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Bacteriophages for managing *Shigella* in various clinical and non-clinical settings

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The control of shigellosis in humans enjoys a prominent position in the history of bacteriophage therapy. d'Herelle first demonstrated the efficacy of phage therapy by curing 4 patients of shigellosis, and several subsequent studies confirmed the ability of phages to reduce *Shigella* based infection. *Shigella* spp continue to cause millions of illnesses and deaths each year and the use of phages to control the disease in humans and the spread of the bacteria within food and water could point the way forward to the effective management of an infectious disease with global influence.

Introduction

The discovery of bacteriophages (phages) approximately 90 years ago initiated a new field of science in which these bacterial viruses were studied for their uses as antimicrobial agents.[1] The practice became known as phage therapy, which is broadly defined as the application of phages to reduce or eliminate populations of bacteria in animals. Phage therapy has now been expanded to include the use of phages to destroy bacteria on organic material destined for foods or inorganic surfaces such as food contact surfaces for example.[2] The latter two applications are now increasingly mentioned as "phage based bio-control." Regardless of the application, phage therapy is based upon several principles, namely, that phages can essentially be found everywhere that their host bacteria are present; They specifically infect bacteria and no evidence exists that phages can cross species barriers and cause infection in animals; They are natural predators of bacteria, and upon infection of their host, can replicate within short periods of time (1 h or less) increasing their numbers by several orders of magnitude; and the diversity of phages with differing physico-chemical characteristics, each possessing specificity for their host bacterial genera, species, or even individual strains, allows for extremely specific cocktails of phages to be developed which will destroy target bacteria while leaving other non-target (and in the case of humans and other higher animals, beneficial) bacteria intact.[3-5]

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In this review, these principles are expanded upon, using the case of phage based control of *Shigella* spp as an example of how phages can be a safe and effective treatment to control a bacterial disease that causes thousands of cases of global diarrheal illness each year.

Food and Waterborne Illnesses Caused by *Shigella* spp

Shigellosis, a major public-health problem in many developing countries, is caused by *Shigella* species. The shigellae cause an estimated 120 million cases and 1.1 million deaths annually around the world.[6,7] The infectious dose is as little as 100 bacterial cells,[8] and contamination occurs primarily via the fecal-oral route, with food, water, fomites, insects and direct person to person contact. Although *Shigella*-contaminated foods and drinks are often the sources of illness, secondary transmission through environmental sources cannot be ignored.[9] Most cases of shigellosis are caused by three species, with *S. dysenteriae* responsible for causing deadly epidemics within the developing world, while *S. flexneri* and *S. sonnei* account for the endemic form of the disease, particularly in industrialized nations.[8] *S. sonnei* is found in all regions of the US and is often endemic within day-care centers and communities of lower socioeconomic status,[10] where large populations reside that are often undernourished, lack proper sanitary facilities, and generally practice poor hygiene.[11]

Each species of the shigellae except *S. sonnei* can be divided into multiple serotypes,[12] and serological subdivision is usually sufficient for tracing the epidemiology of *S. dysenteriae*, *S. flexneri*, and *S. boydii*.[13] Phages have been observed to play a role in serotype differences observed among the shigellae, and have also found use in subtyping schemes. For example, O-antigen alteration and therefore serogroup modification of *S. flexneri* is conferred by temperate phages, and several serogroup converting phages have been isolated and characterized.[14] In addition, several phage typing schemes have been developed for *Shigella* spp[15] with an emphasis placed on phage typing of *S. sonnei*.[16]

Shigellosis usually presents as a locally invasive colitis. Despite their local invasiveness, the shigellae rarely cause bacteremia, and diarrheal disease caused by *S. sonnei* tends to be mild and some patients may be asymptomatic.[17] However, shigellosis occasionally causes hemolytic uremic syndrome (HUS), which is characterized by hemolytic anemia (anemia caused by destruction of red blood cells), acute kidney failure (uremia), and a low platelet

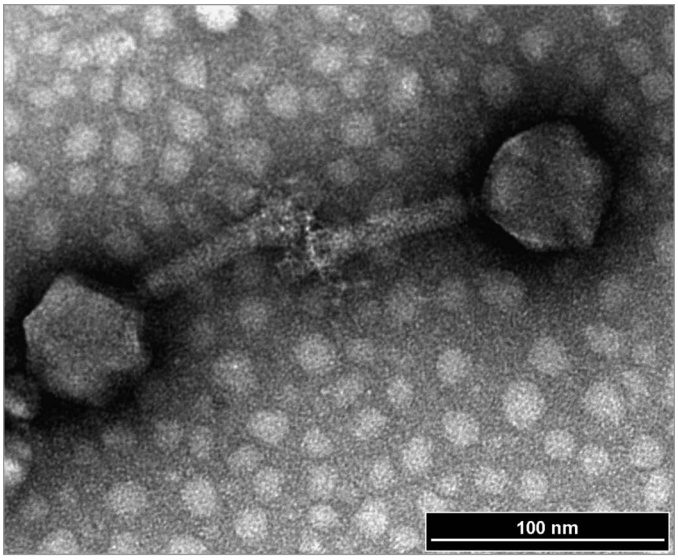

Figure 1. Transmission electron micrograph of the *Shigella* specific bacteriophage ϕSboM-AG3. The bar at the bottom left is 100 nm. TEM image courtesy of H. Anay at the University of Guelph.

count (thrombocytopenia). These manifestations result in part from the actions of Shiga toxin (Stx), which is encoded by temperate bacteriophages (see below) and produced by *S. dysenteriae* type 1.[18,19]

The Relationship between *Shigella* and *Escherichia coli*

The similarities between the shigellae and *E. coli* provide intriguing possibilities for probing the relationship between phages that infect both species. Shiga was the first to discover the dysentery bacillus (now recognized as *S. dysenteriae*) in 1898.[20] This discovery was preceded by that of Escherich, who first described *E. coli* in 1885.[21] Upon both discoveries, it became immediately clear that *Shigella* spp were biochemically and clinically distinct from *E. coli*.[17] Nevertheless, the emergence in the 1980s of *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains that, like the shigellae, cause food-and water-borne outbreaks of hemorrhagic colitis, a dysentery-like illness some-times complicated by HUS,[22] prompted a reinvestigation of the genetic relationship between these genera.[23]

The first information regarding the evolutionary relationships between *Shigella* spp and *E. coli* were provided by DNA-DNA hybridization studies which demonstrated that the shigellae and *E. coli* share > 75% nucleotide similarity. *Shigella* spp appear to be metabolically inactive biogroups of *E. coli*, and both are considered to be a single species, based on DNA homology.[24]

Of importance to the topic of this review, STEC share with *S. dysenteriae* type 1 the production of phage encoded Shiga toxin(s).[18] Since *E. coli* and *Shigella* are so closely related, it stands to reason that their phages would also be similar with respect to physico-chemical properties, and genetic makeup. This means that one can draw upon the enormous scientific literature regarding the morphological, physical, chemical, and genetic characterization of the *E. coli* phages, and apply that knowledge to the *Shigella* phages. Such an approach is immediately useful when one ponders the primary sources from which to isolate *Shigella* phages. There are multiple reports of phages that infect both *E. coli* and *Shigella*,[16,25-27,] providing important insights regarding the nature of somatic antigens on the bacterial cell surface.

