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From Bacteriophage to Antibiotics and Back

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ABSTRACT

Life is a phenomenon, and evolution has given it countless forms and possibilities of survival and formation. Today, almost all relationship mechanisms between humans who are at the top of the evolutionary ladder, and microorganisms which are at its bottom are known. Preserving health or life is not just an instinctive response to threat anymore; rather it is a deliberate action and use of knowledge. During major epidemics and wars which create great suffering, experiences of the man-disease (cause) relationships were examined, so we can note the use of bacteriophages in Poland and Russia before and during the Second World War, while almost at the same time antibiotic therapy was introduced. Since bacteriophages "tracked" the evolution of bacteria, the mechanism of their action lies in the prokaryotic cell, and it is not dangerous for the eukaryotic cell of human parenchyma. It is, therefore, necessary only to reuse these experiences nowadays when we are convinced that bacteria have an inexhaustible genetic and phenotypic resistance mechanism of their own. Antibiotics continue to represent the foundation of health preservation, but now they work together with specific viruses – bacteriophages that we can produce and apply in the context of multi-resistance, as well as for the preparation of new pharmacological preparations. This new approach promises knowledge and possibilities for a new antibiotic-bacteriophage model.

Key words: *antibiotics, antibiotic resistance, bacteriophage, historical overview*

History of Phage Therapy

During the cholera epidemics in 19th century India, around 20 million people lost their lives. Those who drank water or swam in the Ganges rarely contracted cholera[1]. Hindu ritual baths take place in the Ganges River which, due to the monsoons, contains large quantities of phages from the ground. Although in the Hindu world the purpose of these baths is a physical and spiritual purification, their primary effect is therapeutic. In 1896, British bacteriologist Ernest Hanbury Hankin showed that water from the Ganges and Yamuna rivers contain some biological substance which destroys cholera cultures. It could pass through the Millipore filters which retained larger microorganisms such as bacteria[2]. Two years later, Russian microbiologist Gamaley noticed a similar phenomenon[3]. However, it was not until 20 years later that Frederick Twort confirmed that the substance which passes through the filter can completely break bacteria, but cannot grow in their absence[4]. Twort described it as a substance secreted by microorganisms for a purpose unknown at the time[5,6]. Twort's results were published in 1915 in The Lancet journal which is why the discovery of bacteriophage is connected with his name[7]. Twort's work in The Lancet reminded Felix d'Herelle of his previous research conducted in Mexico and Tunis on treating the plague-infected locusts[8]. D'Herelle conducted the first application of bacteriophage as medication on soldiers who suffered from bacillary dysentery[6,9,10]. Even the name 'bacteriophage' was suggested by d'Herelle, a combination of the word 'bacteria' and the Greek *'phagein*' meaning to eat. D'Herelle believed that bacteriophages are viruses which destroy bacteria. His contribution is also evident due to the foundation of several centers for treatment via bacteriophage [11]. For his idea of treating humans and animals against various bacterial diseases with bacteriophages, he was nominated for a Nobel Prize eight times but was sadly never awarded[8].

Although d'Herelle's treatments of patients with bacteriophages conducted in a hospital in Paris were very successful, they were not immediately published[12]. The first published study of bacteriophage treatment dates from 1921 when Richard Bruynoghe and Joseph Maisin announced that they used bacteriophage in the treatment of staphylococcal skin infection[13]. The treatment's positive results provided an incentive for additional research. All

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of this also prompted a few companies to start producing phages commercially and to continue to use them to treat various bacterial infections. D'Herelle's laboratory situated in Paris produced five different phage preparations whose purpose was to treat bacterial infections[6]. In Poland, which also leads the way in bacteriophage research and application to treat humans today, the firsts cases of phage therapy were recorded in 1926/1927 at the Jagiellonian University Medical College Clinic of Surgery in Krakow [10,14]. Despite criticism, research was continued at an institute in Wroclaw, today known as The Institute of Immunology and Experimental Therapy PAS in Wroclaw[15]. The Soviet Union used phage therapy during the Second World War when it was effectively used to treat soldiers[8,16]. Since then in Georgia, a former Soviet Union Republic, phages have been used intensively and with some successful results at the George Eliava Institute in Tbilisi, Georgia. Currently, the Institute has accomplished impressive results in the treatment of wounds and bedsores, ulcers, burns, and purulent osteomyelitis. Phage preparations can also be bought in their pharmacy and used to treat infections caused by *Streptococcus*, *Escherichia coli*, and *Pseudomonas*[17–19]. Phage intended for therapeutic treatments were also produced in the United States of America. In 1940, the Eli Lilly and Co. company produced seven phage preparations intended for human use[6,20].

