# Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
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Review Article

J. Med. Sci., 5 (1): 1-9
January-March, 2005

# Bacteriophage: A Potential Therapeutic Agent (A Review)

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Scientists all over the world are trying to develop new antibiotics against bacteria to overcome the threat of "superbugs" (antibiotic-resistant bacteria). In this context, bacteriophage might be possible alternative weapon. Bacteriophage could use clinically as therapeutic agent for various human infectious complications e.g. surgical wound infections, post burn infections, eye infections, cerebrospinal meningitis etc. and also in many animal disease treatment such as *Klebsiella* infection in white mice, sepsis, pneumonia, colonization of the gastrointestinal tract with Vancomycin-Resistant *Enterococcus faecium* (VRE) in bacteremic mice model etc. Bacteriophage has many other applications and as bactericidal agent in food industries. It also holds a great potential in vaccine development as well as in cancer and HIV research. In this study, identifying the problems of therapeutic applications we discussed their possible solutions. Also detecting, future possible implications of bacteriophage and making comparison with antibiotics and chemotherapeutics, we found it advantageous as a potential therapeutic agent.

**Key words:** Bacteriophage, therapeutic agent

***JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.***

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## INTRODUCTION

The emergence of antimicrobial resistance among a multitude of bacterial and fungal pathogens has become one of the most critical problems of modern medicine[1]. The World Health Organization (WHO) warned very recently that the level of resistance to drugs used to treat common infectious diseases is reaching a crisis point. In this pressure situation, renewed research works on bacteriophage therapy is already started in many western countries. It can be used as a stand-alone therapy when bacteria are fully resistant to antibiotic and as a valuable adjunct to antibiotics when the bacteria are still susceptible[2].

Bacteriophage (Bacterial viruses, also called "phages") invades bacterial cells, disrupt bacterial metabolism and cause the bacterium to lyse. The first observation of phage activity was reported in India[3]. Hankin[3] noticed a marked anti-bacterial activity in the waters of Indian rivers Ganga and Yamuna against *Vibrio cholerae*. Hankin[3] demonstrated this activity on bacterial cultures and proved that this unidentified substance may be responsible to protect cholera outbreak along these riversides. A similar phenomena was observed by a Russian scientist Gamaleya only after two years later[4]. At the beginning of the 20th century, Fredreick Twort form England and Felix d'Herelle from Canada, working at the Pasteur Institute in Paris reported that isolated filterable entities were capable of destroying bacterial cultures and producing small cleared areas on bacterial lawns. Considering many possibilities they concluded this may be a virus[5]. After 2 years, Felix d'Herelle, a French-Canadian microbiologist officially named these ultra microbes as "Bacteriophages" (bacteria eater)[6].

During the first World war hemorrhagic dysentery outbreaks among troops in 1915. That time d'Herelle cultured the bacteria *Shigella* from patients fecal and studied their growth on agar cultures, which he initially called taches, then taches vierges and later, plaques[6]. d'Herelle findings were presented during 1917 meeting of the Academy of Sciences and they were subsequently published[7] in the meeting proceedings. D'Herelle made aware and considered prior discoveries but he mentioned that it is viruses and not "enzymes"[8,9].

**Past and present studies of bacteriophage as therapeutic agent:** The nature of bacteriophage was not clearly known at the earlier days of it's therapeutic uses. It was widely used around the world in the 1930s and 1940s and after the discovery of antibiotics, the bacteriophage research was continued in Russia, Poland, Georgia and Eastern Europe[10].

The first attempt to use bacteriophages therapeutically is the d'Herelle's attempt to the treatment of dysentery[6]. The first reported application of phages to treat human infectious diseases came in 1921 from Richard and Maisin[11], who used bacteriophages to treat *Staphylococcal* skin diseases. The bacteriophages were injected into and around surgically opened lesions and regression of the infections is found within 24 to 28 h[6].

In Tblisi, Georgia (1963-64), phages were used to treat bacterial dysentery[12]. It was proved very effective not only to treat dysentery but also diarrheal diseases of unknown origin among children. It was argued that the phage preparation, although developed specifically against *Shigella* species, was also active against some additional gastrointestinal pathogens[10]. Recently, a combination of phages and antibiotics was used in treating bacterial dysentry (by *Shigella*) and salmonellosis (by *Salmonella*)[13]. In an earliar study, bacterial dysentry (by *E. coli.* and *Proteus*) immunosuppressed leukemia patients were treated with combination of phages and bifidobacteria[14].

