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Review

Phages and their application against drug-resistant bacteria

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Abstract: At the beginning of the 20th century the phenomenon of spontaneous bacterial lysis was discovered independently by Twort and d'Herelle. Despite the suggestion at that time by d'Herelle that these agents might be applied to the control of bacterial diseases in the west this idea was explored in a desultory fashion only and was eventually discarded largely due to the advent of extensive antibiotic usage. However, interest was maintained in countries of the former Soviet Union where bacteriophage therapy has been applied extensively since that time. Central to this work was the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia, which was founded in 1923 through the joint efforts of d'Herelle and the Georgian George Eliava. Ironically, given his contributions to public health in the Soviet Union, Eliava was branded as an enemy of the people in 1937 and executed. d'Herelle never again returned to Georgia. In spite of these tragic events this institute remained the focus for phage therapy in the world and despite being continuously active in this field for 75 years, now struggles for its financial life. In the Eliava Institute, phages were sought for bacterial pathogens implicated in disease outbreaks in different parts of the Soviet Union and were dispatched for use in hospitals throughout the country. Although infections caused by a wide variety of bacterial pathogens have been treated, much of this has been published in Russian and is not readily available in the west. Work has also been carried out in Poland over many years and this has only recently been published in English. By contrast, interest in the west has been limited to a small number of enthusiasts and academics and until very recently little interest has been shown. The main reason that the medical and scientific communities are now beginning to take notice, is the continuing world-wide rise in the incidence of multiply-antibiotic-resistant bacterial pathogens and the absence of effective means for their control. Recent publicity over the work of the Eliava Institute has concentrated the minds of the western world on the potential for infectious disease control that bacteriophage offer, a procedure that is biologically more acceptable than antibiotic use and which has been in use for several decades already.

Keywords: bacteriophage therapy; disease control; phage–vaccine therapy; prophylaxis

1 THE PRINCIPAL ACTIVITIES OF BACTERIOPHAGES RELEVANT TO THEIR APPLICATION IN THERAPY

Bacteriophages are viruses for which bacteria are the natural hosts. Most of the Eubacteria, amongst which human and animal pathogens occur, are hosts to specific phages. There are exceptions, eg phages have not yet been isolated for *Neisseria meningitidis* or *N gonorrhoeae*. The initial stage of infection is adsorption of an individual phage particle to a susceptible bacterium. This occurs usually through the interaction between the phage tail and a specific attachment molecule on the bacterial surface (Fig 1). There is great specificity in this attachment and a phage will not generally attach to unrelated bacteria. The phage injects its nucleic acid into the cell where it either replicates itself or, as in the case with some phages, integrates into the bacterial chromosome. Replication results in formation of mature particles around the newly synthesised phage nucleic acid followed by lysis of the cell and release of the new daughter phages (Fig 2). Integration of the nucleic acid into the host chromosome results in suppression of free replication and prevents further infection by the same type of phage. The phage nucleic acid becomes an integral part of the chromosome and does not excise, except under certain conditions when it reverts to a replicative cycle. Phages for clinical use therefore must be selected from virulent, non-lysogenic phages.

The relevance of this is related to the specificity of

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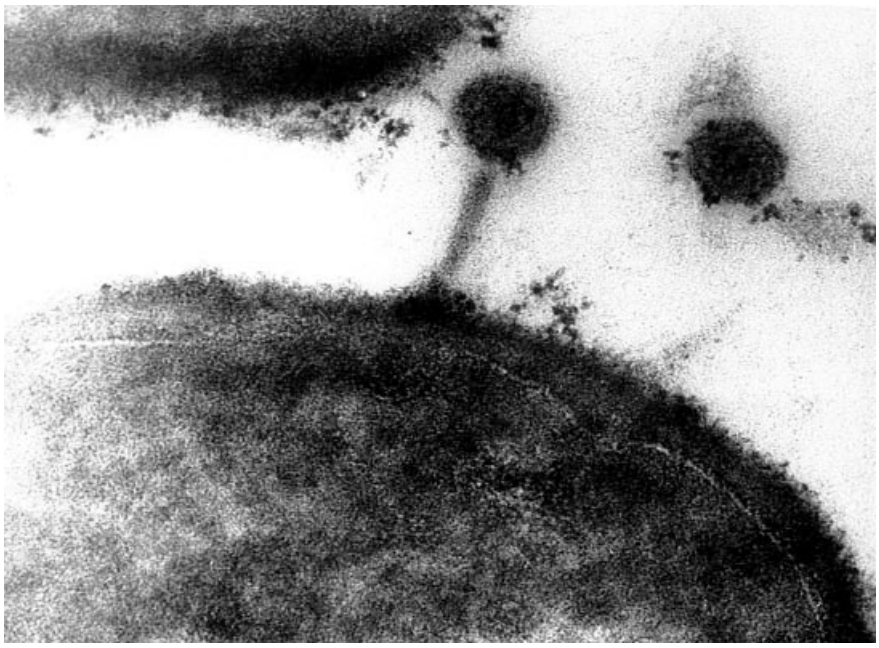

Figure 1. Phage Sb-1 attaching to *Staphylococcus aureus*.

phages in that they may be specific not just for bacterial species but for clones/strains possessing the attachment antigen. They have no effect on inert bacteria that may also be present, ie they do not affect the normal flora.