The majority of early bacteriophage research was based on preventing bacterial infections[10] and were conducted in the former Soviet Union or Poland. It was not until the end of the 1980s that bacteriophages were discovered 'again' and research on animals started[21,22]. Research on humans began in the 2000s. Results of the first phase of randomized clinical research carried out with bacteriophage in the USA were published in 2009[23].

Emergence of Antibiotics

By imitating the relations in microbiocenosis, complex pharmaceutical products were created – drugs that in very small doses and concentrations suppress or destroy pathogens in humans and animals. They are called antibiotics, and their mechanisms of action includes: damaging the cell wall, disabling production of essential bacterial protein, blocking transporting cell wall's mechanism, blocking cell reproduction, blocking bacterial enzymes, etc.[24,25]. Antibiotics are drugs that are selectively toxic to bacteria, and nontoxic or acceptable toxic for the host's organism.

The founder of modern antimicrobial therapy is Alexander Fleming who in 1928 published his research on growth inhibition of *Staphylococcus aureus* on a feeder contaminated with molds *Penicillium notatum*. The discovery of penicillin was followed by the discovery of sulphonamide in 1932, streptomycin in 1943, chloramphenicol in 1946, erythromycin in 1948, vancomycin in 1953, rifampin in 1957, ciprofloxacin in 1961, daptomycin in 1986, bedaquiline in 1997[24].

More than 4000 antibiotics were isolated from microbial sources, and more than 30000 synthetic and semisynthetic ones were produced by natural origins (of same or similar compositions and operating principles). In clinical practice only about 100 various antibiotics are used because of the harmful consequences they provoke in the patients or because their wider usage would be too expensive[26,27]. Therefore, the theoretical value of antibiotics depends only upon their activity, i.e., their efficiency, speed, simplicity, availability, and safety which can fall within these characteristics: selective toxicity (bacterial cell versus host cell), therapeutic ration, tolerance, pharmacokinetics, and pharmacodynamics[28]. Antibiotics are divided according to their mode of operation, effect, mechanism of action, and chemical composition.

Antibiotics had soon become an irreplaceable cure for an increasingly large number of bacterial diseases while research and creation of new antibiotics promised a carefree future[24]. However, already after twenty years of the systematic antibiotic application, it was noted that infections with some agents responsible for diseases (gram positive and gram negative bacteria) could not be overcome with existing antibiotics anymore, and synthesis of new, mostly selective antibiotics ensued[29]. Combinations or alternative antibiotics were used, which led to panic at the end of the last century. In fact, some types of bacteria, using their genome which was upgraded during billions of years, create resistance against the most commonly used antibiotics, and soon afterward against all available ones[26].

Countless attacks, both biological and environmental, 'teach' the bacteria which then create a resistance mechanism against most antibiotics by producing new proteins which block the antibiotics or repair damaged systems in the cytoplasm, and can also change cell wall structure as the first line of defense (the most effective barrier). Therefore, bacterial resistance and understanding its mechanisms becomes the subject of study while new antibiotics are synthesized simultaneously[30,31].

Resistance may be natural, i.e., permanently displayed through the 'wild' phenotype, or acquired, displayed through mutations or new gene acquisition[24]. The inherited – natural resistance is exhibited by bacteria by making it more difficult for antibiotics to enter the gram-negative cell of a bacteria. In that way, lipopolysaccharide membranes stop the entry of penicillin 6, macrolides, and vancomycin while some inactivate antibiotics via bacterial enzymes (e.g., *Klebsiella* which has beta-lactamase). An example of natural resistance is the absence of affinity between antibiotics and the target location or the absence of the target location. Therefore mycoplasmas, which do not have a cell wall, are resistant to beta-lactamase, antibiotics which influence cell wall synthesis[32].

