

Bacteriophage therapy: experience from the Eliava Institute, Georgia



Nina Chanishvili

George Eliava Institute of
Bacteriophage, Microbiology and
Virology,
Tbilisi, Georgia



Richard Sharp

Health Protection Agency
Centre For Emergency
Preparedness and Response.
Porton Down,
Salisbury, SP5 1RJ
United Kingdom
Email: richard.sharp@hpa.org.uk

The lysis of bacteria by bacteriophage was independently discovered by Frederick Twort¹ and Felix d'Herelle² but it was d'Herelle who proposed that bacteriophage might be applied to the control of bacterial diseases. Within the former Soviet Union (FSU), bacteriophage therapy was researched and applied extensively for the treatment of a wide range of bacterial infections. In the West, however, it was not explored with the same enthusiasm and was eventually discarded with the arrival of antibiotics. However, the increase in the incidence of multi-antibiotic-resistant bacteria and the absence of effective means for their control has led to increasing international interest in phage therapy and in the long experience of the Eliava Institute. The Eliava Institute of Bacteriophage, Microbiology and Virology (IBMV), which celebrates its 85th anniversary in 2008, was founded in Tbilisi in 1923 through the joint efforts of d'Herelle and the Georgian microbiologist, George Eliava.

The institute isolated bacteriophage able to lyse bacterial pathogens implicated in disease outbreaks across the Soviet Union, intended for use in hospitals throughout the country; however, much of this work was published in Russian and was unavailable in the West. This paper presents a small part of the scientific literature from the historical publications in the library of the Eliava Institute. The majority of the articles are from the 1930s and 1940s, when this type of therapy was still new and experimental. Many authors describe the methodology and results obtained and provide analyses and comments on the data collected.

In this review, we retain much of the contemporary nomenclature used for the bacteria and the diseases being treated as well as the style of describing the experiments and clinical studies carried by the original authors to provide some understanding of the scientific rationalisation given to the data. In some articles, significant details are absent, preventing a clear explanation for all of the work undertaken. It is often unclear what was meant by the term 'ordinary therapy' describing the treatment given to control groups but it is assumed that, since phage therapy was considered experimental, 'ordinary therapy' must have involved the traditional therapies familiar to the readership of that period.

Our analysis of the literature indicates that phage therapy was used extensively to treat a wide range of bacterial infections in the areas of dermatology³⁻¹⁰, ophthalmology¹¹⁻¹³, paediatrics¹⁴⁻¹⁷, surgery (especially against wound infections)^{18, 19}, urology²⁰, pulmonology²¹⁻²³, otolaryngology²⁴ and stomatology²⁵. We present some examples of phage therapy and prophylaxis in surgery and wound treatment, and in the treatment of intestinal infections. We aim to help stimulate further interest in its application and development at a time when 'alternative' or 'ordinary' therapies are becoming impotent in combating the increasing range of multi-drug-resistant bacteria to which the phages still remain active.

Phage therapy in surgery and wound treatment

The application of phage therapy to surgical and wound treatment began during the Finnish campaign in 1938-1939, with the first review of this work published by Kokin [cited]²⁶, describing the application of mixtures of bacteriophage (produced by the IBMV) infecting anaerobes, staphylococcus and streptococcus

for the treatment of gas gangrene. The mixture was applied to 767 infected soldiers, with a lethal outcome (death) observed in 19% of cases, compared with 42% in the control group of soldiers treated by other methods. Using the same mixture of bacteriophages, other authors observed lethal outcomes of 19% in a group of soldiers treated with phage compared with 54% in a group treated with other medications – Lvov & Pasternak [cited]²⁶.

In addition to its therapeutic use, this phage preparation was also used by the moving sanitary brigades as an emergency aid for treatment of wounds (prophylaxis of gas gangrene). Krestovnikova²⁶ summarised the observations of three moving sanitary brigades carried out over periods of 2-6 weeks following evacuation to front-line hospitals.

The first brigade treated 2,500 soldiers with phages. Only 35 soldiers (1.4%) in this group showed symptoms of gas gangrene while, in the control group of 7,918 wounded soldiers, symptoms were observed in 342 (4%). The second brigade applied phage therapy to 941 soldiers, of which only 14 (1.4%) suffered gas gangrene, in contrast to 7% of the control group who were treated by other alternative methods. The third brigade treated 2,584 soldiers and observed the development of disease in 18 soldiers (0.7%) whilst, in the control group, disease emerged in 2% of cases. Data comparison and observations described by the three independent brigades showed a 3-fold decrease in the incidence of gas gangrene as a direct consequence of the prophylactic treatment of wounds through the application of the phage mixtures²⁶.

