistance

As antibiotic resistance development is a direct evolutionary consequence of exposing bacteria to the selection pressure of antibiotics, could the same happen to phages once phage therapy is applied at a larger scale? Clearly, under laboratory conditions, bacteria will also develop resistance to bacteriophages. The classical Luria–Delbrück experiments by the pioneers of modern phage genetics illustrated that point with cycles of resistance development and evolutionary interplay between *E. coli* and its phages [32]. Will phage resistance then make phage therapy quickly useless? Not necessarily, as suggested by recent experiments with *P. aeruginosa* [33]. In an *in vitro* fibrin-clot model, *P. aeruginosa* showed first a titer decrease after phage treatment followed, as predicted, by an outgrowth of phage-resistant bacteria. This outgrowth was prevented when phage was combined with antibiotics, while antibiotics alone caused no cell death. When Oechslin and colleagues repeated the experiment in a rat endocarditis model, phage or antibiotics reduced the *P. aeruginosa* load in the vegetations on valves by 100- to 1000-fold. Combining phage with antibiotics resulted in a 10^6-fold decrease in pathogen

Table 2 Comparison of distinguishing factors between the successful *Shigella* [2,28] and the unsuccessful *E. coli* diarrhea [13] phage therapy trials

Successful *Shigella* trial	Unsuccessful *E. coli* diarrhea trial
High pathogenicity index	Low pathogenicity index
Pathogen uniform	Many E. coli pathotypes and serotypes
Easy phage coverage	Phage coverage is a problem
Monoinfection	Polymicrobial infection
Infection site: colon	Infection site: small intestine
Prevention trial	Treatment trial
Shigella above threshold (?)	*E. coli* below threshold

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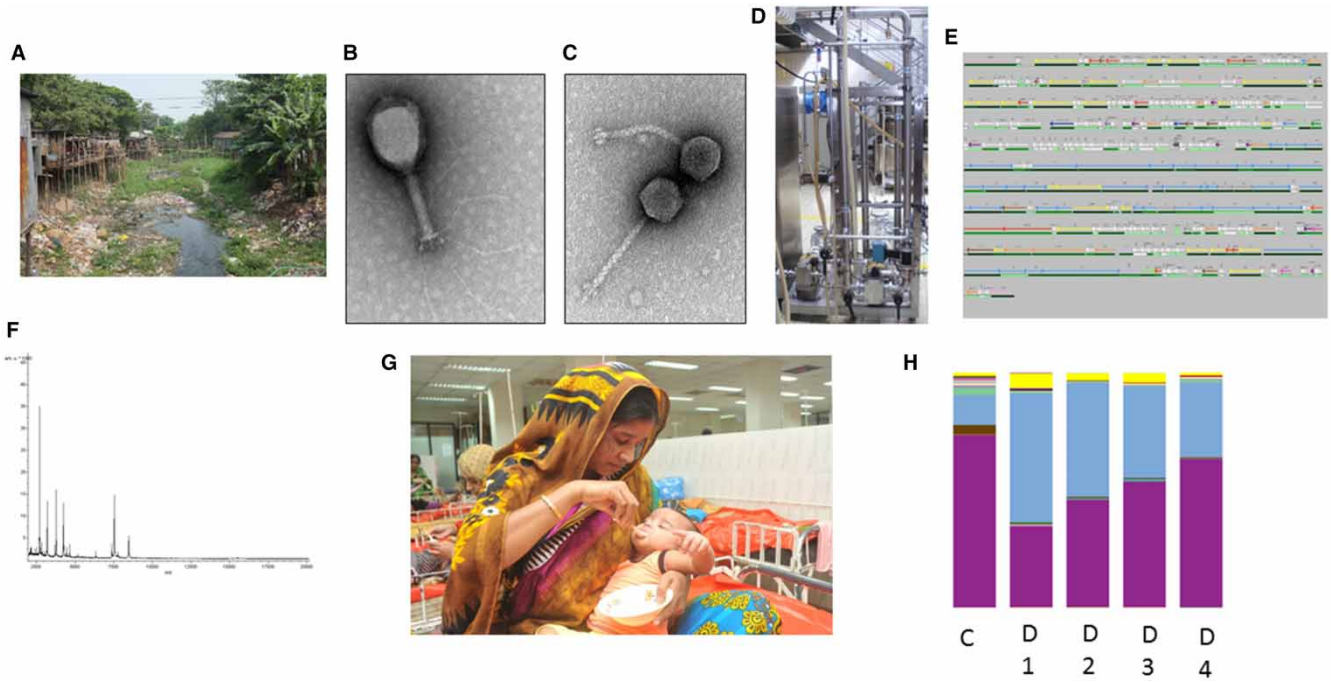