Acquired resistance is far more dangerous than the natural since it exhibits little known mechanisms which bacteria can develop, including gene modification. Cross-resistance is the simultaneous resistance to all antibiotics in the same group (e.g., all beta-lactamase antibiotics)[33]. Multiple resistance is a resistance to various groups of antibiotic combinations of several different and independent resistance mechanisms[25,34].

Bacteria develop acquired resistance through horizontal and vertical gene transfer, and recombination mechanisms. Horizontal gene transfer is a direct transfer of ge-

netic material between biologically unrelated species, while in the vertical gene transfer genetic material passes from the parents to their daughter's cells. Gene recombination mechanism, when bacteria exchange genes via third parties, can occur between two bacteria by the process of transformation, conjugation, and transduction. By recombination, bacteria acquire new genes and therefore become resistant to antibiotics they have never been treated with. Especially 'dangerous' way of gene transfer is conjugation, i.e., gene transfer during sexual reproduction (copulation) between different species of bacteria (parts of plasmids being transferred)[25,35]. Therefore, bacteria acquire new genes which can produce or enhance resistance to antibiotics they never had direct contact with.

Because of all this, in the 21st century antibiotics can be as efficient with treating and stopping the spread of contagious bacterial diseases as they were two centuries ago. More and more multiresistant species appear making likely that even the most simple operations may become fatal because of postoperative infection with the aforementioned multiresistant bacteria[34]. The most dangerous types may be both gram-positive and gram-negative bacteria: *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, Penicillin resistant, *Streptococcus spp* (PRSP), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant, *Enterococcus* (VRE), extended-spectrum beta-lactamases bacteria (ESBLs). However, the biggest danger lies in the resistance of gram-negative (intestinal bacteria) against carbapenems: imipenem, meropenem, ertapenem[36].

Apart from the aforementioned large problems regarding antibiotics (except increasingly larger bacteria resistance), the frequent implementation of inadequately researched or tested antibiotics brings about new dangers. This is why FDA (Federal Agency of the United States) notes that there is a real danger of antibiotics (ciprofloxacin, ofloxacin, norfloxacin, gemiloxacin, moxifloxacin and others) with regard to the permanent nerve, kidney, and eye damage; while fluoroquinolones present a risk for children, senior citizens, and pregnant women[37]. It is therefore obvious that bacteria have a genetic potential and resistance mechanisms against antibiotics because they have encountered similar attacks during their evolution, which resulted in the development of new defense mechanisms[25]. When the questionable and almost inefficient use of antibiotics against bacterial infections from biofilm is added, it becomes evident that a return to the drawing board is needed and the relations in microbiocenosis, in which bacteria have a powerful and well-known enemy – the bacteriophage, need closer attention. Let us then look at how bacteriophage may aid science and medicine to regain balance and to provide the human race with a carefree life and progress.

A Natural Antibiotic – The Return of the Bacteriophage

As always, when we cannot solve existing problems in the usual way, we look for a solution by going back to the drawing board. Luckily, we do not have to go back to the very beginning because there are enough knowledge, experience, and results which claim that bacteriophages can be considered in the change of treating bacterial and viral diseases[38,39]. If we remember the limitations and obstacles to treating diseases with antibiotics, it will become evident that these problems occur because bacteria acquire new attributes. Via transformation, transduction, and conjugation – the mechanisms of gene recombination, bacteria acquire the ability to respond to antibiotics since they are not as adjusted and reshaped as bacteria, i.e., antibiotics are outdated answers.

Bacteriophages are similar to viruses; they can modify their genome twice as fast as prokaryote cells. Treating bacterial diseases on the principle of parasitic antibiosis with the aid of phage preceded the use of antibiotics. Therefore, only the principle of mimicking natural balance can offer incessant search for new right answers. Bacteriophages decipher bacteria molecule's signals in biofilm, hydrolyze the intercellular barrier by disintegrating the extracellular polymeric substances (EPS), adhere to bacterial receptors and "infiltrate" their genes into bacterial protoplasm, and may even disintegrate the protein-protective mechanism in the bacteria itself[40].

In vitro studies of phage relationship on plankton and biofilm cells confirm that the selected course is correct, and the use of bacterial phage in the prevention of biofilm formation on endoprosthesis and catheters suggests a possible effective use of phage *in vivo.* Ethical committees allow the application of bacteriophage in the treatment of animals and humans in the terminal phases of infections with multi-resistant types of bacteria. Due to a series of ethical and moral doubts, such application is termed a test, not a treatment for prudential reasons.