Some applications of bacteriophages as a therapeutic agent by scientists of the Eastern Europe are also found[10]. Peritonitis, osteomyelitis, lung abscesses and post surgical wound infections by *Staphylococcus, Streptococcus* and *Proteus* treated by phages, where antibiotic resistance were found[15]. Immunogenesity of therapeutic phages was analysed and observed it did not impede the effectiveness of therapy in *Staphylococcus*, *Klebsiella*, *E. coli.*, *Pseudomonas* and *Proteus* infections[16]. Intestinal dysbacteriosis by *E. coli.* and *Proteus* was successfully treated by phages together with bifidobacteria in 500 low-birth- weight infants[17]. Also, patients with gastrointestinal tract, skin, head and neck infections by *Staphylococcus, Pseudomonas, E. coli., Klebsiella* and *Salmonolla* were treated with phages. The overall success rate was 92%[18].

In case of suppurative infection caused by *Staphylococcus* and various gram-negative bacteria, orally administration of phages yielded positive results[19]. Letter the efficacy of phage therapy of suppurative and inflammatory complications in oncological patients was assayed by Kolchetkova *et al.*[20]. Incorporation of antibiotics, specific phages and autovaccine was carried out in 1380 patient with infectious allergoses (rhinitis, pharyngitis, dermatitis and conjunctivitis) caused by *Staphylococcus, Streptococcus, E. coli., Proteus, Enterococci* and *P. aeruginosa*[21]. The agents proved effective in 48.0–82.5% of cases. It was established that most effective was the complex of specific phages and antibiotics[20].

Bacteriophage therapy was used in the treatment of recurrent subphrenic and subhepatic abscess with jejunal fistula after stomach resection. In microbiological examination of *E. coli.*, antibiotic-resistance was discovered. After administration of bacteriophages the operation was performed without any antibiotics. During the whole stay at hospital the patient had got bacteriophages. He left the hospital in 33rd day of stay without any abscesses[22]. *Staphylococcus, E. coli.* and *Proteus* generated acute and chronic urogenital inflammation were treated. The efficacy of treatment was 92% (marked clinical improvements) and 84% bacteriological clearance[23]. Very recently orally administered phages were used successfully to treat cerebrospinal meningitis (by *K. pneumoniae*) in a newborn[24]. Similar result was found in another experiment[10]. To normalize Tumor Necrosis Factor alpha (TNF) levels in seum and the production of TNF and interleukin-6 by blood cell cultures and various chronic bacterial disease was the subject for treatment using bacteriophage[25].

It is proposed that bacteriophages can effectively be used for the treatment of post-burn infections, particularly against the ubiquitous opportunistic pathogen *Pseudomonas* spp., known to be notoriously resistant to a variety of antibiotics. In study, phage therapy has an 80% success rate against *Enterococcus* infections and upto 90% against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*[26]. To prevent infection of skin grafts applied to contaminated wounds of burned patients phage could be used effectively. This is supportive by an experiment where infection of split skin grafts in guinea pigs by *Pseudomonas aeruginosa* and *Acinetobacter* 3719 was used and protected by phage therapy[27].

Slopek *et al.*[18,28], reported that the effectiveness of phages against bacterial infections including multidrug resistant mutants[29,30]. Later Laverentz *et al.*[31] summarize the previous studies. The pathogens included were *Staphylococci, Pseudomonas, Escherichia, Klebsiella* and *Salmonella* and the treatment was initiated after the isolation of the etiologic and selecting specific highly potent phages from a collection of more than 250 lytic phages[32].

In many other clinical studies in Soviet papers it was proved that phage is effective in treating lung infections. Phages in combination with antibiotics were successfully used to treat lung and pleural infection caused by *Staphylococcus*, *Streptococcus*, *E. coli* and *Proteus*[33,34]. In an earliar study Meladze *et al.*[35] used only phages to yield 82% success rate. *P. aeruginosa* infections in systic fibrosis patients[36], eye infections[37], neonatal sepsis[38], urinary tract infections[23] and surgical wound infections[39,40] were treated successfully by phages preparation.

Zhukov-Verezhnikov *et al.*[41] compared the effectiveness of "adapted" bacteriophages (i.e., phages selected against bacterial strain isolated from individual patients) to that of commercially available phage preparations. Due to more specificity, the adapted bacteriophages were reported to be five to six fold more effective in curing suppurative surgical infections. Injected phages store themselves elusively in malignant tumors and the erlich carcinoma loses a high percentage of its transplantation ability upon phage addition[42]. Phage interactions with platelet $\alpha v\beta 3$ integrin and platelet-bound CD40L939, may produce effects similar to drugs interfering with their functions (e.g. eplifibatide) and thereby inhibit platelet hyperactivation which appears to play a prominent role in the initiation of atherosclerosis and its complications[43].