The whole idea involves the exploitation and application of a biological system that is already in operation naturally and for which there is good evidence for its ability to determine the outcome of natural and experimental infections. Thus it is likely that conditions exist under which the system operates optimally and all attempts must be made to reproduce these conditions to achieve optimal activity.

2 CLINICAL EFFICACY OF BACTERIOPHAGES IN THERAPY AND PROPHYLAXIS: EXPERIENCE FROM THE ELIAVA INSTITUTE FOR BACTERIOPHAGE, MICROBIOLOGY AND VIROLOGY (IBMV)

Since the discovery of spontaneous bacterial lysis by Twort and by d'Herelle phage therapy has been used extensively with miscellaneous bacterial infections in the areas of oto-laryngology,[3] stomatology,[4] ophthalmology,[5,6] dermatology,[7–11] paediatrics,[12] gynaecology,[13–15] surgery (especially against wound infections),[16,17] urology,[18] and pulmonology.[19–21]

Reviewing the old Soviet literature it becomes obvious that mass application of bacteriophages was

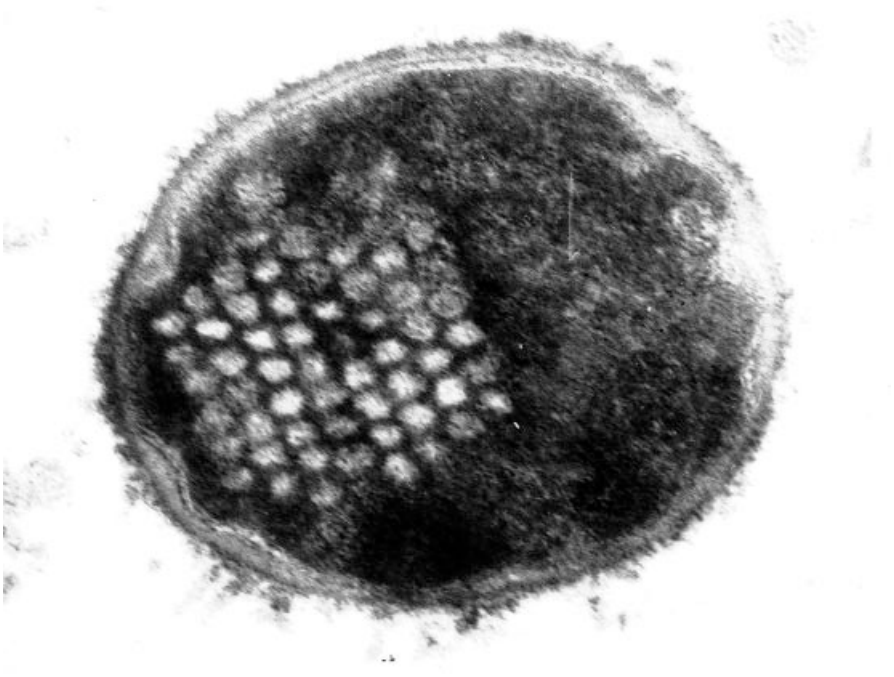

Figure 2. Intracellular propagation of Sb-1 in *Staphylococcus aureus*.

first facilitated in relation to the Finnish campaign (1938–1939) and then during the Second World War (1941–1945). The period between 1930 and 1940 coincides with the peak of a number of scientific publications on phage therapy, this is why it is sometimes difficult to find access to the old data. This in part explains why most of the results of the clinical experiments are not properly designed and registered, the data for the control groups are often missing Migration of patients from one hospital to another did not allow proper monitoring of the clinical effect of phage therapy.

The previous experiences of patients with chronic infections, including non-effective treatment with antibiotics etc, are usually presented as alternative data to conventional control group information. Unfortunately, this erroneous approach to experimental methodology and data presentation continued in the Soviet Union in post-war publications.

Kokin (1947 cited in Ref 22) describes application of the mixtures of anaerobic phage and *Staphylococcus* and *Streptococcus* phage (produced by the IBMV, Tbilisi, Georgia) for treatment of gas gangrene in soldiers. The mixture was applied to 767 cases and resulted in a death rate of 18.8% compared with 42.2% in the control group of soldiers treated by other methods. Other authors have observed death rates of 19.2% in the group of soldiers treated with the same mixture of phages against 54.2% treated with other medications (Lvov and Pasternak, 1947 – cited in Ref 22).