The Second World War and the need for therapeutic preparations inspired the Soviet doctors to perform new trials with phages and develop novel methods for their administration, making this a significant period in the development of phage therapy. Moroz *et al.*²⁷ described the application of phage therapy for the treatment of persistent infections (persisting over 1-3 months) in the soft tissues, as in osteomyelitis following repeated operations and the wounds on the stumps of amputees. The phages were delivered topically as liquid applications, as tampons or through bathing. These methods were easy to perform and less traumatic for patients compared to subcutaneous or intramuscular injections. The bandages were soaked with mixtures of anaerobic and aerobic phages and changed every 2-3 days.

Of the 15 patients treated, 13 were recorded as completely cured after 2-3 weeks, with an improvement observed in the other two cases. In three cases of osteomyelitis, to avoid disturbance of newly operated wounds, the phages were mixed with 0.7% agar and then used as a filling for the wound, providing long-term circulation of phages in the wounds with the phages persisting in the wounds for 7-8 days. Post-operational recovery was considered smooth and painless. Although the study was

performed on a limited number of patients, the results were assessed as highly promising.

Izashvili²⁸ and Vilfenon²⁹ studied the cytological and morphological changes occurring in wounds treated with phages during healing. They demonstrated the positive effect of a specially designed dry phage mixture for treatment of wounds in activation of phagocytosis and in increasing the numbers of neutrophils.

Phage therapy against intestinal infections

Alexandrova *et al.*³⁰ described 87 cases of bacterial dysentery which were treated with polyvalent dysenterial bacteriophage. The majority of patients were small children but the overall ages varied from 4 to over 50 years. Prior to application of phage, the faeces were bacteriologically examined and blood samples checked for presence of anti-phage antibodies. The diagnosis of dysentery was confirmed either bacteriologically or serologically in 69 cases (80%). The phages were administered to 44 patients with moderate symptoms and to 43 with severe symptoms characterised by toxic syndrome, frequent stools, blood in the stools, and other characteristic symptoms. The phages were administered during the early stages of infection, orally in 80 cases, and rectally in seven cases. A single oral dose of 5-6ml or a single rectal dose of 8-10ml of phage suspension was given to patients over 2-3 days. To avoid the action of gastric acid, the phages were given together with carbonated water. Control groups with the same characteristics underwent the 'ordinary therapy'.

Evaluation of the results was based on relief or absence of the main disease symptoms such as stool frequency, bleeding, intoxication, etc (Table 1). It is apparent that the response to treatment began within the first 24-48 hours. The results indicated that phage therapy was 4-10 times more effective than in the control groups. Table 1 shows that, among patients with average severity of infection, 45% had a decrease in the frequency of stools within the first 1-2 days and after 3 days an improvement was seen in a further 41% of cases. In the group of patients receiving the alternative treatment, only 6% saw an improvement in the first 1-2 days and only a further 9% after 3 days; recovery appeared to take 4-5 days by which time a further 85% had recovered. This indicates that the majority of the phage treated patients recovered within 3 days while, for those receiving the alternative treatment, the majority took 4-5 days. The group with severe symptoms also showed an obvious improvement of 50% after the 2nd and 3rd days. In contrast, in the control group, only 4% showed an improvement after 3 days.

One of the most objective studies³¹ involved 219 patients (138 children and 81 adults) with dysentery and haemolytic intestinal

disease. The patients were divided into two groups; the first group was formed of patients suffering dysentery caused by *Shigella flexneri* and *Shigella shiga*, the second group of patients had hemocolitis and colitis caused by an unidentified bacterium. Application of phage therapy in the majority of cases began on the 3rd and 4th days. In each group a number of patients had previously undergone unsuccessful treatment with the other available therapies for 6-10 days, longer in some cases.

A polyvalent polyclonal bacteriophage preparation known as 'intesti-bacteriophage' designed by d'Herelle was applied. This bacteriophage preparation contained phage against *Shigella flexneri*, *Shigella shiga*, *Escherichia coli*, *Proteus* sp, *P. aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* A & B, *Staphylococcus* sp., *Streptococcus* sp. and *Enterococcus* sp. The application of intesti-bacteriophage was considered appropriate since epidemiological studies in Baku (Azerbaijan) had shown that most of the intestinal diseases were caused by this group of bacteria.