By declaring that bacteriophage preparations are a drug, their application is additionally prevented without clinical trials which are too short and too expensive in the search for radical change in the accustomed way of treatment of some bacterial diseases[41,42]. It is to be believed that despite some justified, but also some unjustified limitations, the most optimal solutions will be found because everything else would lead to an increasingly likely defeat against more and more serious forms of a significant portion of bacterial diseases and infections.

Phage is very effective in treating animals, especially fish and poultry, even for industrial purposes, for plant pathogens growth control (bio-controllers), and in food protection before conservation[38,43–46] (Table 1). Experience outside of human application has significantly contributed to the so-called Declaration of Helsinki in 2005, under which bacteriophage therapy is permitted on humans and animals for experimental treatment[47]. Phage therapy allows the treatment of many bacterial infections that have not yet been overcome by the use of traditional pharmacologic agents[14,48]. Some examples of phage application for human and animal treatment or use in preventive protection of endoprosthesis, catheter, and other areas that can be contaminated by biofilms illustrate current experience and knowledge of real possibilities and directions in the treat-

TABLE 1
LIST OF COMPANIES THAT PUBLISH THE USE OF BACTERIOPHAGE AS BASIC TECHNOLOGY[69]

	Company name	Country	Website
Preclinical phage therapy research and development			
1.	AmpliPhi Biosciences	SAD	www.ampliphibio.com
2.	Enbiotix	SAD	www.enbiotix.com
3.	Fixed Phage	UK	www.fixed-phage.com
4.	InnoPhage	Portugal	www.innophage.com
5.	Intralytix	SAD	www.intralytix.com
6.	Pherecydes Pharma	France	www.pherecydes-pharma.com
7.	Technophage	Portugal	www.technophage.pt
The development of products competent phages			
8.	AvidBiotics	SAD	www.avidbiotics.com
9.	Eligo Bioscience	France	http://eligo.bio/
10.	Enbiotix	SAD	www.enbiotix.com
11.	Phico	UK	www.phicotx.co.uk
Phage product distribution			
12.	Biochimpharm	Georgia	http://biochimpharm.ge/
13.	Imbio	Russia	http://home.mts-nn.ru/~imbio/
Patient phage therapy			
14.	Center for Phage Therapy	Poland	www.iitd.pan.wroc.pl/en
15.	Eliava Phage Therapy Center	Georgia	http://eliavaphagetherapy.com/
16.	Phage Therapy Center	Georgia	www.phagetherapycenter.com
17.	Phage International	SAD	www.phageinternational.com
Phage-mediated biocontrol			
18.	APS Biocontrol	UK	http://apsbiocontrol.com/
19.	Epibiome	SAD	www.epibiome.com
20.	Intralytix	SAD	www.intralytix.com
21.	Omnilytics	SAD	www.omnilytics.com
22.	Phage Biotech	Israel	www.phage-biotech.com
23.	Phagelux	China	www.phagelux.com
24.	Technophage	Portugal	www.technophage.pt
Phage lysate products			
25.	Delmont	SAD	https://delmontlabs.com/
Development of enzybiotics			
26.	GangaGen	SAD/India	www.gangagen.com
27.	Lysando GmbH	Germany	www.lysando.com
28.	New Horizons Diagnostics	SAD	www.nhdiag.com
Phage-based bacterial detection technologies			
29.	Sample6	SAD	www.sample6.com
Phage-associated industrial contamination			
30.	Phage Consultants	Poland	www.phageconsultants.com

ment and prevention of bacterial and viral diseases[7,49]. (Table 1)

The problems of phage treatment are that they are recognized by the immune system as foreign matter, and are not able to reproduce with ease. Thus, phage does not succeed in multiplying by a large enough number to infect a sufficient number of bacteria. On the other hand, phage is not used as often largely due to the pharmaceutical indus-

try where antibiotic production is increasing even at the expense of large investments in research and synthesis of new types[50].

Phagotherapy is a term that will be used in further consideration as it implies a variety of procedures that are used in chemotherapy, radiotherapy, which are usually used in treatment[51]. The name of the drug is phage, and the bacteriophage, in the strict sense, will be referred to as therapeutic phage (lithic phage).