Diversity and Distribution of *E. coli* and *Shigella* Specific Phages

Phages are considered to be among the most numerous and diverse entities on Earth, with an estimated 10^{30}–10^{31} phage particles within the biosphere.[28,29] The dsDNA tailed phages, which belong to the order Caudovirales, account for 95% of all the phages reported in the scientific literature.[30] Nineteen families are currently recognized that infect bacteria and archaea. Phages are widely distributed in locations populated by bacterial hosts, such as soil or the mammalian gastrointestinal tract. Sea water represents one of the densest natural sources for phages, where up to 10^8 virions per milliliter have been estimated.[29]

E. coli phages of differing morphology are commonly isolated from sewage, waste water, polluted rivers and fecal samples of humans and other animals.[31] For example, lambda-like siphophages have been isolated from stool samples obtained from healthy subjects,[32] while samples of stools from patients with diarrhea tended to contain T4-like myophages.[33] The mammalian gut appears to be the natural habitat of T4-like phages.[4]

As predicted, similar diversity has also been observed among phages that infect the shigellae. For example, *Shigella* phages exhibiting T4 like genomic and morphological features have been described[34-36] (**Fig. 1**). Other groups have described *Shigella* specific phages that belong to the Podoviridae,[37] and the Siphoviridae,[27] the latter of which is to be expected, as siphophages are known to carry and transduce the Shiga toxin genes.[38] Shigella phages have been isolated from raw sewage,[16] and have also been obtained from environmental sources. Kim et al.[36] isolated a T4-like phage from the Gap River in Korea, and showed that it specifically infected *S. sonnei*. Phage SP18 was morphologically characterized as a myophage, and phylogenetic analysis of major capsid gene (gp23) sequences classified it as a T4-like phage. The phage was able to lyse *S. sonnei*, but could not grow on *S. flexneri*, *S. boydii* or members of the genera *Escherichia* and *Salmonella*. Pyrosequencing of the SP18 phage genome revealed a 170-kb length sequence. Comparative genomic analysis showed that the enterophage JS98, isolated from human stool, is the closest relative of SP18. Both phages appear to be closely related to T4-even phages.[36] Others have isolated *Shigella* phages from environmental water sources in developing countries, where dysentery due to *Shigella* is common.[6] Akter et al.[39] isolated and characterized *Shigella* phages from environmental waters in Bangladesh. Forty-five surface-water samples were collected and tested for *Shigella* bacteria and *Shigella*-specific phages. To investigate phage specificity, different serotypes of *Shigella* strains and other enteric pathogens, including *Salmonella* spp, *Vibrio cholerae*, and *E. coli*, were tested. Isolated phages were characterized by molecular

methods including RFLP, PCR, and PFGE of the phage genome. Lytic phages were isolated that could infect one, two or multiple serotypes of *Shigella*. Based on analysis of the major capsid gene, one of the isolated phages was determined to be T4-like.

Others have used a similar approach to isolate *Shigella* phages. For example, Faruque et al.[37] isolated a phage from surface water samples from Bangladesh that specifically lyses strains of *S. dysenteriae* type 1. This phage, designated SF-9, belongs to the Podoviridae family and has a 41-kb double-stranded DNA genome. Water samples were tested to determine the prevalence of SF-9, and the results indicated that 9 of 71 (12.6%) water samples were positive for the phage. The authors concluded that phage SF-9 may have epidemiological applications as an index of the presence of *S. dysenteriae* type 1 in environmental waters. As with phages that are specific for other bacterial species, *Shigella* specific phages isolated from geographically disparate regions appear to be morphologically and genetically diverse.

In addition to sewage, and environmental waters, phages have also been isolated from a variety of agricultural environments, including soil, water, farm and food processing effluents, manure, and retail foods,[40] and foods of dairy origin.[41] The presence of phages within foods can be regarded as either beneficial (destruction of the host bacteria, which may be pathogenic to humans) or detrimental due to the ability of the phage to transduce virulence genes to host bacteria contained within the food. In an example of the presence of phages that could have potentially detrimental effects, Imamovic and Muniesa[42] recently isolated Stx phages that infect *E. coli* and *Shigella* from samples of beef and salad. Stx phages from the samples were propagated in *E. coli* C600, *E. coli* O157:H7 and *Shigella* strains and further quantified. While the use of temperate phages such as the Stx phages to control the presence of target pathogens is not recommended (see below), this report nevertheless demonstrates the possibility of isolating *Shigella* phages from food samples.

Phage-Based Control of *Shigella:* The Early Years

Summers[1] provides an excellent commentary on the birth of phage therapy. It is interesting to note that the shigellae played a major role in the discovery of phages by d'Herelle, although it is possible that he first observed the phage phenomenon as early as 1910 while studying microbiologic means of controlling an epizootic of locusts in Mexico. Even with the earlier observation, the seminal moment in the discovery and understanding of phages as "eaters of bacteria" is frequently associated with an outbreak of severe hemorrhagic dysentery among French troops stationed at Maisons-Laffitte in the summer of 1915. d'Herelle was responsible for determining the cause of the outbreak, and as part of his investigation, d'Herelle made bacterium-free filtrates from fecal samples obtained from soldiers hospitalized as a result of the outbreak, and mixed and incubated them with *Shigella* strains that were isolated from the patients. The samples were spread on bacterial agar to allow for and observe the growth of the bacteria. It was during these experiments that d'Herelle first observed the appearance of small, clear areas, which would become known as plaques.[1]

It was immediately obvious to d'Herelle that phages could be used as a treatment for bacterial dysentery. The first use of phages to treat human infections was conducted during the late summer of 1919 and consisted of 4 patients, whom d'Herelle treated with phages to cure symptoms of dysentery. On August 2nd d'Herelle injected a patient with 2 ml of an anti-dysentery phage. The patient had come to the hospital the previous day with severe dysentery and 10–12 bloody stools per day. Upon receiving the phage, the patient had three bloody stools in the afternoon, and one more during the night. By the next morning, all symptoms had disappeared.[1] This first phage trial continued in early September, when three brothers were admitted to the hospital, with very severe dysentery. That their situation was dire was reflected by the fact that their sister had died at home after only a few hours of illness. Each patient received the anti-dysentery phage treatment, and all were recovering from symptoms within 24 h.[1]

d'Herrelle's seminal experiment began a period in which the study of phage therapy of dysentery became commonplace, with many experiments reported in the scientific literature over the next 20 years. However, in many cases, these studies have been translated from other languages or excerpted in Bulletins, and salient details of the studies are often lacking. For example, Davison[43] isolated Flexner's bacillus (*Shigella flexneri*) from 10 of 12 cases of dysentery in children. Seven of eight bacterial strains tested were shown to be susceptible to bacteriophage. Phage therapy was used to treat the infections with frequent doses ranging from 5 c.c. to 1,300 c.c. being given to the patients. Seven patients received phages orally, and five were treated by enema. Five of the 12 (42%) patients survived the infection. Failure in the other cases was attributed to the fact that the therapy began too late in the course of the disease.

da Costa Cruz,[44] reported that bacteriophage treatment was clearly the best treatment for bacillary dysentery, with symptoms diminishing considerably within 4 to 8 h, and observed that the illness entered into a convalescent stage in 24 to 48 h following administration of the phage. However, there were no statistics regarding recovery and the number of cases were not reported.

