Phage therapy: present and future

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Abstract. In recent years, bacteriophages are known to have become an effective alternative to antibiotic drugs. The article describes the current and potential applications of bacteriophages and phage endolysins. Also of interest is the devastating effect of phages on biofilms. The development of phage resistance is touched upon as well. Furthermore, the authors discuss the issue of laying down the rules of rational phage therapy.

Perhaps we are seeing the beginning of a new era in the principles of treatment of infectious diseases. An important problem in medicine today is the increasing antibiotic resistance of microorganisms. One solution to this problem is to use bacteriophages.

As an alternative to antibiotics, bacteriophages were included into «The Comprehensive Program for Development of Biotechnology in the Russian Federation through 2020» adopted by the Russian government on April 24, 2012.

In numerous studies bacteriophages are considered as promising anti-infective preparations.

But the question arises: is it safe to use phages, will it not lead to any adverse health consequences?

1. Medical applications

It is known that bacteriophages are found in the places where the relevant target bacteria are accumulated, e.g., in wastewater, soil, etc. Thus, it would be fair to say that if there is a bacterium, there should be a bacteriophage specific to it. This is an extremely useful feature as it makes it possible to provide highly specific treatment of infectious diseases. This justifies the numerous studies on the use of phage-based drugs in various fields of medicine.

The State Register of Medical Remedies (as of October 4, 2016) includes the following bacteriophage preparations: Dysenteric bacteriophage, Salmonella bacteriophage (of groups A, B, C, D, E), Pyobacteriophage, Klebsiella pneumoniae bacteriophage, Klebsiella bacteriophage, Typhoid bacteriophage, E. coli-Proteus bacteriophage, Intesti-bacteriophage, Streptococcal bacteriophage, E. coli bacteriophage, Staphylococcal bacteriophage, Proteus bacteriophage, and Pseudomonas aeruginosa phage.

All these are Russian-made products. The drugs are manufactured by the FSUE «NPO Microgen». Today, it is the only enterprise in the Russian Federation producing bacteriophage preparations as therapeutic agents.
Bacteriophages are specified in the list of medications approved to treat diseases, conditions and monitoring for treatment in the health care standards in the presence of the transplanted lung [1], campylobacteriosis in children [2], shigellosis [3], pertussis [4], etc.

Bacteriophages have acquired particular importance in treating gastrointestinal diseases, including acute intestinal infection, which has been confirmed in several studies. They can be used to correct dysbiosis in children [5]. There is evidence for possible use of bacteriophages in epidemic foci and emergencies as a measure of prevention of shigellosis, salmonellosis, typhoid fever, etc. [6].

Bacteriophages are used in treating ENT disorders. It was found that bacteriophages shorten the duration of treatment during exacerbations of chronic laryngitis. [7].

Bacteriophages are effectively used in surgery. A study performed on the wound infection prevention in a group of patients who had their lower limb amputated has shown that administration of phage therapy reduces the risk of postoperative complications [8]. In a comprehensive treatment of chronic osteomyelitis, the use of bacteriophages has also shown high efficiency [9].

Phage preparations can be applied in dentistry. For example, a study using the drug « Sextaphag » has proven its efficiency in treating gingivitis and parodontium [10]. Bacteriophages have shown a bactericidal effect on E. faecalis even when a root canal biofilm is formed [11].

It is also possible to use bacteriophage preparations in gynecology for the treatment of lower genital tract infections [12].

There is evidence for successful use of phage therapy in urology [13]. Positive results were observed in treating different urinary tract diseases in children [14].

It seems promising to use bacteriophages in pulmonology. Positive results have been reported in respect to pneumonia caused by Pseudomonas aeruginosa [15, 16].

1.1 Biofilms and bacteriophages
A particularly interesting area of phage therapy is the possibility of using bacteriophages to control biofilms. Biofilms pose a major problem in modern medicine. They are formed in the organs, tissues, and implanted equipment. Biofilm formation leads to the occurrence of certain chronic infections. But the most important thing is that antibiotic resistance significantly increases in a conglomerate of bacteria.

Phages produce three types of enzymes which have a devastating effect on biofilms: 1) exerting a hydrolytic effect on the biofilm extracellular polymeric substances; 2) acting on the bacterial cell wall; 3) disrupting the bacterial capsule [17].

The film-forming staphylococcal strains were shown to have high susceptibility (more than 50%) to bacteriophage preparations [18]. Another study proved the efficiency of bacteriophages in the prevention of P. aeruginosa biofilm formation and destruction of those already formed [19].

These findings support the theory of efficient use of bacteriophage preparations against biofilms.

1.2 Phage-associated lysins (PAL)
An important problem in applying phage therapy is to prevent the development of phage resistance. For this purpose, other possible applications of phage preparations are currently being developed. In addition to the possibility of using whole virus bacteriophages, research is being conducted on the use of phage-associated lysins (PAL). The PALs are endolysins, or murein hydrolases, i.e., enzymes which promote cleaving the bacterial cell wall peptidoglycan. Their action occurs only in the presence of the second factor, i.e., choline membrane protein which forms pores in the plasma membrane and provides endolysin with an access to bacterial cells [20].

The efficiency of SPZ7 endolysin against gram-negative bacteria was shown in [21]. It seems promising to use PMG1 endolysin isolated from Pseudomonas aeruginosa [22].