In addition to therapy, this phage mixture has been used by the mobile health brigades as an emergency aid for treatment of wounds (prophylactics for gas gangrene).[22] This paper summarises the observations of the three mobile, brigades. Observation was carried out within 2–6 weeks during the course of patients evacuation to the front-line hospitals. In the first group of 2500 soldiers treated with phages, only 35 (1.4%) showed the symptoms of gas gangrene. In the control group of 7918 wounded solders, 342 (4.3%) were infected. The second brigade applied phage therapy to 941, only 14 (1.4%) of which were infected with gas gangrene, in comparison with 6.8% from the control group treated with other methods. The third brigade dealing with 2584 solders observed the development of the disease in 18 soldiers (0.7%) while in the control group, disease emerged in 2.3% of cases. Comparing the data described by the three independent brigades it becomes apparent that a three-fold decrease in the occurrence of gas gangrene is a direct consequence of the application of phage mixtures for prophylactic treatment of wounds.[22]

Indeed, the war time and lack of therapeutic preparations inspired the Soviet doctors to perform new trials with phages and invent novel methods for their administration. Ironically this time turned into a genuine heyday for creative research in phage therapy. Moroz *et al*[23] describe the application of phage therapy for treatment of old unrecoverable (within 1–3 months) wounds of the soft tissues, repeatedly operated cases of osteomyelitis and wounds on the stumps of limbs.

Phages were administered topically and applied through tampons and bathing. These methods of application are simple and less traumatic for patients, in comparison with subcutaneous or intra-muscular injections. The bandages, soaked with mixtures of anaerobic and aerobic phages, were changed every 2–3 days. Observation of 15 patients indicated a complete cure after 2–3 weeks of treatment in 13 cases and an improvement was observed in the other two cases. It is remarkable that in three cases of osteomyelitis, to avoid disturbance of newly operated wounds, it was decided to mix the phages with 0.7% agar and use this jelly mass as a filling of the wound. This method enabled the long-term circulation of phages in the wounds. Phages remained in the wounds for 7–8 days and post-operational recovery was smooth and painless. Although the study was performed on a limited number of patients the results have been assessed as highly promising.

Others[24,25] have thoroughly studied the dynamic cytological and morphological changes occurring during healing of the wounds treated with phages and demonstrated positive effects of the therapy performed with the dry phage mixture specially designed for treatment of wounds. The authors underline the positive effect of phage therapy in activation of phagocytosis and increase in number of neutrophils, etc.

Tsulukidze[18] describes the application of phage therapy in urology, in particular in cases of acute (13 cases) and chronic cystitis (15), pyelocystitis (5) and purulent paranephritis (4). Duration of chronic cystitis varied from 1 month to several years. The majority of these cases were caused by *E coli* infection (78.4%), the rest by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Treatment was performed with the specific phages and/or Pio-bacteriophage comprising phages against all the above mentioned pathogens. Phages were administered directly into the urinary bladder or into the pelvis of the kidney, in the case of paranephritis by puncture. After treatment of acute cystitis a therapeutic effect was observed within 4–5 h of the first bathing and resulted in pain-relief and decreased incidence of urination. The composition of urine returned to normal. Recovery was achieved after 1–3 days in all 13 cases (100%). Treatment of chronic cystitis was less successful, however temporary improvement was observed. In the third group of patients with pyelocystitis a complete cure was observed in four cases (out of five), re-infection was observed in one case. In the fourth group of patients with paranephritis (four cases) the post-operational recovery was complete and finished in the 2–3 days during which the phages had been sprayed onto the wounds.

Treatment of the deep forms of dermatitis resulting from *Staphylococcus* with specific bacteriophage was shown to be effective and is described in a number of articles.[4,7–10]

More recent studies[11] were performed on 161 patients with chronic and frequently relapsing infections with the following diagnosis: 62 patients with furuncles (boils) and furunculosis, 57 with carbuncles and 45 with hydroadenitis. In all cases antibiotic therapy with penicillin, biomicon, streptomycin had been performed without a positive outcome. Duration of the chronic diseases varied between 2–3 months and 15–20 years. All the patients had high temperatures (37–37.8 °C), headaches, weakness, insomia and movement difficulties. Most of patients with furuncles had 1–12 boils in different parts of the body. Microbial analysis of specimens obtained from 161 patients identified 139 strains of coagulase-positive *Staphylococcus*. Of these strains, 83% appeared to be penicillin-resistant. Phages were administered topically as applications and injected subcutaneously around the infected locus. In total 95% (152 cases) were completely cured, 4.3% (seven patients) showed significant improvement and in 1.3% (two patients) the treatment had no effect. Long-term surveillance was performed over the next 4 years. Relapse was observed after 3–6 months in 8.5% of cases. These patients underwent and additional course of the phage therapy after which they were completely cured. The results are summarised in Table 1.