Spence and McKinley[45] treated 19 out of 20 cases of shigellosis within the first week of infection, with 10 c.c. of bacteriophage administered orally t.d.s. (three times daily, probably in the morning, at midday and at dinner time). The mortality rate was observed to be 10%, and the average stay in hospital was 5.8 d. In contrast, a control group of unknown number in another hospital that did not receive the bacteriophage treatment had a mortality rate of 40% and an average stay in hospital of 12.8 d. These results lend some credence to the hypothesis expressed by Davison[43] that administering phages early in the infection is imperative in order to successfully treat the disease. The need to treat the infection early was also demonstrated when other treatments for dysentery have been employed, including serum-therapy, which when instituted late in the infection seemed to have no effect.[46] Presumably, the reason that both serum and phage therapy treatments fail when administered late in the infection is attributed to the physical effect of the disease on the intestine, with numerous intestinal lesions present late in

Table 1. Clinical Interpretation of the success and failure of patients treated with an anti-dysentery phage preparation (from Compton[46])

Score	Interpretation	Clinical relevance/description
+++	Very good	Stools reduced to 2–3 per day by the 2nd day (counting the initial day as 0), in conjunction with improvement in the general condition of the patient.
++	Good	Stools reduced to 3–4 per day by the third day, or stools reduced to 3 per day by the 4th day, with improved general condition.
+	Moderately good	Stools reduced to 4–5 per day by the 5th day.
(+)	Partial failure	Little or no change in the number of stools or in the general condition within 4 days, but ultimate recovery.
-	Failure	Death, or no change within 1 week of commencing treatment.

the disease, which decreases the chances of the intestine resuming to normal function even if the bacteria are cleared.[46]

Choudhury and Morison[47] described the treatment of 80 cases of bacterial dysentery with a polyvalent bacteriophage which was administered at a dose of 2 c.c. t.d.s. on the 1st day, and b.d.s. (twice daily, probably in the morning and evening), thereafter. The mortality rate was reported to be 4%, but no reference to controls was mentioned.

Taylor et al.[48] reported the treatment of cases in which a short interval had elapsed between onset of the disease and treatment. Two c.c. of polyvalent bacteriophage was given to 14 patients with Shiga dysentery three times daily. A mortality of 14% in the treated patients was observed, in comparison to 12% in a control group. In 6 patients who had Flexner dysentery, and who had been treated with polyvalent bacteriophage, one death was observed. A single death was also observed in the control group.

In one of the most detailed early reports, Compton[46] described the treatment of dysentery in Egypt. In 1927 the author prepared and distributed anti-dysentery bacteriophage preparations for therapeutic use to doctors in the city of Alexandria who were willing to cooperate in an experiment to obtain information on the value of phage therapy to treat dysentery. The distribution was limited to cases which had been diagnosed cytologically as bacillary dysentery. The phage preparation was polyvalent, and consisted of 4 phages including one that had been given to the author by d'Herelle and 3 others that had been isolated in Egypt. The phage preparation was developed by incubating the phages with 16 h broth cultures of 12 local strains of dysentery bacilli, including 2 strains of Shiga, 3 strains of Flexner, 6 strains of Hiss (including one Sonne), and 1 strain of Gay. Complete lysis of the cultures was observed after several hours of incubation at 37°C. After 24 h of incubation, the lysates were mixed and filtered, and the filtrate constituted the therapeutic phage preparation. It was transferred to sterile ampoules in quantities of approximately 2 c.cm. Three ampoules were typically distributed to each patient, complete with instructions on the use of the phage and a questionaire to the doctor. Approximately 200 patients received treatment, including almost 50 in 1927 and 150 in 1928, and phage preparations were returned from 92 cases. Of these, only 66 were full enough for subsequent use. The author hypothesized that the remaining cases from whom no returns were received were likely cured of the disease, since had they required further treatment, they would have returned for subsequent visits to their doctors. Assuming Compton's hypothesis to be correct, a cure rate of 108/200 (54%) was achieved. Still, it should be noted that the patients were mostly poor and left no addresses, meaning that their recovery could not be satisfactorily tracked. One overlooked aspect of phage therapy during this early period that was emphasized by Compton was the fact that providing such a population with phage probably meant to a certain extent the dissemination of anti-dysentery phage in their surroundings, which d'Herelle had previously highlighted as a way to spread the phage treatment beyond those who were directly treated.[46]

A second experiment was apparently conducted using the phage preparations that were returned from the original studies to treat 66 additional patients who had dysentery. For this experiment, the author developed a semi qualitative method of evaluating the recovery of the patients as described in **Table 1**. The results of the experiment showed that 35 of 66 patients had a very good recovery, 10 patients had good recovery, moderately good recovery was observed in 6 patients, partial failure was observed in 5 patients and failure to recover was recorded for 10 patients. Compton suggested that 4 of the 10 failures should be removed from the study, since 2 of the cases had been sick for 2 weeks before treatment, and a 3rd case was dying when the phage treatment was administered. The 4th case was ill for 2 mo before phage therapy. Removing the 4 cases would seem to make sense in light of the above mentioned comments regarding the need to begin phage therapy of dysentery as soon after infection as possible (see additional comments below). Excluding the 4 cases, and then considering the remaining 6 failures, the 5 partial failures, and the 6 moderately good results as a total group of failures, the results of the study indicated that of 62 cases, 45 were successfully treated, indicating a success rate of 72.6%.

The Compton[46] study was an important watershed moment in the early development of phage therapy because the causes of phage therapy failure were analyzed in detail. The author showed for example that the age of the patient, the duration of illness prior to phage treatment, and the bacterial flora could all influence the success or failure of the phage treatment. In particular, both early intervention and the age of the patient played an important role in the success of the treatment. Phage treatment was observed to be least successful with children under 1 year old, while it was three times as successful with children between the ages of 1 and 2, four times better with children between 2 and 4 years old, almost five times as successful when children between the ages of 4 and 10 were treated. In patients above 10 years of age, phage treatment was completely successful (**Fig. 2**).[46]

The experiments also demonstrated that there was an inverse relationship between the success of phage therapy treatment and the amount of days of infection prior to treatment. For instance, if the patient had been ill for three or less days prior to treatment, the success rate of phage therapy was approximately 90%. When treatment was delayed between four and seven days post infection, the success of treatment decreased to approximately 65%. Further decreases in treatment efficacy were observed when phage therapy commenced one to two weeks after onset (55%), and after three weeks of disease onset (50%). Beyond three weeks, and up to two months after infection, an improvement in the percentage of successful treatments was seen (**Fig. 3**).[46]

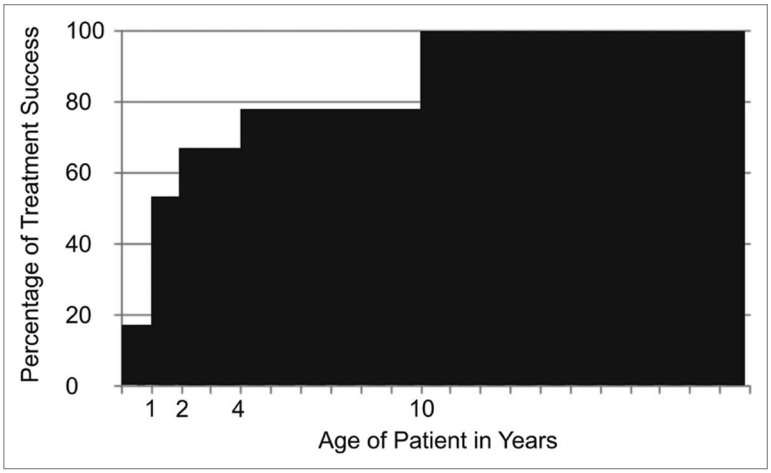

Figure 2. Graph showing the relationship between age and percentage of phage treatment success. Based on the data in the 1929 study by Compton.[46]