PAL-based drugs do have their advantages: firstly, they exclude the presence in the preparation of foreign genetic material which patients may disfavor or try to avoid; secondly, these substances may be obtained synthetically, thus eliminating the multistage process of culturing bacteriophages.
2. Bacteriophages in biomedical nanotechnologies

In studying bacteriophages, it was found that their size ranges from 20 to 200 nm. Thus, most bacteriophages may be qualified as «nanoparticles». These nanoobjects are created by nature; therefore, their distinctive features are complete naturalness and high specificity. For example, it is possible to use bacteriophages as vectors for delivering genes into the cells in order to change their functions [23]. It is particularly important to apply phage peptide libraries for obtaining tissue- and tumor-specific peptides which can be used as independent therapeutic agents or to deliver drugs to specific target organs [24]. In addition, temperate phages can be used to obtain recombinant bacterial strains. A bacteriophage is integrated into the bacterial genome as a prophage, and the microorganism receives additional, new genetic information. This contributes to bacterial synthesis of various substances, such as enzymes, hormones, regulatory proteins, drugs, etc.

3. Other uses of bacteriophages

By now, interest in bacteriophages has increased so much that there are attempts to use them in various spheres of human activity.

A Russian company, Microcosm SPC Ltd., manufactures such products containing phages as «Phagogin» (gel for intimate hygiene), «Otophag» (gel for treating ENT infections), etc. But they do not have a registration number and are not included in the State Register of Medical Remedies.

Bacteriophages can be used in veterinary medicine. They have proven effective in the treatment of colibacillosis in pigs [25]. In another study [26], it has been proven that they reduce the risk of the presence of Salmonella in chicken products.

Bacteriophages are successfully used as dietary supplements for the decontamination of food and for the prevention of a number of infections [27]. They enter into the composition of probiotic dietary supplement «Foodphage» which has proven effective as a drug for correcting dysbacteriosis [28].

Phages are also used in the cosmetic industry. In particular, JSC Faberlic has started introducing bacteriophages into personal and intimate hygiene means. MIRRA Company (Russia), which produces drugs to treat acne and inflammatory skin changes, is also involved in this activity.

4. Phage resistance

Bacteria and bacteriophages have evolved continuously and interdependently, and since these microorganisms are closely interrelated, the changes in them have been aimed at acquiring properties giving the edge over the competitors. On the part of bacteriophages, these properties imply better ways to infect and destroy the bacterial cell. Bacteria, in turn, have developed mechanisms for phage resistance.

Bacteria are able to protect themselves almost at any stage of the infection process. The first mechanism involves blockade of the surface receptors, synthesis of the extracellular matrix and competitive inhibitors to prevent phages from being adsorbed onto the bacterial cell surface. The second mechanism uses phage injection blocking. The third mechanism is triggered after bacteriophage injecting into the bacterial cell genome. Restriction endonucleases recognize and cleave alien DNA. This is known as the restriction-modification (R-M) system. One more way to prevent the development of an infection is an abortive infection when the host bacterium dies and the virus does not spread any further in a population of susceptible strains [20].

A comparative study of the susceptibility of bacterial strains to antibiotics and phages isolated from patients has shown that about 50% are bacteriophage-susceptible. This figure is higher than the susceptibility of these strains to antibiotics but also indicates the presence of phage resistance [29].

The possibility of applying bacteriophages in medical practice depends on the conditions in which phage resistance develops. But, at the moment, this problem is not given due consideration.

This issue became a matter of interest to specialists of the Medical Faculty at the INPE NRNU MEPPhl. Extensive studies are underway at the Departments of Microbiology, Virology and Immunology in order to identify phage-resistant pathogens of pyoinflammatory diseases:
Staphylococcus aureus и Streptococcus pyogenes. However, it is too early to speak about any results at this stage of research.

5. Principles of rational phage therapy
Attempts are made of a wide, large-scale introduction of bacteriophages into practice. However, in the past, we had the imprudence to connive at wide, irrational, uncontrolled use of bacteriophages which resulted in the development of antibiotic resistance in a number of microorganisms. Phage therapy should initially be based on definite standards, i.e., principles of rational use. This problem has been repeatedly addressed in various research works. Based on the acquired knowledge, we can already identify the following main points:
1) Only drugs officially recommended for phage therapy and commercially produced by licensed manufacturers should be used;
2) A drug can be used provided that there are reliable data that it contains only virulent phages but not temperate ones;
3) Administration of bacteriophages can be justified only if antibiotics are found ineffective or cannot be applied;
4) Before administering phage drugs, the phage susceptibility of the target microorganism should be determined;
5) It is necessary to confirm high lytic phage activity against the specific pathogen;
6) Bacteriophages can be used both as monotherapy and in combination with antibiotics;
7) Phages should be applied strictly according to the instruction, following the recommendations on the method of administration and treatment period; the recommended phage titers should be maintained;
8) With regard to phage drugs, it is important to observe the storage precautions; prolonged storage decreases the phage titer and, as a consequence, its activity;
9) Bacteriophages can be prescribed as preventive drugs in epidemic areas in emergencies but not before the phage susceptibility of microorganisms has been determined.

6. Conclusion
Studying the effectiveness of bacteriophages leads as often as not to positive results. Currently, there are all the necessary knowledge, skills and equipment for performing accurate research in this area. Phage drugs are certainly not a panacea for infectious diseases they but represent a promising alternative to antibiotics. Perhaps, we shall have yet to learn about some features of these microorganisms. For example, previously there were no adequate data on the bacterial resistance to phages, which has become a matter of concern nowadays. It is necessary to competently treat these drugs, limiting their potential widespread use. Using bacteriophages requires clear guidelines and rules approved by the WHO.

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