Unusual but interesting data concerning phage therapy were presented by Gvazava[10] who was obliged to apply expired phage preparations for the treatment of deep forms of piodermitis. At the time of application in 1942 the preparations had been expired for 1–5 years. Some ampoules contained no viable phages or had extremely low titre. Experiments however were carried out on 71 patients, 63 of which resulted in a complete recovery, relapse was observed in seven cases and in one case treatment was unsuccessful. Phage lysates were administered as applications and/or subcutaneous injections. Increasing doses of phage lysates, 0.1 cm^3, 0.2 cm^3, 0.5 cm^3, 1.0 cm^3, were injected subcutaneously once per day for about 5–10 days. The control group of patients received sterile broth (placebo) only. The beneficial effect of the therapy derived from the expired phage preparations was considered to be due to the immune effect of the bacterial lysates containing tiny particles of bacterial cells (antigens) within the phage preparations. The immune response and other responses to phage therapy remain to be investigated, including effects similar to vaccination.

Phages are especially efficient for treatment of intestinal infections. Vlasov and Artemenko[26] described the results of treatment of 30 patients with chronic dysentery, many of whom were exhausted by infection and bedridden, having suffered with the infection for 1–2 years. A dry tablet preparation known as 'phage-vaccine' – a combined preparation comprising 10^6 killed cells cm^3 and 10^7 pfu cm^3 of bacteriophages, was applied for treatment. Rectoscopical investigation of the patients showed the presence of bleeding ulcers in 70% of cases. Prior to combined phage–vaccine therapy all the patients underwent multiple courses (1–8) of therapy with antibiotics and sulfonamide preparations and other available means. After the phage–vaccine therapy a complete cure was achieved within 10–20 days in 26 cases (86.7%). Assessment of recovery was based on the overall improvement of the patient, normalisation of coprograms, formation of stool, and recovery of the mucous layer of sigmoidal intestines and rectum.

3 COMBINED PHAGE AND ANTIBIOTIC THERAPY

The end of the 1950s and the beginning of the 1960s was marked by the emergence of a new direction in Soviet medicine with researchers starting to elaborate regimens combining phage and antibiotic therapies.[27–31] From these types of experiment, the work accomplished by Vepkhvadze[32] is especially remarkable. *In vitro* experiments demonstrated that the presence of antibiotics in the broth increases the phage titre 10^1–10^4-fold. However, the author could not explain the mechanism of this observation. *In vivo* studies were carried out in a mouse model with groups of mice (20 in each group) infected with antibiotic-resistant *Staphylococcus aureus*. Groups were then treated with the specific *Staphylococcus* phage (a product of the IBMV, Tbilisi, Georgia) alone, with antibiotics alone and in combination with different antibiotics including dichlortetracycline, erythromycin, pasomycin, oxacylin plus phage. In the first series of experiments antibiotics and phages were administered immediately after infection. In the second series, phages were administered immediately after infection, while antibiotics were added at different intervals of

Table 1. Effect of phage therapy for treatment of some forms of dermal infections caused by *Staphylococcus aureus*

Group	Diagnosis	Number of patients	Type of therapy	Duration of treatment (min–max)	Complete cure (%)	Improvement (%)	Temporary effect (%)	No effect (%)	Re-infection (%)
Exp Gr1	Furunculosis	62	Phage	3–10 days	97.7	3.3	–	0	4.8
Control 1	Furunculosis	62	Antibiotics	3 months–16 years	0	53.3	20.9	25.8	100
Exp Gr2	Carbunculosis	54	Phage	5–10 days	66.7	20.0	–	13.3	11.1
Control 2	Carbunculosis	54	Antibiotics	2 months–15 years	0	7.4	92.6	0	100
Exp Gr 3	Hydroadenitis	45	Phage	5–10 days	88.9	6.7	–	4.4	6.6
Control 3	Hydroadenitis	45	Antibiotics	2 months–20 years	0	24.4	75.6	0	100

Note: Control groups used were the same groups of patients previously treated with antibiotics.[11]

time (4, 8, 24h after infection). In the third series, antibiotics were administered immediately after infection, while the phages were given at 4–8–24h.

It was shown that the results of treatment performed separately with either sub-therapeutic doses of phages (app titre 1×10^7 pfu cm^3) or antibiotics (3200 μg cm^3), are quite similar (Table 2). However, for the combined use of antibiotics and phages the best results were achieved when the antibiotics were administered 24h prior to phage treatment. It was recommended that the bacteriophages were used in combination with antibiotics but with a difference of 24h. The data are presented in Table 2 and indicate greater efficacy when phage were administered 24h after administration of antibiotic and microbial challenge, with 75% of mice surviving to day 15.