Bacterial flora also played a role in treatment outcome as several of the causes of *Shigella* dysentery in children under 2 years of age may have actually been cases of *Bacillus proteus*, which would have rendered the phage treatment inactive.[46]

Other, less documented studies continued after Compton's[46] work. In 1930, Riding[49] reported 60 cases of bacillary dysentery over a 2 year period in Khartoum, Republic of Sudan. Records were only maintained for 48 of the cases. Thirty-five were treated orally with a bacteriophage received from d'Herelle, and 13 cases were controls. The author first tested the efficacy of the phage in vitro against strains of bacteria that were isolated from patients, including Shiga, Flexner, Flexner Y, Sonne and Schmitz. Forty eight of the 60 strains of dysentery bacilli isolated were susceptible to the phage, and 41 of 43 strains of serologically confirmed *B. dysenteriae* were susceptible to the phage. Following the in vitro experiment, the phage treatment was administered to the patients. It was concluded that phage therapy was not effective in eliminating the infection when compared with controls. However, it is difficult to interpret what endpoint was selected to determine phage efficacy. Furthermore, while 35 cases were apparently given the phage treatment, data are presented for 39 patients. Data was only presented for 8 of the 13 control cases, and some of the control cases received alternative treatments such as saline and serum treatment which could have affected disease outcome. If one assumes that the presence of normal stools in patients denoted a clearing of infection, then normal stools were observed in 14/39 (36%) of phage treated cases as compared with 4 of 8 (50%) control cases. The average day following hospital admission that normal stools were observed was 11.6 d in the phage treated patients and 12.5 d in the controls. Riding suggested several reasons why the phage treatment was unsuccessful in clearing the infection including the probability that oral ingestion leads to quick elimination or destruction of the phage by the human body, the fact that the contents of the intestines during an infection of dysentery do not appear to be a suitable medium for the process of bacteriophagy, and the clinical course of acute bacillary dysentery is not altered by the oral administration of bacteriophage. It is impossible to comment on these findings as no information was reported regarding dose, duration, and the number of times the phage preparation was administered, all of which would affect the clinical results. Also, most of the patients had been ill for some days before treatment was started, which as previously discussed can also affect the clinical outcome.

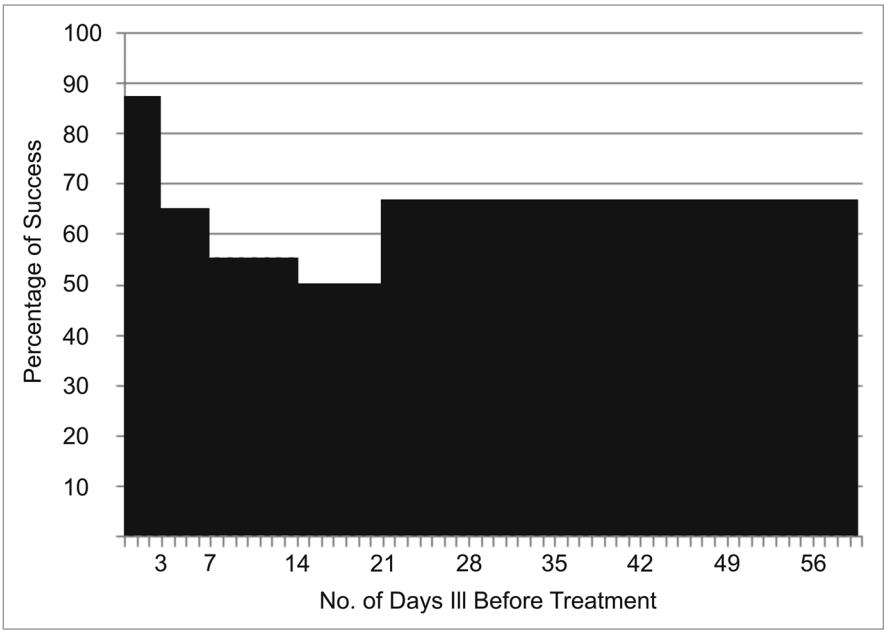

Figure 3. Graph showing the relationship between the number of days of illness prior to treatment and the percentage of phage treatment success. Based on the data in the 1929 study by Compton.[46]

Querangal des Essarts[50] treated 190 cases of bacillary dysentery during a 29 d outbreak on board two ships at Brest, France. Fifty-nine of the cases were confirmed, with 16 cases of Shiga, 38 cases of Flexner and 5 cases of paradysentery. One hundred and 80 five of the cases were treated with polyvalent Shiga-Flexner bacteriophage, prepared from convalescent stools. The phage treatment was administered orally, with 5 c.c. in alkaline water administered on the 1st day, 10 c.c. on the 2nd and 3rd days, and 5 c.c. the 4th day. The author reported "remarkable" results. For example, after the 2nd or 3rd day, blood and mucus disappeared, and after 4 d the stool appeared normal microscopically. There were no controls in the study. The author also claims to have arrested an outbreak of dysentery among infants at a holiday camp by prophylactic administration of bacteriophage. As with the first report, there were no controls.

Kessel and Rose[51] reported 68 cases of dysentery. Thirty-four cases were given bacteriophage treatment, while the other 34 cases served as controls. A phage preparation was given at a dose of 3 to 5 c.c. orally, every 12 h. There were three deaths reported in the control group and four deaths in the treated group. The period of hospitalization was slightly but not significantly lower in the treated group.

Johnston et al.[52] observed that bacteriophage therapy did not affect the clinical course of dysentery when 70 infants under 2 years of age were treated with 1 ounce of bacteriophage at hourly intervals. The treatment failure may have been due to the fact that only 17 out of 94 (18%) bacterial strains tested in vitro were susceptible to the bacteriophage employed.

Vaill and Morton[53] treated 200 cases of dysentery with bacteriophage in New Jersey, USA, but only report on the records of 22 cases. Of the recorded cases, only one case is cited as a control. The report is interesting because the authors used a strain specific bacteriophage which had been adapted to the patient's strain of bacillus by serial passage, while at the same time they emphasize the importance of beginning phage therapy as soon as possible after the onset of the disease. No explanations are given regarding the reconciliation of these two mutually exclusive statements.

Murray[54] treated 146 cases of bacillary dysentery with bacteriophage for 6 years, between 1931 and 1937. The treatment usually took 2 weeks, and was seldom longer than 3 weeks. No controls are reported, nor are there any details recorded with respect to susceptibility of the phage on isolated bacteria. No details regarding origin and characteristics of the phage are recorded. Notwithstanding the lack of these important details, the author concludes that bacteriophage is "by far the best method of treating bacillary dysentery and that failure in treatment can be attributed to the fact that a reliable bacteriophage has not been used." Murray[54] also recommended that a controlled series of experiments will be required to prove the value of phage therapy.