Figure 1. Development of a T4-like phage cocktail.

An ecological survey was conducted with polluted environmental water from a suburb of Dhaka/ Bangladesh and stool from healthy and diseased children (**A**), filtrates were plated on *E. coli* and individual plaques were selected if T4-like phages were detected (**B**) and rejected if less well-characterized phages were encountered (**C**). T4-like phages were screened for their host range on diarrhea-associated *E. coli* and suitable phages were amplified at the fermentation unit of NRC (**D**) and underwent genome sequencing (**E**). The purity of the amplified phages was controlled by mass spectrometry (**F**) and a cocktail of phages was constituted. This cocktail was tested at icddr,b first in a series of safety tests in healthy subjects of decreasing age and then in a combined phase I/II treatment trial in children hospitalized with acute *E. coli* diarrhea (**G**). Surprisingly, *E. coli* diarrhea is characterized more by a fecal *Streptococcus* (blue) dysbiosis than by an *Escherichia* outgrowth (yellow) (mauve: *Bifidobacterium*) (**H**). Here C is control children, and D1 to D4, diarrhea patients at days 1–4 of hospitalization; the histogram shows bacterial stool abundance determined by 16S rRNA gene sequencing. Figure credit: O. Sakwinska, NRC for (**A**), G. Bourdin, NRC and B. Schmitt, NRC for (**B**–**F**), and R. Hossain and T. Islam, icddr,b for (**G**) (permission from mother was obtained).

titer or, in many cases, sterilization of the infection site. Sterilization was never achieved with any antibiotic treatment, therefore suggesting a strong synergistic effect between these two antibacterial agents. Even more interestingly, no phage-resistant bacteria developed in the vegetations of the endocarditis model treated with phage alone. Close scrutiny of the *in vitro*-obtained phage-resistant bacteria revealed that the mutants had lost either pilus-mediated twitching motility or LPS synthesis capacity and thereby important virulence factors. A very similar phenomenon has been described 30 years ago by researchers in calves infected with a bovine diarrhea pathogen, *E. coli* strain O18:K1. They used an oral anti-K1 phage, which caused a rapid decline in the intestinal pathogen load and resolved diarrhea. Phage-resistant mutants evolved by loss of the K antigen, but since the capsular K antigen is a virulence factor, these mutants had lost pathogenic potential for calves [34]. If phages can be used to select for the loss of bacterial virulence genes, it should also be possible to use phages as selective agents to prevent antibiotic resistance development. This was actually achieved for *P. aeruginosa* with a phage that uses the outer membrane porin of a multidrug efflux system as a receptor for infection [35]. When phage was added, phage-resistant mutants evolved that became, in parallel, sensitive to antibiotics. While these are still *in vitro* observations, evolutionary biologists already point to innovative ways how to extend the lifetime of current antibiotics by phage use (for a recent review, see ref. [36]). In fact, phage–antibiotic synergy (PAS), where their combined effect is greater than the sum of the individual effects, have been described for many pathogens, namely *E. coli* [37], *E. coli* biofilms [38], *S. aureus* [39], *Burkholderia cepacia* [40] as well as *P. aeruginosa* [41] and its biofilms ([42], but see also ref. [43]). So far, PAS has mostly been studied *in vitro* or in a few animal models, but credibility for phage therapy will only come from