The bacteriophage preparation was administered orally once a day. Adults were given 10ml of phage and children 2.5-5.0ml prior to meals together with carbonated water. For young children, an alternative rectal administration of 50-100ml phage was the recommended dose following an enema with carbonated water. No side effects were observed after either oral or rectal administration of phages. During the treatment with phage other forms of therapy were stopped. Evaluation of the results was based on relief or full disappearance of the main disease symptoms such as high temperature, stool frequency and consistency bleeding and intoxication. Where relief was observed during the first 3 days, the result was considered to be good, improvement within 4 days was considered average with the symptoms completely gone within 10 days. Where no effect was

observed within the first 4-5 days, the treatment was replaced with the 'ordinary therapy' and the impact of phage therapy was evaluated as negative.

Table 2 illustrates the decrease in stool frequency following phage therapy, with an improvement observed in 50% of cases within 1-3 days; 24% of cases showed a decrease in the frequency of stools within the first 24 hours of phage application. In 25% of cases there was no stool improvement observed or relief of other disease symptoms such as bleeding. Of 113 cases with blood in the stools, 28% showed relief of this symptom within the 24 hours of phage administration and a further 27% of patients showed an improvement within 2-3 days. A further 25% of patients showed no evidence of bleeding after 4-10 days of phage therapy, with only 20% showing no relief of symptoms. Overall, 74% patients out of the 219 treated with bacteriophages showed an improvement or were completely cured, with only 26% showing no improvement.

Lurie³¹ underlined the difficulty in drawing objective conclusions from the results described since the majority of patients were already receiving a variety of emergency treatments at home including cleansing enemas, change of diet, and relief of dehydration or receiving a purgative treatment. This mixed background made it difficult to conclude that the therapeutic effect was due solely to phage therapy.

Phage preparations are generally considered to be particularly efficient for the treatment of intestinal infections. Thirty patients with chronic dysentery, many bedridden and exhausted by infection, were treated with a dry tablet preparation known as 'phage-vaccine', a combined preparation comprising 106 killed cells/ml and 10⁷pfu/ml of bacteriophages³² (Table 3). The patients had suffered with infections for 1-2 years and, in 70% of cases, rectoscopical investigation indicated the presence of bleeding ulcers. Prior to combined phage-vaccine therapy all

Table 1. Stool frequency and phage treatment³⁰.

Disease cases (n)	Decrease in stool frequency / improvement							
	Day 1-2		Day 3		Day 4-5		No improvement	
	Absolute no.	%	Absolute no.	%	Absolute no.	%	Absolute no.	%
Exp. Group 1 (n=44) moderate symptoms treated with phages	20	45	18	41	0	0	6	14
Control Group 1 (n=53) moderate symptoms	3	6	5	9	45	85	0	0
Exp. Group 2 (n=43) severe symptoms treated with phages	11	26	10	23	0	0	22	51
Control Group 2 (n=45) severe symptoms	0	0	2	4	29	64	14	31

the patients underwent multiple courses (1-8) of therapy with antibiotics and sulfonamide preparations and other available treatments. After the phage-vaccine therapy, complete cure was achieved for 26 patients (87%) within 10-20 days. Assessment of the results was based on improvements in the general condition of patients including normalisation of coprograms, formation of stools and recovery of the mucous layer of sigmoidal intestines and rectum.

Gnutenko ³³ reported the results from using bacteriophage to treat workers in the food processing industry who were identified to be carriers of *S. typhi* or *S. paratyphi*. Twenty one staff, mainly over 40 years of age and comprising 18 women and three men, underwent the treatment during 1940-1941 and in 1944. Previous treatments using other methods had proved unsuccessful and it was decided to apply a combined phage therapy by intramuscular phage injections, and rectal and duodenal administrations of phage. In those cases where duodenal treatment was not possible, oral administration was used. The duodenal administration was considered to have two important advantages: the phages were transported directly into the area colonised by the bacteria and the phage preparation was not affected by the gastric acids and therefore retained its activity. The rectal treatment was believed to provide a supplementary treatment since it was considered that those bacteria surviving in the gall bladder would be killed in the rectum. To evaluate the potential for the migration of phages from the rectum to the gall bladder, samples of bile were checked for the presence of both the administered therapeutic phages and naturally occurring phage.