In cancer treatment, bacteriophage showed promising potentiality. Bacteriophage treatment was applied in 20 cancer patients (age: 1-66 years), 17 with solid tumors, 3 with hematological malignancies, all with concurrent bacterial infections (by *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *K. oxytoca* and *E. coli.*). The cure of infection was achieved in all cases (cessation of suppuration, closure of wounds, eradication of pneumonia etc.)[44]. Phages also could contribute to immunosurveillance against cancer by blocking $\beta 3$ integrin activity of neoplastic cells thus preventing their growth *in situ* and metastasis formation. Also, by occupying the $\alpha v\beta 3$ intigrin receptor, phages could deprive neoplastic cells from growth signals provided by extracellular matrix proteins[45].

In USA, colonization of the gastrointestinal tract with vancomycin-resistant *Enterococcus facium* (VRE) has become endemic in many hospitals and nursing homes. In an experiment to find the solution of this problem bacteriophage therapy was tested to rescues mice bacteremic from a clinical isolate of VRE. In conclusion, it was found that the phages have functional capability to rescue mice and it is not due to the nonspecific immune effect[46].

By many experiments, on animal disease treatment bacteriophage proved a successful and potential therapeutic agent. In an experiment Smith and Hugging[47] used anti KI phage *in vitro* and *in vivo* against a 018:KI1+7ColV+ *E. coli* strain, designated as MW. Another experiment was conducted treating *E. coli.* diarrhoea in calves, piglets and lambs[48]. A further experiment in calves lead to the isolation of seven phages highly active *in vitro* and *in vivo* against one or other

seven bovine enteropathogenic strains of *E. coli* belonging to six different serotypes isolated form sewage. Seven experimentally induced *E. coli* diarrhoea in calves was cured by a single dose of $10^5$ phage organism[49]. In another very recent study, it is showed that the Smith and Hugging phage and antibiotic therapy results from experimental infections with a capsulated *E. coli* (KI) in mice are qualitatively and quantitatively robust[50].

Bogovazova *et al.*[51] showed that intraperitoneal introduction of bacteriophage preparation was effective in the treatment of generalized *Klebsiella* infection in white mice. Later the monovalent preparation of *Pneumoniae* bacteriophage and the polyvalent bacteriophage preparation was used for the treatment of infections caused by *K.ozaene*, *K. rhinoscleromatis seleromatis* and *K. pneumonia sensu lato*. Recently in Norway, Olssenl *et al.*[52] reported that clinical trials in Eastern Europe, phages have been used successfully in treatments against antibiotic resistant bacteria, for instance in suppurative wound infections, gastroenteritis, sepsis, osteomyelitis and pneumonia. This encouraging data are supported by recent findings in well-controlled animal models demonstrating that phages can rescue animals from a variety of fatal infections.

Bacteriophage could be used in food industries. Bacterial food poisoning remains a major worldwide health problem. Much of human *Salmonella* infection originates from poultry, thermophilic *Campylobacter* spp. are the most frequently identified human enteric pathogen in England and Wales[53]. Poultry meat is a major source of *Campylobacter jejuni* infection [54,55] and up to 90% of flocks appear to be colonized with this organism at the time of slaughter. Surface contamination of carcasses by *Salmonella* and other enteric pathogens have been reduced by the application of lytic phages[56].

The use of naturally occurring, lytic phages to reduce contamination of fresh-cut produce with foodborne pathogens has several advantages over the use of chemical sanitizers and washes[31]. Methods commonly used in industry, such as aqueous washes containing chlorine formulations or plain water, are nonspecific and can achieve a less-than-10-fold reduction in *Listeria monocytogenes* populations on cut-produce surfaces[57]. Alternatively, specific phages attack the targeted pathogens only, thus preserving the competitive potential of the indigenous microflora[10]. They also can reduce the bioburden on produce of those bacteria that are resistant to antibiotics[58]. Homologous phages and bacteriophage pools have been used against spoilage bacteria on meat. To reduce the potential for the development of phage-resistant mutants, a cocktail of different phages is applied. Lytic bacteriophages may provide an effective alternative for decontaminating fresh-cut fruits that may contain various bacterial pathogens[59].