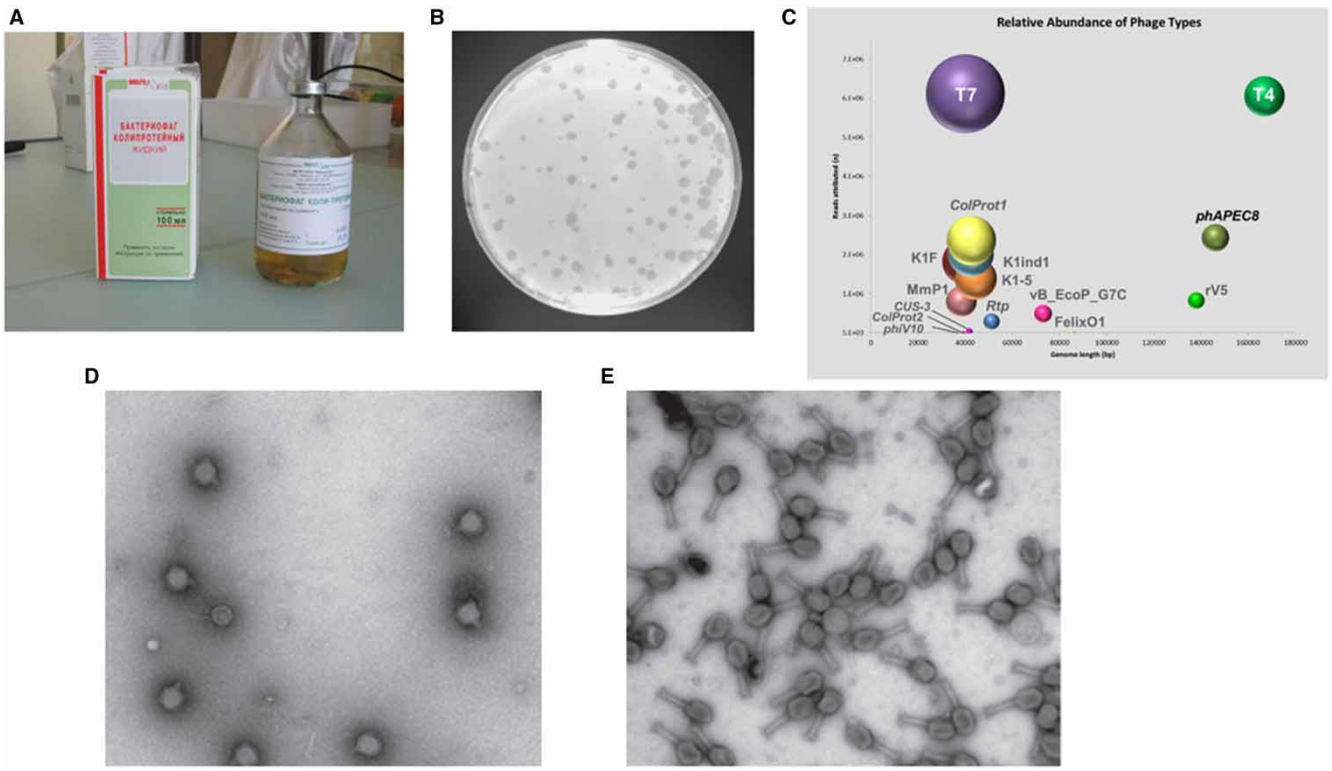

Figure 2. Characterization of the commercial Microgen ColiProteus phage preparation.

A commercial *E. coli*/*Proteus* phage preparation was purchased from Microgen/Russia (**A**). On an *E. coli* indicator strain, different phage plaque types were identified (**B**). Large plaques yielded T7-like phages (**D**) and small plaques T4-like phages (**E**). Metagenome sequencing identified more than a dozen of different phage types with T7 and T4 phages dominating (**C**). Figure credit: G. Bourdin, NRC.

