Bacteriophage precision drug against bacterial infections

K. Sandeep

Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity against *Vibrio cholerae*, which he observed in the waters of the Ganga and Yamuna rivers in India, and he suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics[1].

Frederick Twort in 1915 and Felix d'Herelle in 1917 recognized that some viruses infect bacteria. By 1920 they isolated bacteriophages from several bacterial species and started using them as anti-bacterial agents. In the 1930s and subsequent decades, virologists such as Luria, Delbruck and many others utilized these viruses as model systems to investigate many aspects of virology, including virus structure, genetics, replication, etc.

Phage therapy products were licensed for sale in the United States in 1930s. The US firm Eli Lilly marketed seven different phage preparations to fight *staphylococcus*, *streptococcus* and *E. coli*. The Ministry of Health of the former Soviet Union routinely licensed active phage preparations for use in humans. German and Red Army soldiers were said to carry vials of phages in their medical kits, during war time.

By the early 1940s, antibiotics were introduced as a cure for all bacterial infections. Bacteriophage therapy came to a halt in most of the world, except in the former Soviet Union and parts of Eastern Europe. Several hundred reports on phage therapy are available. Majority of these were from the former Soviet Union and Eastern European countries.

Now there is a widespread development of antibiotic resistance in pathogenic bacteria, so the need for new antibiotics and alternative strategies to control microbial infections is of increasing urgency.

Bacteriophages are the viruses that infect bacteria. There are thousands of different bacteriophages, each of which may infect one or several types of bacteria. Some bacteriophages infect several related species of bacteria, however they do not infect antigenically unrelated bacteria. Bacteriophages are common in all natural environments and are directly related to the numbers of bacteria present. There are at least 12 distinct groups of bacteriophages, which are diverse structurally and genetically.

When a bacteriophage meets a suitable host bacterium, its tail fibres bind to the precise molecules that distinguish that particular bacterium as a suitable host. Bacteriophage then injects its strand of genetic material into the cell.

There are two main groups of bacteriophages – lytic and lysogenic. They have two different strategies for getting their host to replicate them. Lytic bacteriophages instruct the machinery in the host cell to make more bacteriophages. Fully viable progeny bacteriophages burst out and kill the bacteria. The released bacteriophages attack new bacteria. Each of these cycles takes an average of 30 min and produces about 50–400 phages. This process continues until all the bacteria are eliminated from the system.

The lysogenic bacteriophages attach their strands of genetic instructions to the DNA of the bacteria. The bacteriophage DNA gets replicated along with the bacteria, generation by generation. This group of bacteriophages can also cut their piece of DNA free from the host's DNA at any time, and instruct the host cell to produce a large number of bacteriophages. This leads to burst of bacterial cell and release of newly formed bacteriophages.

Only the lytic phages are a good choice for developing therapeutic phage preparations. Lysogenic phages are inappropriate candidates for phage therapy as it may not destroy bacteria immediately. They can also transfer virulence genes and those mediating resistance to antibiotics to other bacteria.

Western scientists have now re-discovered bacteriophage therapy as a potent weapon against antibiotic-resistant bacteria. Eliava Institute of Bacteriophagey, Microbiology, and Virology of the Georgian Academy of Sciences, Tbilisi, Georgia, was a leading centre for investigation, especially dangerous bacterial organisms. The Institute also worked in isolation and selection of active bacteriophages against the pathogenic bacteria, and in creation of therapeutic phage preparations. The preparations of specific phages have been successfully used in burns, sepsis, gastroenterological infections, paediatric infections and also in surgical departments, against the primary and nosocomial infections.