Haler[55] reported the treatment of an epidemic of dysentery in a home for children that housed 32 children and 17 staff. Seven cases of Sonne infection were observed, but the author also reported an atypical organism which was believed to have evolved from the Sonne bacillus by the action of the bacteriophage. No experimental evidence was given for this conclusion. Everyone was administered bacteriophage (dose unknown) three times daily for 2 weeks, followed by a single daily dose afterwards (duration unknown). The epidemic ceased 2 d after bacteriophage administration and no cases were observed for a year. As with many of the previous reports, there were no controls and the author concludes that the "the cessation of the epidemic may have been a coincidence."

Collectively, these reports reveal much diversity in result and conclusions. Comparisons of the studies are impossible due to lack of information regarding concentration of phage, numbers of different phages employed, method of preparation, method of administration, and the fact that in many of the reports no controls were included.

Phage Based Control of Dysentery in the Military

Throughout history, military populations have experienced great morbidity and mortality due to gastroenterological disease. Dysentery remained an important cause of mortality through World War I and into World War II.[56] Dysentery was a big problem in the trenches due to poor hygiene and contaminated water supplies. It should not be surprising that phage therapy was investigated by military authorities as a method by which to treat outbreaks of the disease. Guthof[57] shared the report of a battalion in a German infantry regiment that was suffering from bacillary dysentery and subsequently treated with Dysentery Polyfagen (polyvalent phage). Fifty two adults showed good recovery within 2 to 4 d, and three children with severe infections also recovered. No controls were mentioned.

The British army conducted four small-scale trials of phage therapy in the Middle East, two of which were unpublished and did not report promising results. The third trial[58] was published in the British Medical Journal and also reported unconvincing results. The fourth trial was a carefully controlled experiment in which 32 cases were enrolled, of which 18 were in the control series, and 14 were treated by bacteriophage. The results of this study showed that the bacteriophage group made slightly better progress toward cessation of the infection than the control group. However the author concluded that the difference was so small that had an additional dozen cases been treated, the result might easily have been reversed.[59]

Perhaps in relation to the Vaill and Morton[53] report, during which a reference regarding the need to adapt the phage preparation to the specific bacterial strain to be treated was made, Kliewe and Helmreich[60] emphasized the importance of ensuring that the bacteriophage used to treat a dysentery infection is potent against local strains of bacteria. In Poland, it was observed that many of the local strains of dysentery bacilli were not susceptible to German bacteriophages. An evaluation of the prophylactic value of locally prepared bacteriophage mixtures was accomplished by giving 113 soldiers a dose of sodium bicarbonate followed by 10 c.c. of the phage mixture in half a cup of tea or coffee on three successive mornings. Two hundred and 50 men of the same unit served as untreated controls. Over the following 8 weeks, no cases of dysentery developed among the 113 bacteriophage-treated men, while ten cases occurred among the controls. Furthermore,

phage therapy was observed to be particularly effective in cases of mild or moderately severe Flexner Y dysentery. However, in cases of severe illness there was frequently an exacerbation of symptoms and only occasionally improvement. Still, 16 carriers were cured of disease after they had received bacteriophage therapy on 3 successive days.

Phage-Based Control of *Shigella*: Later Studies

In 1942, interest in the use of phages to treat clinical disease led the US National Research Council/CMR to sponsor a variety of successful and interesting animal studies with phages targeting *S. dysenteriae*. In one such study, Dubos et al.[61] demonstrated in vivo lysis of bacteria, and multiplication of phages as being protective against experimental infection of mice with *S. dysenteriae*. These experiments showed that phages can be carried to wherever they are needed and multiply there, when in the presence of an appropriate bacterial host, at levels that are far higher than those in blood. When 10^5 phage were applied intraperitoneally, approximately 10^2 phage arrived in the brain of control mice. When the experiments were conducted with mice that were intracerebrally inoculated with *Shigella*, a massive increase of phage was observed in the brain to 10^9 phage after 8 h, indicating amplification of phage in vivo in a tissue that is protected by a tight barrier. When 10^9 phage were injected intraperitonally, phages appeared with titers as high as 10^7 in the blood, but began to decrease several hours after injection. A survival rate of 72% was achieved if the mice were treated with 10^7 to 10^9 phages, as compared with only 3.6% with no treatment. When phages that were heat-inactivated or that did not target the infecting bacteria were injected into the mice, no protective effects were observed. Morton and Perez-Otero[62] reported that phages multiply within animals when in the presence of their bacterial hosts, with their demonstration of an increase in phage concentration, in vivo, during experimental infections with *Shigella paradysenteriae*. If the mice received a *Shigella* strain that was not susceptible to the phage in vitro, the in vivo phage amplification was not observed.[62] It was also demonstrated that the protective effect of the phage could be diluted. Limiting efficiency was reached at a 10:1 bacterium-phage injection ratio. The treatment could be delayed for up to 3 h after bacterial challenge, and phage treatment could precede the bacterial challenge by 4 d and still prevent mortality.[63]

Another attempt to treat human shigellosis with phages was made by the Hirszfeld Institute in 1957.[3] The Institute was instrumental in developing and producing phages for the treatment of many diseases, including infections caused by antibiotic resistant bacteria that were refractory to conventional treatment with antibiotics.[3] Others have also described the efficacy of phages against antibiotic-resistant members of the Enterobacteriaceae, including the genera *Escherichia*, *Proteus*, *Salmonella*, *Shigella*, *Serratia*, and *Klebsiella,* as well as multidrug resistant *Pseudomonas* and Streptococci.[64] Miliutina and Vorotyntseva[65] combined phage therapy and antibiotics to treat shigellosis and salmonellosis and reported that the combination of phages and antibiotics was effective in treating cases where antibiotics alone were ineffective.

An extensive clinical evaluation of the efficacy of phage therapy as a treatment for shigellosis was conducted in Tbilisi, Georgia, during the 1960s.[66] The study lasted for 109 d and involved 30,769 children between the ages of 6 mo and 7 years. In the study, children on one side of the streets (17,044 children) were given dried *Shigella* phages in tablet form orally once every 7 d, while the children on the other sides of the streets (13,725 children) received a placebo. The children were monitored weekly at the time of receiving the phages. Fecal samples from all children exhibiting gastrointestinal disorders were assessed for the presence of *Shigella* spp and other, unspecified diarrhea-causing bacteria. The results of this study showed that the incidence of dysentery was 3.8-fold greater in the untreated children. Furthermore, based on bacterial-culture confirmed cases, the incidence of dysentery was 2.6-fold greater in the untreated children. One surprising conclusion from the study was that there was an overall 2.3-fold reduction in diarrheal diseases of unknown origin among children treated with the phages relative to the untreated group. The reason for this is unclear but may be partly due to the ability of the phage preparation to destroy other non-*Shigella* bacterial gastrointestinal pathogens. As previously described, many phages have been isolated that can infect bacterial isolates from closely related genera including *Shigella* and *Escherichia*.

There are other examples of the use of phage therapy against shigellosis. For example, Mikeladze and coworkers[67] described the treatment of acute colitis caused by *Shigella* or *Salmonella*. One ampoule (5 mL) of a phage product called bacti-intestiphage was diluted in boiled and cooled water and was administered orally to infected patients every 2 h. Each patient ingested a total of 8–10 ampoules of the phage product, while placed on a liquid diet. Phage treatment led to a decrease in fever and an improvement in the patient's symptoms. Even when patients received treatment late in the infection, considerable improvement was still observed, although the colitis tended to persist. When a second series of ampoules was ingested following a day of rest, further decrease in symptoms was observed. The mortality rate of patients with dysentery was reduced by 50% of what is typically observed, while all patients that had colitis were cured.