successful clinical trials in humans. To achieve that goal, efficacy trials of phage as an adjunct to the established antibiotic therapy were proposed in the format of 'non-inferiority' trials [42]. If one considers the use of modified phages, the PAS concept could be extended by using phages that carry additional, engineered antimicrobial genes. An example of this is provided by the filamentous coliphage M13 overexpressing the *lexA3* repressor of the bacterial SOS response. By preventing DNA repair processes, this modified phage enhanced the impact of bacterial antibiotics such as quinolones, which lead to hydroxyl radical formation and DNA damage [44]. While this genetic approach was, until recently, limited to filamentous phages, e.g. delivery of lethal-addiction toxin phagemids with coliphage M13 [45], the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR-associated protein) system allows now for the efficient genome engineering of virulent phages from Gram-positive [46] and Gram-negative bacteria [47], therefore extending the possibility of using phages alone or in combination with antibiotics for therapeutic goals. The CRISPR-Cas system offers still other possibilities: an antibiotic-resistant bacterium was infected with a modified temperate lambda phage containing a CRISPR-Cas cassette directed against both an antibiotic resistance-conferring plasmid and a lytic phage. In the resulting lysogenic bacterium, the resistance plasmid is degraded, creating an antibiotic-sensitive derivative that is protected against infection with the lytic phage. Non-lysogens that are antibiotic-resistant are, however, killed by the lytic phage [48]. The researchers proposed this strategy for hospital surfaces and in hand sanitizers to replace antibiotic-resistant pathogens with sensitive ones. Once modified phages are accepted as part of phage therapy approaches, the technological possibilities with the CRISPR-Cas system extend the options well above what was possible. However, achievements with filamentous phages are already promising; for example, by knocking out an export protein in a filamentous *Pseudomonas* phage made the derivative phage non-replicative, yet still lethal to the infected cell. Since this derivative phage induced only limited cell lysis, it also reduced the release of toxic bacterial products and thus possible side effects of phage therapy [49].

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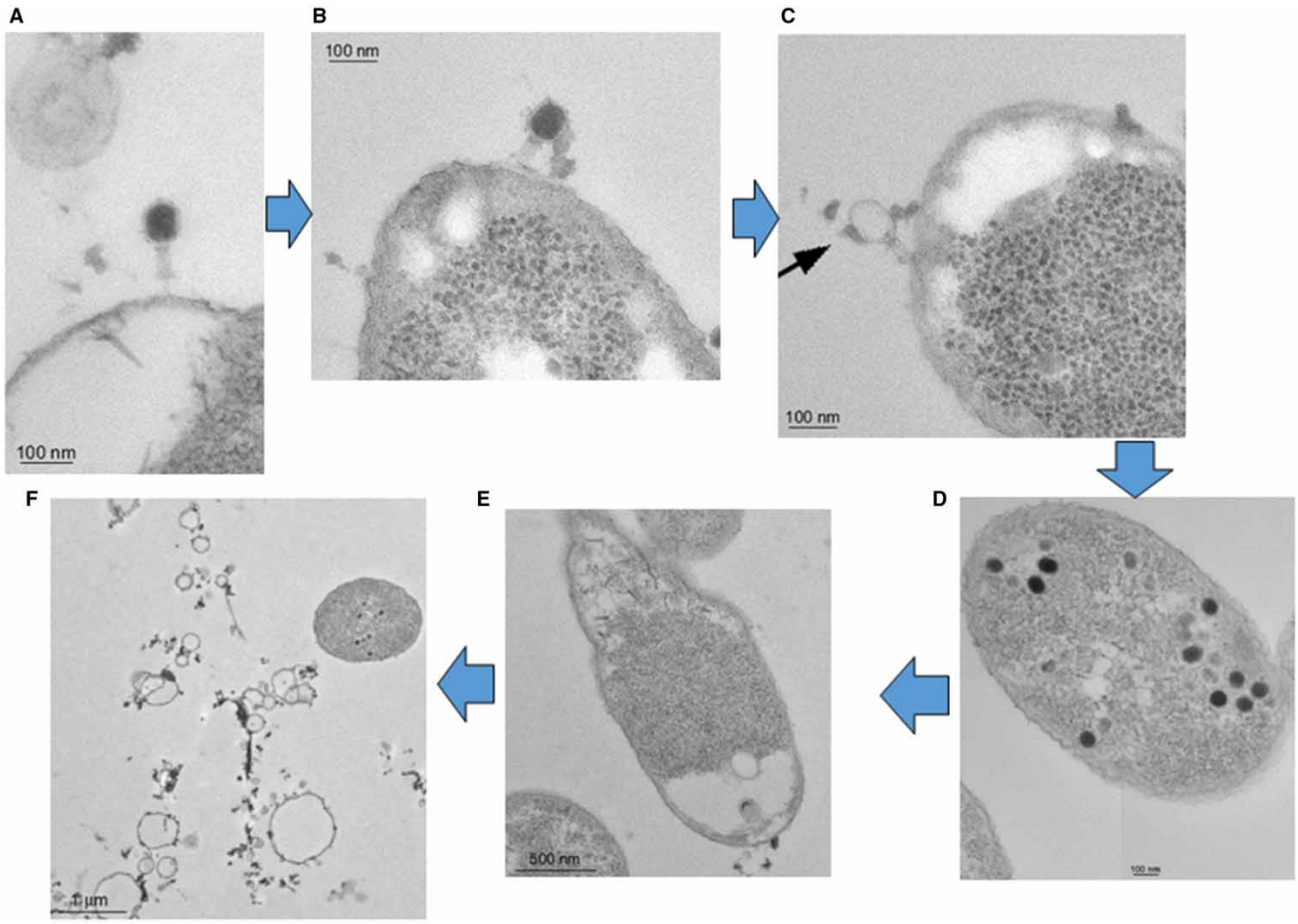