With an 8h and 4h delay in administration of phage 65% and 40% of mice survived. Controls where only erythromycin was administered simultaneously with the challenge indicated 45% survival and where only phage were administered at 24h the survival rate was 35%.[32]

Other combined therapies include studies in which recovery from pulmonary *S aureus* infections were recorded in groups of patients given antibiotics or antibiotics together with bacteriophage against which the *S aureus* strain was sensitive. Patients (152) who had undergone lung operations were involved in the study. Forty-five patients (Gr I) received antibiotics together with phages and a control group (Gr II), comprising 107 patients, was treated with antibiotics only. Remission and stabilisation of the suppuration process in the experimental group (Gr I) was observed in 93% of cases, whilst in the control group (Gr II) it was 80%. The post-operative re-infection rate of the pleural cavity in the group of patients receiving both antibiotics and phages (Gr I) was 24%, in contrast to 67% in the control group administered antibiotics alone.[22]

Phage have been administered by various routes including local administration (via tampons and bathing of cavities), inhalation, oral administration and also parenteral administration, including intramuscular and intravenous injection.

The institute has had particular success for the treatment of *S aureus* septicaemia of newborns where the prognosis is otherwise poor. The poor development of the immune system may have been involved in the persistence of the phages in these cases and opens up opportunities for treatment and prevention of diseases in immuno-compromised patients. In one study recovery occurred after 7 days of antibiotic therapy and after 3 days of combined antibiotic and phage therapy.[5] Samsygina[12] obtained similar results more recently when treating miscellaneous rhinitis, dermatitis or conjunctivitis. Infections were caused by *S aureus, S epidermidis* or mixed infections including these together with members of the Enterobacteriaceae and treatments applied, phage, antibiotics or a combined therapy. Clinical improvements were obtained in 86%, 48% and 82% of patients in the three treatment groups respectively.

Although phages were administered intravenously at

Table 2. Combined application of phages and antibiotics

Preparation	*Administration*	*Number of mice*	*Number of surviving mice the 15th day*		*P*	*Average life expectancy (days after manipulation)*
			Abs Number	*%*		
Phage + Ery	Simultaneously with infection After 4h	20	8	40	>0.5	6.7
Ery alone	After 4h	20	10	50	>0.5	7.7
Phage + Ery	Simultaneously with infection After 8h	20	7	35	>0.5	5.6
Ery alone	After 8h	20	9	45	>0.5	6.6
Phage + Ery	Simultaneously with infection After 24h	20	8	40	–	5.8
Ery alone	After 24h	20	8	40	–	6.6
Ery + Phage	Simultaneously with infection After 4h	20	8	40	–	6.4
Ery alone	Simultaneously with infection	20	9	45	>0.5	7.3
Phage alone	4h after infection	20	8	40	–	3.2
Ery + Phage	Simultaneously with infection After 8h	20	13	65	–	8.1
Ery alone	Simultaneously with infection	20	9	45	>0.2	7.3
Phage alone	8h after infection	20	7	35	<0.05	3.6
Ery + Phage	Simultaneously with infection After 24h	20	15	75	–	9
Ery alone	Simultaneously with infection	20	9	45	<0.05	7.3
Phage alone	24h after infection	20	7	35	<0.01	2.4

the very early stages of clinical experiments in the 1930s and 1940s, this type of therapy was rejected due to the unpleasant side effects including rising temperature, up to 38–39 °C shivering, headaches, etc. However, a lethal outcome has never been reported.[3,18,33] The most important and the most recent achievement among the activities of the Eliava Institute of Bacteriophage is manufacture of the apyrogenic Staphylococcus phage for intra-venous use,[34–36] the action of which was proved in clinical experiments on adults and children. Altogether 900 patients were involved in this study, among them 247 children aged between 1 day and 15 years and 653 adults aged 15–72. A total of 494 people were treated with phages alone or in combination with antibiotics and/or immune-stimulating means; 406 persons from the control group were treated with antibiotics only. Intravenous use of phages did not show any significant side effect.

After this experiment the *Staphylococcus* phage was produced by the industrial part of the Eliava Bacteriophage Institute and successfully applied in many clinics throughout the whole territory of the former Soviet Union. The phage was applied for intra-venous use in infusions, transfusions and injections. It was mostly used for treatment of chronic septicaemia, for treatment and prophylactics of eye, ear, throat and lung diseases, for healing burned wounds, for the bacterial consequence of surgical operations on bones and skull, for infertility problems in women related to bacterial inflammation problems, and so on.

Recently our group performed a random study, the purpose of which was to analyse the practical outcome of the usual treatment accomplished by two different regimens: (a) application of antibiotics only, and (b) antibiotics and therapeutic cocktails of the phages with the broad action. These comprised the Pio- and Intesti- bacteriophages (produce of the Eliva IBMV), containing phage against *Staphylococcus, Streptococcus, E coli, Pseudomonas, Proteus* and others.