A 30ml dose of phage preparation was administered as an enema after washing the intestines with a cleansing enema. After 12-

14 hours a probe was introduced directly into the gall bladder to take a bile sample followed by the administration of a 20ml phage preparation. After 5-6 hours the patients were given an intramuscular injection of phages (5-10ml). The above procedures were repeated 3-5 times in one course. This complete course was then repeated 2-3 times. After each course of treatment, the faeces were examined for presence of bacteria. After this series of treatments six of the 10 carriers of *S. typhi* were found to be clear of the organism while four showed no change. Eight of the 11 *S. paratyphi* carriers were cleared, while three did not respond to the treatment. It was recommended that such treatments should be carried out under hospital conditions and at least three courses of treatment should be carried out over 10-15 days.

Phage prophylaxis

Phages have also been used extensively in the FSU for prophylaxis, especially in communities where the rapid spread of infections may occur such as in kindergartens, schools and military accommodation etc. An experiment on the prophylactic use of phages was successfully carried out in 1935 on thousands of people in the regions with a high incidence of dysentery ²⁵. The results were reported at scientific conferences in 1934 and 1936 in Kiev and in 1939 in Moscow after which the dysenterial phage preparation was finally approved as a preventive measure for mass application. It was recommended that repeated seasonal prophylactic 'phaging' be carried out in areas where it was endemic. Later modifications included the supply of the dysenterial phages in dry tablet forms; this also began to be included in clinical studies.

Table 2. Stool frequency and phage therapy ³¹.

Groups (n)	Decrease in stool frequency following phage treatment / improvement							
	Day 1-2 Absolute no. %		Day 3 Absolute no. %		Day 4-5 Absolute no. %		No improvement Absolute no. %	
Children (n=54) with unidentified infection	10	19	11	20	15	28	18	33
Children (n=84) identified with Shigella infection	19	23	18	21	19	23	28	33
Adults (n=54) with unidentified infection	16	30	22	41	12	22	4	7
Adults (n=27) identified with Shigella infection	8	30	4	15	9	33	6	22
TOTAL (n=219)	53	24	55	25	55	25	56	26

One of the later studies describes the results of preventive treatment carried out with the phage tablets having an acid-resistant coating³⁴. Experimental and control groups were selected at random; one soldier provided an 'observation unit'. The populations in the experimental and control groups were located in different geographical zones; however, they were all placed in similar types of endemic area. Bacteriophage and placebo were coded and were given when there was a rise in morbidity (threatening to become an epidemic) particularly in June-July and September-October. Two tablets of the coded preparations were given to the people in the experimental and control groups 1.5 hours prior to meals. One group was given the tablets once every 3 days, while another group of the same size received the preparation once every 5 days. Calcium gluconate was used in placebo experiments. The efficiency of prevention using phage prophylactics given once every 3 days was 75%, and 67% when given every 5 days, leading to a recommended use of the phage tablets once every 3 days.

During the course of this study coincidental outbreaks of dysentery related to water contamination with *Sb. dysenteriae* were observed in two separate communities. Analysis of morbidity showed great difference between the experimental and control groups, with morbidity in the control group appearing to be 5.7 to 9.5 fold higher than in the experimental group receiving phage tablets once every 3 or 5 days. The phage tablets therefore provided a high level of prophylactic action³⁴.

For the prevention of typhoid epidemics specific phages were also used, with two tablets administered once every 5-7 days during the outbreak season^{35, 36}. Intestinal colonisation and overgrowth with *Pseudomonas aeruginosa* in young hospitalised children was prevented successfully by phage administration³⁷.

Phage preparations have also been found to be very useful in the disinfection of surfaces and facilities in hospitals. Walls in wards, different surfaces, and even instruments and wounds, (the source of some pathogens) have also been treated, with

a considerable effect found in children's clinics³⁸. Recent IBMV studies demonstrate a high eradicating effect of *P. aeruginosa* and *S. aureus* phages in a laboratory environmental model^{39, 40}.

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Table 3. Comparison of the efficacy of ordinary treatment and phage therapy³².

Disease cases (n)		Lingering illness		Lethal outcome	
		Absolute no.	%	Absolute no.	%
Children up to 2 years receiving ordinary treatment					
Average severity	(n=60)	12	20	7	12
Severe	(n=47)	5	11	37	79
Children up to 2 years receiving phage therapy					
Average severity	(n=48)	5	10	1	2
Severe	(n=36)	3	8	22	61

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Dr Nina Chanishvili is head of the Laboratory for Genetics of Microorganisms and Bacteriophages at the George Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia. Her association with the bacteriophage studies at the Institute extends over 30 years.

Prof Richard Sharp coordinates Business and Strategic planning for the Health Protection Agency, he has had a long interest in the ecology and biotechnological exploitation of bacteriophage. He is a visiting Professor at the University of Liverpool.

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