Finally, phage-based prophylactic treatment of shigellosis resulted in a 10-fold decreased incidence of dysentery among patients that received phages.[68]

Collectively, these later studies have established the use of phage therapy as a viable option to treat gastrointestinal distress, and specifically show the efficacy of the treatment against *Shigella*.

Much less attention has been afforded to the use of phages to control of *Shigella* in foods. Still, a recent study indicates that phages can be effective at reducing the presence of *Shigella* in poultry. Zhang and colleagues[69] recently investigated the ability of *Shigella* specific phages to inhibit the growth of *Shigella* spp in ready-to-eat chicken. Samples of spiced chicken were inoculated with individual *Shigella* species (1×10^4 cfu/g) or a cocktail of *S. flexneri* 2a, *S. dysenteriae*, and *S. sonnei* at a concentration of 3×10^4 cfu/g. Phages, either individually or as a cocktail, were added to the chicken samples at concentrations of 1×10^8 PFU/g or 3×10^8 PFU/g, respectively, and samples were incubated at

4°C for 72 h. The authors noted that the application of a higher concentration of phages (3×10^8 PFU/g) led to more effective bacterial reductions. The phage cocktail could reduce bacterial concentrations by up to 2 $\log_{10}$/g after 48 h incubation when treated with the cocktail, and after 72 h, the bacterial host was undetectable. Similar results were obtained when a single phage was used. The results indicated that *Shigella* specific phages can effectively reduce or eliminate the presence of *Shigella* spp in ready-to-eat chicken products.

Clinical Safety of Phage Therapy

No discussion of phage based control of bacterial pathogens is complete without a discussion of the safety of the approach. Several safety issues have been raised over the years with respect to the use of phages as a therapeutic, including questions regarding phage-phage recombination[70] and the presence of toxin genes within phages. One safety issue that is less discussed, is the fact that a number of human bacterial pathogens owe their virulence factors to prophages integrated into the bacterial genome. This is true for a number of foodborne pathogens that are members of the Enterobacteriaceae including *Shigella* and *E. coli*, which contain prophages encoding a major virulence factor, the Shiga-like toxin. O-antigen modification (serotype conversion) in *S. flexneri*, which is an important virulence determinant, is also conferred by temperate bacteriophages.[71] Another member of the Enterobacteriaceae, the salmonellae, often carry prophages that confer antibiotic resistance.[72] *Vibrio cholera*, a major cause of diarrheal illness worldwide,[73] causes illness through production of a (cholera) toxin, which is encoded by a prophage.[73] A survey of phage and prophage genomes showed that many siphophages contain proven or potential virulence factors.[74,75] These examples explain why temperate phages should not be selected for phage therapy. As previously discussed, the immense knowledge generated from the study of phages that infect *E. coli* can be directly applied when evaluating phages to develop appropriate cocktails for control of *Shigella*.

Conclusion

The use of phages to control bacterial dysentery by d'Herelle, and the subsequent literature detailing the efforts of other workers in this area has highlighted some of the successes and failures of early phage therapeutic approaches. The use of phages to control bacterial infections has progressed to the point where effective methods have been developed that address the issues of broad bacterial infectivity and phage resistance. As such, less emphasis is placed on the need to adapt phage preparations to specific strains of bacteria isolated from each patient, as was described in some of the early phage literature. These methods include the combination of broad host range phages, and phages that can alter their host range in situ[76] within cocktails that can be used to treat bacterial disease. The use of properly designed phage treatments represents a practical and safe solution to control presence of established bacterial pathogens, and the rapid emergence of new infectious diseases. As interest in phage therapy has increased, so to have well designed, blinded studies, which have answered many of the questions derived from the early phage studies. One potential challenge to the advance of phage therapy is regulation of the treatment. For example, all of the phages in a given cocktail will need to be appropriately characterized before they can be used in clinical treatment; fortunately, the constant improvement of rapid and cost effective genome sequencing technologies ensures that all phages used in phage therapy approaches will be properly assessed for safety. The use of rational and logical methods to produce phage products, the ever growing number of peer-reviewed publications[77-83] that indicate the safety of clinical phage use, and the effective use of phages as antimicrobials, as well as the growing number of commercialized phage products will ensure that the interest and enthusiasm in phage therapy continues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Summers WC. Felix d'Herelle and the origins of molecular biology. Yale University Press 1999; New Haven, Conn
2. Abuladze T, Li M, Menetrez M, Dean T, Senecal A, Sulakvelidze A. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef with *Escherichia coli* 0157:H7. Appl Environ Microbiol 2008; 20:6230-8; PMID:18723643; http://dx.doi.org/10.1128/AEM.01465-08
3. Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. Antimicrob Agents Chemother 2001; 45:649-59; PMID:11181338; http://dx.doi.org/10.1128/AAC.45.3.649-659.2001
4. Brüssow H. Phage therapy: the *Escherichia coli* experience. Microbiology 2005; 151:2133-40; PMID:16000704; http://dx.doi.org/10.1099/mic.0.27849-0
5. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. Bacteriophage 2011; 1:66-85; PMID:22334863; http://dx.doi.org/10.4161/bact.1.2.15845
6. Niyogi SK. Shigellosis. J Microbiol 2005; 43:133-43; PMID:15880088
7. Shigellosis: disease burden, epidemiology and case management. Wkly Epidemiol Rec 2005; 80:94-9; PMID:15807074
8. Phalipon A, Sansonetti PJ. Shigella's ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? Immunol Cell Biol 2007; 85:119-29; PMID:17213832; http://dx.doi.org/10.1038/sj.icb7100025
9. Levine OS, Levine MM. Houseflies (*Musca domestica*) as mechanical vectors of shigellosis. Rev Infect Dis 1991; 13:688-96; PMID:1925289; http://dx.doi.org/10.1093/clinids/13.4.688
10. Eichner ER, Gangarosa EJ, Goldsby JB. The current status of shigellosis in the United States. Am J Public Health Nations Health 1968; 58:753-63; PMID:4868332; http://dx.doi.org/10.2105/AJPH.58.4.753
11. Gangarosa EJ, DuPont HL. Bacillary dysentery, In H. F. Conn and R. B. Conn.,Jr., (ed.), Current diagnosis 1973; W. B. Saunders Publishing Co., Philadelphia. p. 203-204
12. von Seidlein L, Kim DR, Ali M, Lee H, Wang XY, Thiem VD, et al. A Multicentre Study of *Shigella* Diarrhoea in Six Asian Countries. Disease Burden, Clinical Manifestations, and Microbiology 2006; 3:1556-69
13. Centers for Disease Control and Prevention. *Shigella* surveillance report no. 36, 1st and 2nd quarters 1975; Center for Disease Control and Prevention, Atlanta
14. Allison GE, Verma NK. Serotype-converting bacteriophages and O-antigen modification in *Shigella flexneri*. Trends Microbiol 2000; 8:17-23; PMID:10637639; http://dx.doi.org/10.1016/S0966-842X(99)01646-7
15. Bergan T. Chapter V: Bacteriophage Typing of *Shigella*. Methods in Microbiology 1979; 13:177–286
16. Pruneda RC, Farmer JJ 3rd. Bacteriophage typing of Shigella sonnei. J Clin Microbiol 1977; 5:66-74; PMID:319112
17. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Win WC Jr. The Enterobacteriaceae. Diagnostic Microbiology, Fourth Edition. 1992. p105-184. J.B Lippincott Company, Philadelphia
18. Sansonetti PI. *Shigella* plays dangerous games. ASM News 1999; 65:611-7
19. Keusch GT. The rediscovery of Shiga toxin and its role in Human Illness. Infect Dis Clin Pract 1997; 6:533-6; http://dx.doi.org/10.1097/00019048-199706080-00008
20. Trofa AF, Ueno-Olsen H, Oiwa R, Yoshikawa M. Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. Clin Infect Dis 1999; 29:1303-6; PMID:10524979; http://dx.doi.org/10.1086/313437