In addition to this, 32 case records of the same diseases accomplished by the two medical doctors have been studied. Thus, the two groups of 16 patients comprising 11 boys and 5 girls aged 1 day to 3 months were selected. They underwent different methods of therapy as described above. Most of the patients from the two groups (50%: 68% accordingly) suffered with the generalised form of septicaemia caused by coagulase-negative *Staphylococcus* and/or *E coli*. In the first group phage cocktails were given after the implementation of antibiotic therapy to combat the residual infection. In the second group the therapy was implemented by application of a variety of last generation antibiotics only. The average duration of hospital days in the first group was 24, while in the second group this index went up to 28. Significant improvement in the first group was apparent on the 4th–7th days following onset of phage therapy. Since the doctors did not know that their records would be checked and the results analysed we assume that the results obtained can be considered as objective evidence for the efficiency of the combined antibiotic and phage therapy.[37]

Many authors underlined the importance of *in vitro* phage spot-tests for prediction of the *in vivo* results of treatment, however these results are not completely in agreement.[5,10,11,32,38] Thus, Shvelidze[11] reports the results of *in vitro* screening of 161 strains of *Staphylococcus aureus* against specific therapeutic bacteriophage which showed 78% effectiveness (108 cases). In *in vivo* tests an additional 22% (31 cases) were cured although the corresponding *in vitro* tests were negative.[11] No correlation has been ever observed between the drug and phage-resistance, on the contrary most of the antibiotic-resistant strains revealed high sensitivity towards the specific phages.

4 PHAGE FOR DRUG-RESISTANT BACTERIA

For prognosis of the future effectiveness of phages for treatment of current troublesome drug-resistant bacteria our group has performed *in vitro* screening of a number of MRSA, VRE, imipenem-resistant *Ps aeruginosa* (kindly provided by Dr G Morris, UMAB, USA) and *E coli* O157 strains (kindly provided by Professor S Ulitzur, Technicon, Israel) with the phages from the IBMV collection. The results obtained are very promising. Ninety three MRSA and 186 *Enterococcus* strains have been examined in the study, in particular, 41 *E faecalis*, 140 *E faecium* and 5 *E gallinarum*. Culture samples were isolated in 1995–1997 from respiratory routes, extremities, blood, urine, wounds and other sources. The *in vitro* efficiency of the phage Sb-1, which is the basic component of the intravenous phage preparation, was 84% against clinical MRSA strains.[39–40] Phage Ent-6 indicated 98% efficiency towards VRE strains.[41]

With the *Ps aeruginosa* the situation is different. Three groups of *Ps aeruginosa* strains derived from clinical and environmental sources were tested against 39 clones of *Ps aeruginosa* phages (15 from the collection of the Eliava IBMV and 24 recently isolated from sewage waters, clinical strains and other sources).

Group I originated from the IECS hospital (Republic of Georgia) and comprised 39 clinical strains isolated in 1998–2000 (23% from respiratory routes, sputum, throat, bronchus, pleural liquid, chest wounds and the rest from urine, bile, wounds and the hospital environment). Group II comprised 20 imipenem-resistant *Pseudomonas* strains (55% from respiratory routes), isolated in 1994–1997 (provided by Dr G Morris, UMAB, USA), Group III comprised 19 mucoid cultures isolated from CF patients in the USA and Belgium in 1998–1999 (provided by Dr E Kutter and Dr M Vaneechoute).

The observed *in vitro* efficacy against the strains was 27–46% (Group I), 53–76% (Group II) and 33–53% (Group III).[42–44]

5 PROPHYLAXIS

Phages have also been used extensively in the former Soviet Union for prophylaxis, especially for communities where the spread of infections may be rapid, such as kindergartens, schools, military accommodation, etc. According to the review written by Krestovnikova,[22] an experiment on prophylactic use of phages was successfully accomplished in 1935 on thousands of people in the regions with high incidence of dysentery. The results were reported at scientific conferences in 1934, 1936 (Kiev) and 1939 (Moscow) after which the dysenterial phage preparation was finally approved as a preventive mean for mass application. It was recommended that repeated seasonal prophylactic 'phaging' in the epidemic centres be performed. Later on different modifications of the dysenterial phages, namely dry tablet forms were included in the clinical studies. One of the later studies describes the results of preventive treatment performed with the phage tablet forms having an acid-resistant coverage.[45]

Experimental and control groups were formed according to accidental selection, one soldier was taken as an observation unit. Persons in the experimental and control groups, that were located in different geographical zones of the USSR, however were placed in similar epidemic conditions. Bacteriophage and placebo were coded. The coded preparations and placebo were given during the rise of morbidity (threat of epidemics), in particular in June–July and in September–October. Coded preparations were given to the persons from experimental and control groups 1.5–2 h prior to meals, two tablets each time. One group of people was receiving these tablets at intervals of 3 days, while another group was receiving them at 5 day intervals. Calcium gluconate was used in placebo experiments. Phage and placebo was given to every second person, thus providing equality of quantity and quality between the experimental and control groups. The Efficiency of prevention of the phage prophylactics taken once per 3 days was 75%, and 67% when taken once per 5 days. Thus, a conclusion was drawn out according to which it was recommended that the phage tablets were used once per 3 days.