Clinical Application of Bacteriophage

Resistance of some gram-negative bacteria (*P. aeruginosa, E. coli, Klebsiella*) and some gram-positive ones (*S. aureus, Streptococcus sp, Enterococcus*), as a cause of severe diseases and septic states, was provoked, among other things, by inadequate and unsuitable therapeutic doses, mutation ability, or as a result of the difficult circumstances of treatment of immunocompromised patients[52]. These are causal reactions which significantly reduce the overall resistance of infected patients and increase their likelihood of secondary infections with the same resistant types. The application of phagotherapy is already apparent today in people with diabetes infected with MRSA, those infected with *Burkholderia cepacia* (BCC), and those suffering from cystic fibrosis (CF)[53–56].

Cystic fibrosis is a disease of the lower respiratory system with chronic duration, and it is genetically conditioned[57]. CF is a result of the mutation of the CFTR (cystic fibrosis transmembrane conductance regulator) gene with more than 1,500 known mutations. The most common result is the loss of amino acid phenylalanine in the protein regulating the transport of chlorine through the cell membrane[58]. Due to this process, a very viscous and sticky secretion occurs in the lower airways and prevents normal coughing and removal of bacteria. In such patients, therefore, infections with gram-negative bacteria are very common, predominantly with *Pseudomonas aeruginosa*[59]. It has been shown that the cause of these infections is *Burkholderia cepacia*, also a gram-negative bacteria, which is also resistant to antibiotics[60]. BCC infections are the second leading cause of death among hospital patients (the mortality rate is around 20% in Canada)[61]. It spreads rapidly among lying patients, especially patients suffering from CF, and in the acute phase it results in septicemia, heart failure, and significantly reduces life expectancy. Phagotherapy was performed on mice infected with isolated strains of BCC from immunocompromised patients with cystic fibrosis. Isolated strains of BCC are highly resistant to antibiotics, and completely to rifampicin, ampicillin, cefepime, and others[62].

Inflammation of *B. cepacia* and phagotherapy using the aerosol in the lung was first performed with several different phages[54]. Aerosol *B. cepacia* was applied 24 hours before the aerosol phage, and two days after treatment, significant and dramatic reduction in the number of bacteria (logarithmic reduction rate) was observed. Before infection, the mice were immunocompromised with cyclophosphamide (CPA) which causes leukopenia, and the significant reduction of *B. cepacia* could not be attributed to the immune system of the host[63]. Phage therapy in mice infected with BCCs strains from patients with CF is very effective and could be applied to humans.

In mice, co-therapy of lithic bacteriophage and linezolid were also implemented in the treatment of diabetic foot infection caused by MRSA[56]. In annual diabetic patients, there is a chance that the "diabetic foot" disease will occur in 1%, and the pathogenesis mechanism includes neuropathies, infections, and ischemia. The person with diabetes does not feel pain in the area of the lesion, and the anti-inflammatory response of the organism is suppressed, making patients aware of infection only at the wound drainage stage[64]. Exceedingly wide-spread mixed type infections are a major problem because the cause of the infection is very often resistant (multi-resistant). One of the most common causes is MRSA.

A very effective antibiotic – linezolid (oxazolidinone)[64] can be used for the treatment of MRSA purulent infections in the heel for diabetics, and the experiment was intended to confirm the existence of a similar agent – the therapeutic phage, which is best suited for *in vivo* administration[56]. This research was also performed on mice. The rear heels of diabetic mice were infected with MRSA clinical isolates, identified by gram response and biochemical probes. Therapeutic phage MR-10 was injected into the rear foot of diabetic mice. Chhibber et al., through this study demonstrated the efficacy of phage and antibiotic linezolid therapy[56]. Pharyngeal and linezolid therapy gives the best results, and it is possible to conclude that combined therapy reduces the occurrence of resistance, simplifies and reduces the application. Also, it has been shown that phages allow antibiotics to be more effective, i.e., they accelerate their entry into the bacteria and thus become an effective alternative to the treatment of MRSA infections in people with diabetes.