Figure 3. The principle of phage therapy: as seen *in vitro*.

Stages of phage T4 infection of *E. coli* strain K-12, starting with phage adsorption, preparation for DNA injection (**A** and **B**), after injection of phage DNA (**C**), formation of progeny phage in the infected cell (**D**) and lysis of the infected cell (**E**), leading to cell ghosts (**F**) (clockwise, from top left to bottom left, thin-section electron microscopy). Figure credit: M.-L. Dillmann, NRC.

Phages in the microbiome

Disturbances in gut microbiome maturation in young mammals and infants have been associated with negative health outcomes, ranging from growth impairment to immune maturation defects and allergy development [50]. Microbiome research is still rather descriptive and, until recently, mostly limited to bacteria and archaea. Now the gut virome, largely dominated by phages, has come into focus [51–53] and, since phages infect and lyse bacteria, this analysis has the potential to explain part of the observed dynamics and disturbances of the gut microbiota development. The Gates Foundation has issued a Global Grand Challenges call, 'Addressing newborn and infant gut health through bacteriophage-mediated microbiome engineering'. The focus is environmental enteropathy, a poorly described inflammatory condition of the gut, which might be a cause for stunting in children from developing countries [54]. The underlying idea is to use phages not only against enteric pathogens, but also against a dysbiosis of the gut microbiota. An emerging concept is that when bacterial commensals are present out of physiological proportions, they may become pathobionts and induce inflammation [55]. The idea of microbiome engineering with phages was stimulated by concepts of marine ecologists who developed the 'kill the winner' hypothesis [56]. According to this hypothesis, phages come into play when one microbial member of a given ecological niche grows to dominate due to an ecological disturbance. Passing over the replication threshold, they become the targets for phage control. If after a bout of phage infection, bacteria slip under the threshold, they escape from phage pressure. In this way, phages might play an important role in