21. Escherich T. The intestinal bacteria of the neonate and breast-fed infant. 1885. Rev Infect Dis 1989; 11:352-6; PMID:2649968; http://dx.doi.org/10.1093/clinids/11.2.352
22. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. N Engl J Med 1995; 333:364-8; PMID:7609755; http://dx.doi.org/10.1056/NEJM199508103330608
23. Johnson JR. Shigella and Escherichia coli at the crossroads: machiavellian masqueraders or taxonomic treachery? J Med Microbiol 2000; 49:583-5; PMID:10882082
24. Brenner DJ. 1984. Family I. Enterobacteriaceae, p. 408-420. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore, Md
25. Chang HW, Kim KH. Comparative genomic analysis of bacteriophage EP23 infecting *Shigella sonnei* and *Escherichia coli.* J Microbiol 2011; 49:927-34; PMID:22203555; http://dx.doi.org/10.1007/s12275-011-1577-0
26. Goodridge L, Gallaccio A, Griffiths MW. Morphological, host range, and genetic characterization of two coliphages. Appl Environ Microbiol 2003; 69:5364-71; PMID:12957924; http://dx.doi.org/10.1128/AEM.69.9.5364-5371.2003
27. Strauch E, Lurz R, Beutin L. Characterization of a Shiga toxin-encoding temperate bacteriophage of *Shigella sonnei.* Infect Immun 2001; 69:7588-95; PMID:11705937; http://dx.doi.org/10.1128/IAI.69.12.7588-7595.2001
28. Hatfull G. Bacteriophages: nature's most successful experiment. Microbiology Today 2008; November:188-91
29. Suttle CA. Viruses in the sea. Nature 2005; 437:356-61; PMID:16163346; http://dx.doi.org/10.1038/nature04160
30. McAuliffe O, Ross RP, Fitzgerals GF. The New Phage Biology: From Genomics to Applications. In Mc Grath S and van Sinderen D (editors) Bacteriophage: Genetics and Molecular Biology (1st ed.) 2007. Caister Academic Press, Norfolk, Engand. pp 1-42
31. Ackermann HW, Nguyen TM. Sewage coliphages studied by electron microscopy. Appl Environ Microbiol 1983; 45:1049-59; PMID:6847179
32. Dhillon TS, Dhillon EK, Chau HC, Li WK, Tsang AH. Studies on bacteriophage distribution: virulent and temperate bacteriophage content of mammalian feces. Appl Environ Microbiol 1976; 32:68-74; PMID:987749
33. Furuse K. Distribution of coliphages in the general environment: general considerations. In Phage Ecology 1987; Edited by S. M. Goyal, C. P. Gerba & G. Bitton. New York: Wiley
34. Zamchuk LA, Nikolozishvili MT. Funktsional'nye vzaimodeĭstviia genomov fagov Shigella sonnei i faga Escherichia coli T4 pri smeshannoĭ infektsii. Genetika 1979; 15:1767-74; PMID:387521
35. Anany H, Lingohr EJ, Villegas A, Ackermann HW, She YM, Griffiths MW, et al. A Shigella boydii bacteriophage which resembles Salmonella phage ViI. Virol J 2011; 8:242-53; PMID:21595934; http://dx.doi.org/10.1186/1743-422X-8-242
36. Kim KH, Chang HW, Nam YD, Roh SW, Bae JW. Phenotypic characterization and genomic analysis of the Shigella sonnei bacteriophage SP18. J Microbiol 2010; 48:213-22; PMID:20437154; http://dx.doi.org/10.1007/s12275-010-0055-4
37. Faruque SM, Chowdhury N, Khan R, Hasan MR, Nahar J, Islam MJ, et al. *Shigella dysenteriae* type 1-specific bacteriophage from environmental waters in Bangladesh. Appl Environ Microbiol 2003; 69:7028-31; PMID:14660345; http://dx.doi.org/10.1128/AEM.69.12.7028-7031.2003
38. Strockbine NA, Marques LR, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986; 53:135-40; PMID:3522426
39. Akter M, Azmi IJ, Mondol AS, Aslam M, Dutta DK, Endtz HP, et al. Characterization of *Shigella*-specific Bacteriophages Isolated from Environmental Waters in Bangladesh. 12th Annual Scientific Conference (ASCON XII) Health Systems Research: People's Needs First. 9-12 February 2009, ICDDR,B, Dhaka, Bangladesh Abstract 174 (150)
40. Greer GG. Bacteriophage control of foodborne bacteria. J Food Prot 2005; 5:1102-11
41. Brüssow H. Phages of dairy bacteria. Annu Rev Microbiol 2001; 55:283-303; PMID:11544357; http://dx.doi.org/10.1146/annurev.micro.55.1.283
42. Imamovic L, Muniesa M. Quantification and evaluation of infectivity of shiga toxin-encoding bacteriophages in beef and salad. Appl Environ Microbiol 2011; 77:3536-40; PMID:21441341; http://dx.doi.org/10.1128/AEM.02703-10
43. Davison WC. The Bacteriolysant Therapy of Bacillary Dysentery in Children: Therapeutic Application of Bacteriolysants; d'Herelle's Phenomenon. Am J Dis Child 1922; 23:531-4
44. da Costa Cruz J. Le traitement des dysenteries bacillaires par le bacteriophage. Compt. rend. Soc Biol 1924; 91:845-6
45. Spence RC, McKinley EB. Therapeutic Value of Bacteriophage in Treatment of Bacillary Dysentery. South Med J 1924; 17:563-8; http://dx.doi.org/10.1097/00007611-192408000-00005
46. Compton A. Anti-dysentery Bacteriophage in the Treatment of Bacillary Dysentery: Record of 66 Cases Treated, with Inferences. Lancet 1929; 2:273-5; http://dx.doi.org/10.1016/S0140-6736(01)02171-7
47. Choudhury BKP, Morison J. The Spread of Dysentery in a Khasi Village and Its Treatment with Bacteriophage. Ind Med Gaz 1929; 64:66-7
48. Taylor J, Greval SDS, Thant U. Bacteriophage in Bacillary Dysentery and Cholera. Indian J Med Res 1930; 18:117-36
49. Riding D. Acute Bacillary Dysentery in Khartoum Province, Sudan, with Special Reference to Bacteriophage Treatment: Bacteriological Investigation. J Hyg (Lond) 1930; 30:387-401; PMID:20475071; http://dx.doi.org/10.1017/S0022172400010512
50. Querangal des Essarts J. Le bactériophage dans une épidémie de dysenterie bacillaire (Applications thérapeutiques et prophylactiques). Bull Soc Path Exot 1933; 26:979-81
51. Kessel JF, Rose EJ. Bacteriophage Therapy in Bacillary Dysentery of the Flexner Type. Ann Intern Med 1933; 6:1193-9; http://dx.doi.org/10.7326/0003-4819-6-9-1193
52. Johnston M, M' Ebbs JH, Kaake MJ. Bacteriophage Therapy in Acute Intestinal Infection (Summer Diarrhea). Can Public Health J 1933; 24:443-6