During the course of this experiment coincidental outbreaks of dysentery related to water contamination with *Sh dysenteria* were observed in two separate communities. Analysis of morbidity in this epidemic showed a great difference between experimental and control groups. Morbidity in the control group appeared to be from 5.7- to 9.5-fold higher than in the experimental group that was receiving the phage once at 3 or 5 day intervals indicating a significant level of prophylactic action.[45]

For prevention of typhoid epidemics the specific phages were also administered via two tablets once every 5–7 days during the season when outbreaks are prone to occur.[46,47]

Intestinal colonisation and overgrowth by *Pseudomonas aeruginosa* in young hospitalised children has been prevented successfully by phage administration.[48]

Phage preparations have also been found to be very useful in the disinfection of surfaces and fomites in hospitals, the walls in hospital wards, and instruments and wounds themselves. A considerable effect in children's clinics has been observed.[49]

The published work from Poland claims very high rates of recovery from a variety of bacterial conditions (between 75% for ulcerated varicose veins to 100% for intestinal infections). However, no control groups were included so these results are very difficult to assess properly.[49–53]

6 PHAGE THERAPY/PROPHYLAXIS HAS A NUMBER OF ATTRACTIVE ADVANTAGES

Bacteriophage are highly specific for the target bacteria without affecting the normal microflora and are effective against multiple drug-resistant bacteria.

Following numerous studies in animals and from clinical experience no adverse side effects have been observed. Purified phage preparations do not cause significant side effects such as allergy, intoxation, etc.

Because of their ability to multiply in the target hosts a single dose treatment can be envisaged for many diseases.

The environment provides an almost inexhaustible source of new active phages when required. Ecological safety of phages is implied by their high specificity towards the target bacteria. Bacteriophages will therefore not accumulate in the environment.

Production of bacteriophages is economical. It does not require sophisticated and expensive equipment or complicated and multi-stage technologies.

7 EXPERIENCE IN THE WEST: EARLY CLINICAL FAILURES, RECENT EXPERIMENTAL SUCCESSES AND PERCEIVED POTENTIAL

In contrast to eastern Europe, in the west phages got themselves a bad name early on, largely as a result of ignorance and poorly planned experiments. At that time the nature of phage and phage–bacterial interactions, including lysogeny and DNA restriction, was as poorly understood as was the mechanism of disease production by the major bacterial pathogens.

This was demonstrated by the attempts to control cholera with phage.[54] In this case little attempt was made to isolate suitable phages able to effect efficient lysis, which in the late 1920s was hardly surprising. Use in the field was largely uncontrolled. On one occasion the field trial consisted of pouring an undisclosed amount of phage down a drinking well in an area where the infection/disease was endemic. Assessment was based on enumeration of the number of cases occurring before and after treatment. Whether the strain was susceptible to that particular phage was not even disclosed and one cannot help thinking that it was not considered. These are some of the problems in

the early literature that which led to discrediting of the technology. In addition, a number of very dubious commercial preparations appeared in the 1930s which claimed effectiveness against a number of inappropriate infections, some of which, such as herpes infections, eczema and urticaria, did not even have a bacterial aetiology.

However, experimental work continued albeit in a very limited way between the 1940s and the 1980s, some of which demonstrated efficacy, while other work did not. Success was often dependent on a combination of the pathogen–host system chosen and the skill of the experimenter.[55–58]

More recent Work in the United Kingdom started with the demonstration by Williams Smith and colleagues that individual phages, chosen carefully, could be very effective against experimental *Escherichia coli* infections in animals. The systems chosen were septicaemia in mice[59] and neonatal diarrhoea in calves, sheep and pigs[60–62] where disease could be prevented or treated by specific phages isolated from sewage and phages were found to be more effective than antibiotics. Experimental work has been continued by his colleagues using *E coli* septicaemia in chickens and calves[63] and *Pseudomonas* colonisation of skin preparations,[64–65] major practical health problems in animals and man. Attempts by these research groups to obtain further funding for this very applied scientific work, which nevertheless shows great potential, has, however, been very unrewarding.

The recent experimental work carried out by these two UK groups led to them drawing a number of conclusions from their work regarding its potential.[66] They concluded that bacterial diseases are most likely to be amenable to phage therapy or prophylaxis where a stage of the pathogenesis mimics conditions under which phages multiply optimally *in vitro*. Thus, in nutrient broth phages multiply and spread readily between susceptible cells, conditions which are mirrored, to some extent in blood and cerebro-spinal fluid. Thus some of the true septicaemias caused by *Pasteurella* spp in the veterinary field and human bacterial meningitis and septicaemia may be amenable to this approach. Phages will also spread between bacterial cells by direct contact, as occurs on the surface of an agar plate and as may occur in the semi-confluent pallisades of bacteria that colonise the small-intestinal mucosae in some of the enterotoxigenic bacterial enterites caused by *E coli* and *V cholerae* or on the skin, as in the colonisation of burns by *Pseudomonas* or the anterior nares by *Staphylococcu aureus*. In addition, DNA restriction by the targeted bacterium will mean that of any collection of bacterial strains, which are potentially susceptible, only a fraction of them will be lysed at the first attempt. The remainder may be lysed after short-term passage of the phage *in vitro* such that the efficiency of plating increases, as occurs in adaptation of Vi phages for typing new strains of *S typhi*. However, the practical consequences of this are that in many cases treatment of acute infections will rely on the aetiology being known and that the pathogen in an outbreak is clonal.