In the former Soviet Union and Eastern Europe, physicians routinely used phage therapy in the general practice, extensively in paediatric, burn and surgical hospital settings. Phage preparation was carried out on an industrial scale till the break-up of the Soviet Union. Tonnes of tablets, liquid preparations and spray containers of carefully selected mixtures of phages for therapy and prophylaxis were produced and used. The largest use was in hospitals, to treat both primary and hospital-acquired infections, alone or in conjunction with antibiotics. The problems of bacterial resistance were overcome by the use of well-chosen mixtures of phages with different receptor specificities against each type of bacteria. The results were further improved whenever the clinicians typed the pathogenic bacteria and monitored their phage sensitivity. In many cases, using phage in conjunction with other antibiotics showed better results than either the phage or the antibiotic alone.

The Eliava Institute isolated new broader-acting phage strains in 1985–86. In the years since, there have been continued improvements in the formulation based on further studies, and phages against *Klebsiella* and *Acinetobacter* have been isolated and developed into therapeutic preparations. One of the latest developments is the Intestiphage preparation, which includes 23 different phages active against a range of enteric bacteria. An enteric-coated pill was also developed, using phage strains that could survive the drying process. In the last decade the Institute focused on nosocomial infections, where multi-drug-resistant organisms have become a particularly lethal problem. Many clinical studies confirm the high effectiveness of bacteriophage therapy in combating bacterial infections which do not respond to treatment with the available antibiotics[2].

In Poland and Europe bacteriophages were successfully used in the treatment of purulent meningitis in new-born caused by *Klebsiella pneumoniae*[3], subphrenic abscess[4], suppurative bacterial infec-

tions[5], chronic suppurative infections of the skin caused by *Pseudomonas, Staphylococcus*, *Klebsiella, Proteus* and *Escherichia*[6], postoperative septic *Staphylococcal* infections[7], septic arthritis of the knee and osteomyelitis[8].

In the former Soviet Union phage therapies were successfully used to treat infections caused by *Pseudomonas aeruginosa*, *staphylococcus* and other bacteria[9]. Phage therapy was also found to be effective in *Klebsiella* infections[10]. Bacteriophages had no effect on normal microflora and did not aggravate dysbiotic disturbances. For this reason, bacteriophages may become one of the alternative antimicrobial remedies, selectively affecting infective agents[11].

Antibiotic therapy is now facing a new challenge in the rise of bacterial strains resistant against many antibacterial substances. This necessitates the search for new highly active and safe antibacterial preparations. It is interesting to note that all presently available antibiotic groups were developed more than 40 years ago with the exception of one group, oxazolidinone, which was introduced into the market in the late nineties. Bacteria got enough time to develop resistance against many of these drugs. In the 1990s drug-resistant bacteria increased rapidly, whereas development of new antibiotics lagged behind. Resistant strains of bacteria are present in all the available antibiotics. Therapeutic application of the bacteriophages on the background of spreading of the multi-resistant microorganisms should be viewed as the alternative for available antibacterials.

Many biomedical research institutions around the world are developing phage treatments that fight infectious diseases such as tuberculosis and salmonella, as well as anthrax. The Johns Hopkins University has expanded its tuberculosis biopharmaceutical development program by establishing a collection of clinical isolates to *Mycobacterium tuberculosis* from the key geographic regions of the world, where tuberculosis represents a major public health challenge. The emerging bacteriophage product candidate for tuberculosis is currently in the early stages of manufacturing and pharmaceutical development. The bacteriophage therapy may revolutionize the treatment of tuberculosis in the near future.

Phages cannot infect the cells of organisms more complex than bacteria because the surface properties of these cells are not susceptible to the bacteriophage invasion. Bacteriophages can be isolated wherever that particular bacterium grows. They are present in water, skin, air, food, body surface, throat, intestine, faeces, sewage, soil, ocean depths and hot springs.

Advantages of bacteriophage therapy

1. For every type of bacteria known in nature, there is at least one complementary bacteriophage that specifically infects a single bacterial species. So bacteriophage therapy is possible in all bacterial infections.

2. If a suitable bacteriophage is introduced onto an infected wound, it will continue to increase in numbers as long as there are bacteria to infect and destroy. However, as soon as all the bacteria have been destroyed, the action of the phage will cease and the dormant phage particles will disperse harmlessly.