Nisin, a broad-spectrum, pore-forming bacteriocin, produced by lactic acid bacteria, active against *L. monocytogenes*[60] that are often found on produce combination of phage treatment with the application of the bacteriocin nisin to control the *L. monocytogenes* on fresh cut apple and honey dew melon has been proved very effective[61,62]. Using the combination of a phage cocktail and nisin, *L. monocytogenes* populations decreased by 5.8 log units (to a final population of 1.4 CFU $mL^{-1}$) and by 3.5 log units (to a final population of 0.6 CFU $mL^{-1}$) on honeydew and apple slices, respectively[63]. The storage life of adipose tissue could be increased from 4 days in controls to 8 days in phage-treated samples by preventing the development of off-odors associated with the growth of *B. thermosphacta*[58].

**Genetically engineered phage as therapeutic agent:** The application of genetic engineering to modified phage gene and other purposes is proved possible. The ability of the phage to transfer virulence genes has important implications on the development of vaccines against bacteria. For example, a non virulent *Vibrio cholera* without toxin gene could be used as vaccine but it turn into a fully virulent one if infected by CTX phage. In addition, using phage in the prophylaxis and treatment of bacterial infections in humans, lethal-agent delivery systems also have immense potential at the preharvest stage in the biocontrol of *E.coli* O157:H7 in animals and fresh foods[64] and could play a role in preventing transmission of fish pathogens[65]. Non-lytic phages can be used to deliver DNA encoding bactericidal proteins to bacteria. In this process phage is used as a lethal agent delivery vehicle, the M13 phagemid system and the addiction toxins Gef and ChpBK. The phage delivery of the lethal-agent phagemids pGef and pChpBK, resulted in significant reduction in circulating bacteria compared to the time-matched control, pUPRIP[66].

**Factors affecting the efficacy of bacteriophage therapy:** Efficacy of phage was determined almost exclusively by quantitative clinical criteria and also the efficacy depends on the pharmacokinetics of phages. Other several factors could hamper the efficacy of phage therapy or may cause troublesome to the patients under therapy[20,67].

- One of the important factors in therapeutic success is the ability of a phage to multiply faster than the bacterial pathogen can spread.
- Interpretation of the information as part of a kinetic process of "ecological interactions".

- The type of proinflammatory complications (wound infections management result is the best).
- The microflora pattern of corresponding monoinfection.
- Characteristic of the therapeutic phages (*P. aeruginosa* phase is characterized by the highest therapeutic activity, as compared to *Staphylococcal* and other phages.)
- Knowledge of detailed information on specific infections via molecular biology and gene manipulation techniques.

**Route of administration:** It is already supported that bacteriophage could be detected in the blood and internal organs of the animals within 24 h irrespective of the route of its administration, e.g., intraperitoneal, intravenous or intranasal[68]. Bacteriphage is also administered orally, rectally, locally, intravenously, intramouscularly and sometimes directly as spray on wounds or pleural mucosa[10,47].

**Types of Administration of Bacteriophage:** Kolchetkova *et al.*[20] administered bacteriophage parallel with antibiotic, after long ineffective antibiotic therapy, phages alone starting from the onset of the purulent complication. Complex treatment of specific phages with antibiotics is also reported[21].

**Problems associated with bacteriophage therapy and possible solutions:** The major disadvantage of phages is their narrow host range, which could overcome by using 'multivalent' phages or a cocktail containing a range of lytic phages against the major strains of pathogen. Another drawback of phage therapy is that the phages can be susceptible to host restriction enzymes where DNA methylation of phages could be a solution. Purification of phage particles is also necessary to avoid the presence of toxin in crude phage lysates. Reticuloendothelial syetem clearance of phages is another problem, which could be reduced by a serial passage technique, e.g., *Escherichia coli.* Phage mutants and *Salmonella typhimurium* phage P22 is long-term circulation mutant phage[69].

Another problem associated with phage therapy research that the pharmacokinetics of self-replication agents differs from those of normal drugs. Treatment outcome depends critically on various density dependent thresholds, often with apparently paradoxical consequences. An ability to predict these "thresholds" and associated "critical time points" is necessary of phage therapy is to become clinically practicable[67].

In therapeutic use of lytic phages, phage-resistant develop frequently. It is well documented that phage neutralizing antibody present in circulation[16] after the administration of phage to the patient. Antiphage antibodies can also be detected in the sera of patients traced with these phages. Suring regression of infection, a titre fall has been observed. This data suggest that naturally occurring phages can induce humoral immunity[70,71]. Furthermore, *in vitro* humoral responses to phage phiX174 have been used for more than 30 years in clinical immunology as a measure of T helper cell dependent antibody production[72]. Also some lysogenic phage carry genes that can enhance the virulence of the bacterial host. For example, the CTX phage integrates into the chromosome of *V. cholerae* and the lysogen expresses cholera toxin. Lastly, by genetic engineering we can change the bacteriophage properties to expand the spectrum of their lytic activity and to eliminate therapeutic drawbacks of some natural phages[73].