In addition to the problem of clonality due to restriction other objections are raised, the most frequent of these being the development of resistance to the phage in use. Ideally phages should be selected which have surface virulence determinants, such as capsules or adhesive fimbriae, as phage attachment sites. In this case resistant mutants generally show reduced virulence.[59,60] However, it might not be possible to find such phages in which case the problem can be overcome by use of a combination of phages.[61,62] The problem of resistance development to individual phages is more likely to occur where a large population of bacteria is exposed to the phage in use. Thus resistance is least likely to develop where the individual patient treated is an epidemiological 'dead end' with no or little chance of the pathogen and phage spreading to other hosts. This could happen by careful treatment and isolation of patients in clinics or wards or where a part of a patient, such as a burnt limb, may be isolated. Antibodies will eventually develop against the phage used but this is not likely to be a major consideration in treatment of acute disease.

Phages are less likely to be efficient where the phages cannot attach to and spread between the susceptible target organisms. This would be the case where the pathogen had already become intracellular as a part of its infection cycle, where inflammatory debris may be present or where the pathogen was outnumbered by much larger numbers of inert bacteria as may occur in the large intestine.

However, these limitations do not preclude their use where conditions for replication may not be optimal. Thus it is possible to administer phage in numbers that overwhelm the pathogen, resulting in lysis without multiplication.[67]

These extrapolations from British experimental work might be useful pointers for suggesting the most promising avenues of research arising from the above work and the extensive clinical experience in Georgia and the former Soviet Union. There are a number of reasons why the development of phages for those diseases which are amenable to this approach should be carried out by government-funded agencies rather than commercial enterprises. These arise primarily out of a perceived requirement for the search for novel phages as novel virulent strains of a particular pathogen are found and the need to monitor the development of resistance where this might occur. The potential of this 'new-old' approach to control some of the bacterial pathogens which may become increasingly difficult could be explored with just a small fraction of the vast amounts of national and international financial support for basic and applied immunological research and commercial pharmaceutical development.

8 PHAGES VERSUS ANTIBIOTICS

One of the areas that has made the idea of phage

therapy attractive once again is the increasing prevalence of antibiotic-resistant bacteria. The widespread use of antibiotics in modern medical practice is related to their rapid antibacterial action and broad spectrum of activity. This last characteristic is crucial in the treatment of diseases where the pathogen has not been identified but therapy is required immediately. However, antibiotic usage has negative effects, some of which are becoming increasingly important including:

Generalised, non-specific antimicrobial activity that damages the normal microflora enabling colonisation by opportunistic pathogens.

Side effects, such as allergy and toxicity, including effects on the immune system.

Selection of antibiotic-resistant bacteria and enhanced rates of transfer in the absence of the normal flora

Enhanced spread of fungal and yeast infections.

9 OTHER APPLICATIONS

Bacteriophages, of course, have been central to the development of molecular genetics because individual phage plaques could be cloned so easily. In addition to this, they have been an invaluable component in the epidemiology of bacterial infections by phage typing, the system where the clonal identification of a strain is made by its sensitivity to a range of phages, susceptibility or resistance depending on whether a receptor is present or whether the bacterium is lysogenised by related or unrelated phages.[68]

A further novel application for the future is connected to the potential use of antisense technology. Recent work has identified ribozymes which have been shown to be useful in reducing antibiotic resistance in laboratory strains of *E coli* by this method.[69] In this work the antisense ribozyme DNA was introduced into the bacterium on a plasmid by transformation. In the clinical situation such a procedure would be very difficult but introduction by incorporation of the ribozyme DNA into the chromosome of a phage might be more effective. This would also allow a two-pronged approach to control of diseases or infections caused by multi-resistant bacteria by using phage. In those bacterial cells that may be mutants resistant to the phage in use lysis by phage could be replaced by increased susceptibility to antibiotics by expression of the ribozyme DNA.

10 SUMMARY

In summary, despite the early failures in the west, countries in the former Soviet Union have continued to use bacteriophages for disease therapy and prophylaxis and sterilisation purposes with apparent success. Given the increasing concern over multiple antibiotic resistance in a number of bacterial pathogens this is an area of disease control whose potential is proven and which should be reviewed.

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