3. Because phages are so specific to the bacteria they infect, they will not harm other beneficial bacteria present in the intestine and other parts of the body and will not affect the microbial ecosystem in the body. There is no chance of super infection with other bacteria. The bacterial imbalance caused by treatment with many antibiotics can lead to serious secondary infections involving relatively resistant bacteria, often extending hospitalization time, expense and mortality. This will not occur with specific bacteriophage therapy.

4. Some people are allergic to antibiotics so phage therapy could be a useful alternative for these patients. No patient has ever been known to suffer an allergic reaction to bacteriophages. That may be because phages are omnipresent living organisms on earth, found in soil, water, plants and humans.

5. Phage therapies can be administered to patients in different ways which include pills, injections, enemas, nasal sprays, ointments, etc.

6. Each phage infects a specific bacteria or range of bacteria. A person in hospital, where bacterial infections abound, can be treated with a range of phages targeted at several types of bacteria. They can be given a cocktail of phage types to attack one type of bacteria or they can be given a combination of phage and antibiotic treatment.

7. Phages are considered safe for therapeutic use. No major side effects have been described so far. Only a very few side effects have been reported in the patients undergoing phage therapy. This might be related to extensive liberation of endotoxins from dead bacteria as the phages were destroying the bacteria most effectively. This type of reaction can also happen when antibiotics are used.

8. Because bacteriophages grow exponentially, a single dose is often sufficient to treat an infection. It is able to self-reproduce as long as corresponding host-bacteria are present in the environment. Therefore the need for repeatedly administering the phage is greatly reduced.

9. An important feature of phage therapy is that bacteriophages do not infect human or animal cells.

10. Bacteria can develop resistance against both antibiotics and phages. Since phage is targeted to receptors on bacterial membrane or capsule, which are important virulence determinants, development of phage resistance usually means changes in those structures and may, therefore, lead to attenuation of the strains in virulence. Unlike antibiotics, bacteriophages can mutate in step with evolving bacteria. If bacteria become resistant to bacteriophages, other bacteriophages species can attack those new resistant strains. Mutations that enable bacteria to resist antibiotics do not enable bacteria to resist bacteriophages. Development of a new antibiotic is a very expensive and time-consuming process; it can take over 10 years and several million dollars to develop a new antibiotic. Developing of a new phage, on the other hand, can potentially be accomplished in days, at a much lower expense.

11. Since selection of active phages is a natural process, evolutionary arguments support the idea that active phage can be selected against every resistant bacterium, by an ever ongoing process of natural selection.

12. Production is simple and relatively inexpensive. So the treatment costs of bacterial infections will be reduced. This facilitates their potential applications to underserved populations.

Problems associated with bacteriophage therapy

1. Because of the high specificity of phages, the disease-causing bacterium has to be identified before the administration of phage therapy. One phage kills only a specific subgroup of bacteria. One species

of bacteria may contain many subgroups. But one antibiotic may kill many different species and subgroups of bacteria simultaneously. So a physician would need to make a specific diagnosis before prescribing a phage treatment.

2. Absences of bacteriophage action efficacy in certain cases were reported. It may be due to insufficient diagnostics and incorrect choice of the method for implementation of a specific phage.

3. The gastric acidity should be neutralized prior to oral phage administration.

4. Bacteriophage with a lytic lifecycle within a well-defined *in vitro* environment does not ensure that the bacteriophage will always remain lytic under normal physiological conditions found in a body. It may change to adapt lysogenic cycle in some circumstances.

5. Bacteriophages are viruses and, in general, viruses tend to swap genes with each other and other organisms with which they come into contact. So there is a chance of spread of antibiotic resistance in bacteria.

6. Many doctors are scared to give live bacteriophage to the patients.

Another specific treatment for bacterial infections under experimental stage is the use of bacteriophage enzymes. One type of bacteriophage enzyme acts only on specific species of bacteria without affecting normal bacterial flora. All double-stranded DNA bacteriophage contains a lytic system consisting of 'lysin' which is capable of degrading the bacterial cell wall to allow phage release. In Gram-positive bacteria, exogenously added lysin can lyse the cell wall and bacterial death.