**Future possible implications in bacteriophage therapy:** Phages hold a great potential future not only as a solution to antibacterial antibiotic crisis (the problems of super bug) but also to contribute in may branches of science. Cellular interaction with phage is still unrevealed and such interactions should be investigated[25,74]. Once phages are detected in animal sera and suggested that interactions between phages and mammalian cells should be investigated[75]. In fact the preferential accumulation of phages in tumor tissues and inhibition of tumor growth was already demonstrated in the 1940s and binding to neoplastic cells was confirmed[42,76]. $T_2$ phages also bind *in vivo* to guinea pig leukocytes (but not erythrocytes). In addition it is suggested that phages can attach to the plasma membrane of lymphocytes[77].

Bacteriophage therapy is arises a new horizon in cancer treatment. Bacteriophage can upregulate depressed immune responses when administered in-patients with infections[25]. Short incubation of human lymphocytes and monocytes with bacteriophage *in vitro* may induce intracytoplasmatic cytokine synthesis in lymphocytes and monocytes[71]. It indicates that patients who had undergone bacteriophage treatment may acquire increased resistance to subsequent bacterial and viral infections[28]. For these reasons, bacterial infections in immunocompromised cancer patients should be a special target for bacteriophage treatment. Also, very recently Polish Scientists proposed that phages should bind cells expressing $\alpha$IIb$\beta$3 (and to some extent, $\alpha$v$\beta$3), i.e., platelets, neoplastic cells (positive for $\beta$3 ) and activated T cells. If this interaction can fully revealed phages will in future not only be use as a antibacterial agent but also in

the potential treatment of cardiovascular and autoimmune disease, cancer and transplant rejection[45]. KGD+ phages could provide local immunosuppressive and thereby prevent the development of autoimmuno colitis[78].

Phages could contribute in progress of HIV research. It engages in a combat zone in the viral battlefield, at the level of plasma membrane and HIV infection[79] and thereby help our immune system that usually does not prevent the reinfection but does prevent clinical disease[80]. Recently, Japanese Scientists reported that bacteriophage therapy could be very effective to treat infectious disease in aquaculture[73].

Some companies are trying to exploit different eccentricities of phages in their quest for new drugs. For instance, PhageTech in St. Laurent, Canada, is studying a myriad of phage "killer" proteins that derail the host metabolism to make it easier for the phages to reproduce. Again, Schaak and her colleagues launched "MicroStealth Technologies", which aims to use phages as delivery vehicles for antimicrobial peptides that are only active inside bacterial cells[81].

**Advantages of phage therapy over chemotherapeutics:** Bacteriophages have been proved more effective than antibiotics in treating certain infections in human and experimentally infected animals[10]. Phages are very specific to their targeted bacterial species where antibiotics target both pathogenic and normal microflora[82]. Phages also replicate at the site of infection and are thus available where they are most needed[47]. Antibiotics and chemotherapeutics causes multiple side effects including intestinal disorders, allergies and secondary infections (e.g., yest infections)[83] but a few minor side effects of therapeutics phages is reported[18,84] which may have been due to the liberation of endotoxins from bacteria lysed *in vivo* by the phages. Such effects also may be observed when antibiotics are used[85]. Antibiotics are selected for many resistant bacterial species, not just for resistant mutants of the targeted bacteria but[86] phage-resistant bacteria remain susceptible to other phages having a similar target range. Developing a new antibiotic (e.g., against antibiotic-resistant bacteria) is a time-consuming process and may take several years[87,88]. Instead, selecting new phages (e.g., against phage-resistant bacteria) is a relatively rapid process that can frequently be accomplished in days or weeks[10]. Production cost of therapeutics doses of bacteriophage is low in comparison to antibiotics and sometimes only a single dose administration is sufficient to get proper response[10].

Phages are very abundant in nature and the most frequent life forms of earth, being virtually omnipotent. In the former Soviet Union and Poland as well as in the USA, phage preparations were licensed for sale in 1930's[10,44]. Despite pharmaceutical companies are actively trying to develop new antibiotics, today's bacteria have developed supergenes. But if phage properties, like exponential growth and the ability to mutate and overcome bacterial mutations and resistance can be optimized, phage therapy may be a way forward in the fight against antimicrobial resistance[81].

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