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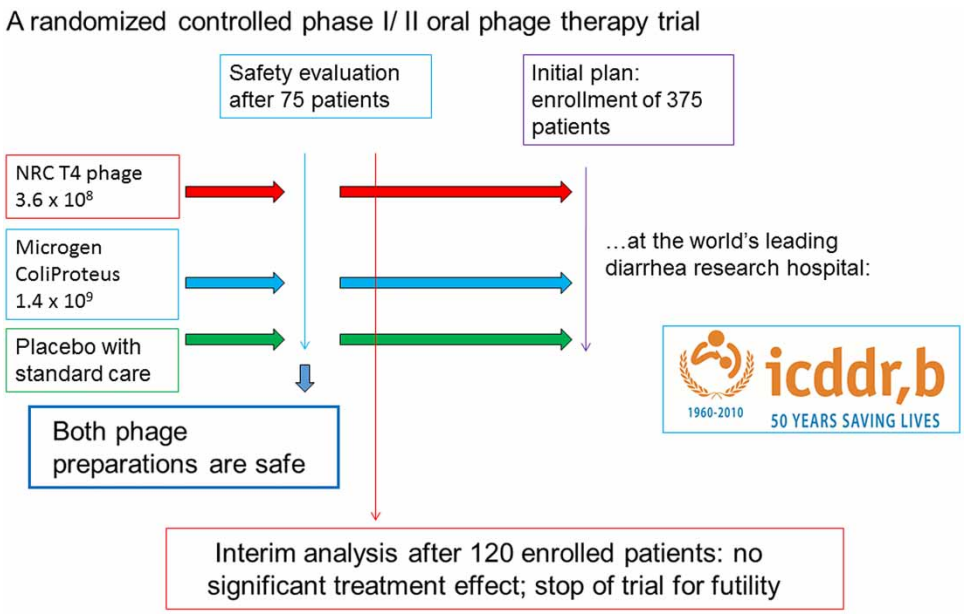

Figure 4. Flow scheme for the three-arm oral phage therapy trial at icddr,b in children hospitalized with *E. coli.* diarrhea.

Standard care in all three arms was reduced-osmolarity glucose-based oral rehydration solution supplemented with zinc.

assuring the maintenance of diversity in a bacterial population. Since microbial diversity has been postulated as an indicator of gut health, intestinal phages might have a beneficial role for gut health in young children. The future will tell whether biotechnologists can use phages as an oral adjunct to re-establish lost diversity in the gut microbiome, which would extend phage therapy into a much wider application field.

Conclusion

Phage therapy has a substantial potential as an antimicrobial agent over antibiotics since phage can be specifically targeted to defined bacterial pathogens without affecting the bystander commensal microbiota. However, the potential of phage has to be proved in controlled clinical trials to convince the medical community about its practical value. The specificity of phage is also a drawback since, to succeed, it necessitates a knowledge ranging from microbiological diagnosis of the pathogen in the individual patient to a sound literature knowledge about the disease involvement of the given pathogen. These constraints limit phage therapy to a still to be defined set of bacterial infectious diseases. Part of these drawbacks can be alleviated with complex phage cocktails covering many different pathogens. Combining phages with antibiotics might lead to interesting synergistic effects, not the least with respect to resistance development. The use of phages for gut and skin microbiome modulation needs still more basic research about the ecophysiological role of the human virome and its effect on bacterial microbiota composition.

Summary

- Since phages do not interact with human cells and as demonstrated by numerous phase I clinical trials, phages are safe for clinical use as long as temperate and transducing phages are excluded.
- Phages infect bacteria in a species-, even strain-specific way, which necessitates an adaptation of a phage preparation to the targeted pathogen and its epidemiological specificities.

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- Not all bacterial infections are suitable for a phage therapy approach. Success will be dictated by accessibility of the target bacterium in the patient and a better understanding of phage–bacterium interaction within the patient.
- Polymicrobial infections and pathogens whose involvement in disease has not been firmly proved are unlikely targets for phage therapy.
- Combining phage with antibiotic treatment offers possible synergic actions, might prevent resistance development and needs to be explored in clinical trials.
- A better definition of the role of the human virome and its impact on the bacterial microbiota is needed before one can consider using phages to modulate a dysbiotic gut or skin microbiome.

Abbreviations

CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; EMA, European Medicines Agency; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; FDA, Food and Drug Adminstration; GMP, Good Manufacturing Practice; LPS, lipopolysaccharide; LT, labile toxin; NRC, Nestlé Research Center; PAS, phage–antibiotic synergy; pfu, plaque forming unit; ST, stable toxin.

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Competing Interests

H.B. is an employee of the Nestlé Research Center, but has otherwise no financial involvement in phage therapy.

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