These bacteriophage enzymes limit the spread of infection and kill targeted bacteria on contact. These enzymes could be used as spray, lozenge, mouthwash, suppository, inhaler, bandages and eye drops. These enzymes are in the experimental stage and will be available for human use within few years.

Recently, highly evolved enzymes from bacteriophage were tested to eradicate the carriage of pathogenic group A streptococci in mice. The researchers examined the killing activity of one of these enzymes on group A streptococci using lysin, from the streptococcal bacteriophage C1. It is also showed that giving C1 phage lysin does not harm the normal mucosal bacteria. The use of phage lytic enzymes offers a safe alternative to antibiotics. It may also be used to prevent the spread of streptococci to classmates and family members. The therapy could be important in developing countries like India, where streptococcal throat infection may lead to rheumatic fever.

Vincent Fischetti and colleagues at Rockefeller University in New York isolated the enzymes, called Pal and Cpl-1, from a bacteriophage that targets *S. pneumoniae*[12]. A nasal spray containing the enzyme could be an effective alternative to conventional antibiotics, and could help to eliminate human reservoirs of the bacteria. In *in vitro* experiments, Pal killed 15 strains of *S. pneumoniae* within seconds of contact, but did not interfere with human cells or other, harmless, bacteria that grow in the nose and throat.

1. Sulakvelidze, A., Alavidze, Z. and Morris, G. J., *Antimicrob. Agents Chemother.*, 2001, **45**, 649–659.
2. Weber-Dabrowska, B., Mulczyk, M. and Gorski, A., *Arch. Immunol. Ther. Exp.*, 2000, **48**, 547–551.
3. Stroj, L., Weber-Dabrowska, B., Partyka, K., Mulczyk, M. and Wojcik M., *Neurol. Neurochir. Pol.*, 1999, **33**, 693–698.
4. Kwarcinski, W., Lazarkiewicz, B., Weber-Dabrowska, B., Rudnicki, J., Kaminski, K. and Sciebura, M., *Pol. Tyg. Lek.*, 1994, **49**, 535.
5. Slopek, S., Weber-Dabrowska, B., Dabrowski, M. and Kucharewicz-Krukowska, A., *Arch. Immunol. Ther. Exp.*, 1987, **35**, 569–583.
6. Cislo, M., Dabrowski, M., Weber-Dabrowska, B. and Woyton, A., *Arch. Immunol. Ther. Exp.*, 1987, **35**, 175–183.
7. Slopek, S., Kucharewicz-Krukowska, A., Weber-Dabrowska, B. and Dabrowski, M., *Arch. Immunol. Ther. Exp.*, 1985, **33**, 261–273.
8. Lang, G., Kehr, P., Mathevon, H., Clavert, J. M., Sejourne, P. and Pointu, J., *Rev. Chir. Orthop. Reparatrice. Appar. Mot.*, 1979, **65**, 33–37.
9. Kochetkova, V. A., Mamontov, A. S., Moskovtseva, R. L., Erastova, E. I., Trofimov, E. I., Popov, M. I. and Dzhubalieva, S. K., *Sov. Med.*, 1989, **6**, 23–26.
10. Bogovazova, G. G. *et al.*, *Zh. Mikrobiol. Epidemiol. Immunobiol.*, 1992, **3**, 30–33.
11. Bogovazova, G. G., Voroshilova, N. N. and Bondarenko, V. M., *Zh. Mikrobiol. Epidemiol. Immunobiol.*, 1991 **4**, 5–8.
12. Loeffler, J. M. and Fischetti, V. A., *Antimicrob. Agents Chemother.*, 2003, **47**, **1**, 375–377.

K. Sandeep is in Sector Reform Cell, Directorate of Health Services, Thiruvananthapuram 695 035, India.
e-mail: sandeepkerala@hotmail.com