53. Vaill S, Morton GL. Bacteriophage Therapy of Bacillary Dysentery. J Lab Clin Med 1937; 22:594-600
54. Murray JE. The Treatment of Bacillary Dysentery with Bacteriophage. Practitioner 1938; 141:199-201
55. Haler D. Use of the Bacteriophage in an Outbreak of Dysentery. Br Med J 1938; 2:698-700; PMID:20781774; http://dx.doi.org/10.1136/bmj.2.4056.698
56. Philbrook FR, Gordon JE. Army Experience with Diarrheal Disorders before World War II in Preventive Medicine in SB Hayes, JB Coates, EC Hoff and PM Hoff (Eds.) Communicable Diseases, Transmitted Chiefly Through Respiratory and Alimentary Tracts. U.S. Army Medical Department Office of Medical History. 2009. Available at http://history.amedd.army.mil/booksdocs/wwii/PM4/DEFAULT.htm
57. Guthof O. Die Behandlung der Ruhr bei der Truppe mit Bakteriophagen, Deutsche med. Wchnschr 1941; 67:375-6; http://dx.doi.org/10.1055/s-0028-1119086
58. Wilson DC, Jameson JE. British medical Journal 1942 July, p. 81. As referenced in Boyd JSK, Portnoy B. Bacteriophage Therapy in Bacillary Dysentery. Trans R Soc Trop Med Hyg 1944; 37:243-62
59. Boyd JSK, Portnoy B. Bacteriophage Therapy in Bacillary Dysentery. Trans R Soc Trop Med Hyg 1944; 37:243-62; http://dx.doi.org/10.1016/S0035-9203(44)90037-4
60. Kliewe H, Helmreich W. Ueber Ruhrbakteriophagen, München. med. Wchnschr 1941; 88:617-9
61. Dubos RJ, Straus JH, Pierce C. The multiplication of bacteriophage in vivo and its protective effects against an experimental infection with Shigella dysenteriae. J Exp Med 1943; 78:161-8; PMID:19871319; http://dx.doi.org/10.1084/jem.78.3.161
62. Morton HE, Perez-Otero JE. The increase of bacteriophage in vivo during experimental infections with *Shigella paradysenteria*, Flexner, in mice. J Bacteriol 1945; 49:237-44; PMID:16560915
63. Morton HE, Engley FBJ. The protective action of dysentery bacteriophage in experimental infections in mice. J Bacteriol 1945; 49:245-55; PMID:16560916
64. Kumari S, Harjai K, Chhibber S. Topical treatment of *Klebsiella pneumoniae* B5055 induced burn wound infection in mice using natural products. J Infect Dev Ctries 2010; 4:367-77; PMID:20601788
65. Miliutina LN, Vorotyntseva NV. [Current strategy and tactics of etiotropic therapy of acute intestinal infections in children]. Antibiot Khimioter 1993; 38:46-53; PMID:8060185
66. Babalova EG, Katsitadze KT, Sakvarelidze LA, Imnaishvili NS, Sharashidze TG, Badashvili VA, et al. Preventative value of dried dysentery bacteriophage. Zh Mikrobiol Epidemiol Immunobiol 1968; 2:143-5
67. Mikeladze C, Nemsadze E, Alexidze N, Assanichvili T. Sur le traitement de la fievre typhoide et des colites aigues par le bacteriophage de d'Herelle. La Médecine 1936; 17:33-8
68. Anpilov LI, Prokudin AA. Preventive effectiveness of dried polyvalent Shigella bacteriophage in organized collective groups. Voenno-Med Zh 1984; 5:39-40
69. Zhang H, Wang R, Bao H. Phage inactivation of foodborne *Shigella* on ready-to-eat spiced chicken. Poult Sci 2013; 92:211-7; PMID:23243250; http://dx.doi.org/10.3382/ps.2011-02037
70. Repoila F, Tétart F, Bouet JY, Krisch HM. Genomic polymorphism in the T-even bacteriophages. EMBO J 1994; 13:4181-92; PMID:8076614
71. Allison GE, Verma NK. Serotype-converting bacteriophages and O-antigen modification in *Shigella flexneri.* Trends Microbiol 2000; 8:17-23; PMID:10637639; http://dx.doi.org/10.1016/S0966-842X(99)01646-7
72. Schmieger H, Schicklmaier P. Transduction of multiple drug resistance of *Salmonella enterica* serovar typhimurium DT104. FEMS Microbiol Lett 1999; 170:251-6; PMID:9919675; http://dx.doi.org/10.1111/j.1574-6968.1999.tb13381.x
73. Faruque SM, Albert MJ, Mekalanos JJ. Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae. Microbiol Mol Biol Rev 1998; 62:1301-14; PMID:9841673
74. Canchaya C, Proux C, Fournous G, Bruttin A, Brüssow H. Prophage genomics. Microbiol Mol Biol Rev 2003; 67:238-76; PMID:12794192; http://dx.doi.org/10.1128/MMBR.67.2.238-276.2003
75. Boyd EF, Brüssow H. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. Trends Microbiol 2002; 10:521-9; PMID:12419617; http://dx.doi.org/10.1016/S0966-842X(02)02459-9
76. Fogg PC, Rigden DJ, Saunders JR, McCarthy AJ, Allison HE. Characterization of the relationship between integrase, excisionase and antirepressor activities associated with a superinfecting Shiga toxin encoding bacteriophage. Nucleic Acids Res 2011; 39:2116-29; PMID:21062824; http://dx.doi.org/10.1093/nar/gkq923
77. Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol 2010; 28:591-5; PMID:20810181; http://dx.doi.org/10.1016/j.tibtech.2010.08.001

78. Merril CR, Scholl D, Adhya SL. The prospect for bacteriophage therapy in Western medicine. Nat Rev Drug Discov 2003; 2:489-97; PMID:12776223; http://dx.doi.org/10.1038/nrd1111
79. Harper DR, Enright MC. Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections. J Appl Microbiol 2011; 111:1-7; PMID:21410851; http://dx.doi.org/10.1111/j.1365-2672.2011.05003.x
80. Stone R. Bacteriophage therapy. Stalin's forgotten cure. Science 2002; 298:728-31; PMID:12399562; http://dx.doi.org/10.1126/science.298.5594.728
81. Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, et al. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 2005; 11:211-9; PMID:16258815; http://dx.doi.org/10.1007/s10156-005-0408-9
82. Bruttin A, Brüssow H. Human volunteers receiving Escherichia coli phage T4 orally: a safety test of phage therapy. Antimicrob Agents Chemother 2005; 49:2874-8; PMID:15980363; http://dx.doi.org/10.1128/AAC.49.7.2874-2878.2005
83. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care 2009; 18:237-8, 240-3; PMID:19661847