This experience of *in vivo* co-therapy is consistent with *in vitro* experiments on the combined effect of bacteriophage and antibiotics on resistant strains of *E. coli* and *P. aeruginosa* in particularly hard conditions - in biofilm[65]. Biofilm is a community of prokaryotic cells coated with the extracellular polymer matrix. It is protected from antimicrobial drugs and acts as a matrix for the establishment and development of slow-growing subpopulations of resistant bacteria and specific gene expression[66]. In order to reduce the risk of developing resistant cells, combined therapy is often used. This primarily refers to the combination of multiple antibiotics (tobramycin + clarithromycin, daptomycin + clarithromycin). Likewise, phage cocktail used as pre-treatment can significantly reduce the development of biofilm[66]. Combinations of antibiotics and phage in therapy more effectively prevent the development of biofilms or reduce already formed biofilm matrix for one to two logarithms[66]. There are no reports of resistance in biofilm treated with phage and antibiotic combination.

Study of co-therapy of bacteriophage and the antibiotic tobramycin on the biofilm of mixed culture of *E. coli* and *P. aeruginosa* has shown that such combined treatment reduces the number of resistant bacteria[65]. *E. coli* and *P.*

aeruginosa cultures were used, T4 bacteriophage was used to infect *E. coli*, and bacteriophage PB-1 was used to infect *P. aeruginosa.* Tobramycin was used as the antibiotic. Tobramycin is an aminoglycoside bactericide derived from *Streptomyces tenebrarius*, (tetrasaccharide structures, streptamine structures). It is an inhibitor of bacterial protein synthesis and an inactivator of microbial enzymes. It is more effective against gram-negative bacteria, but in a broader spectrum also against some gram-positive bacteria[66]. Coulter et al. have shown that phage and antibiotics act to reduce resistant bacteria. In monoculture of *E. coli* treated with tobramycin or T4 phage, the reduction is about 39%, and their combination reduces the number of resistant bacteria by 99%[65]. In monoculture of *P. aeruginosa*, the individual reductions for both T4 phage and tobramycin are about 60%, while their combination reduces the number of resistant bacteria by about 99%[65]. Since the study was conducted in pure bacterial cultures, the conclusion should also include the results of the combined effect of tobramycin and the associated specific therapeutic phage for treatment in polymicrobial biofilm infection.

In some studies, it has been observed that in biofilms with mixed cultures, specific phages are not fully effective, probably due to the different types of EPS produced by those types, and the specific phage does not have an effective response to that nonhomogeneous matrix. This is why the research done in the Qvin Astrid military hospital in Belgium on patients with colonized burns is very insightful[67]. Infected wounds were treated with phage cocktail (phage active against *P. aeruginosa* and *S. aureus*). In controlled conditions, the examination was conducted on 9 persons and 10 burns. Selected phage cocktail BFC-1 intended for the treatment of *P. aeruginosa* and *S. aureus* (phage from the families *Myoviridae, Podoviridae*, and ISP *Myoviride*) was at a concentration of 109 units/ml purified from endotoxin. Previously classical bacteriological identification had established that the wounds were infected with *P. aeruginosa* and *S. aureus*. Directly before applying BFC-1 phage to the colonized burns, the wounds were divided into two equal parts, one half being treated with standard therapy. Thus, patients with suspected MDR strains of *P. aeruginosa* received amikacin in combination with ceftazidime and meropenem, and patients with suspected MDR strains of *S. aureus* were treated with vancomycin and linezolid. The other half of the same patients' wounds were treated with BFC-1 phage cocktail at 1 ml sterile and endotoxin purified by spraying system and on average 0.03ml per square centimeter of the wound (or about 10^7 phages). The surface of the wounds was approximately 95 cm^2 on average, and after 2 to 3 hours the biopsy was performed on each. After the initial colonization of the wounds from the homogenized sample after the final biopsy, taken after the BFC-1 phage treatment, only a small bacterial load was observed while unchanged bacterial loads were observed in wounds treated with antibiotics. Rose et al. undoubtedly confirmed the effectiveness of phagotherapy on burns[68].

Conclusion

The question is whether phage can be the second line of defense, the line that all the more powerful pathogens cannot overcome? Of course they can, because their desirable and positive effect is incomparably greater than the possible unwanted consequences. Science is constantly discovering new forms and preparations of phagotherapy, gene therapy, antibiotic co-therapy, diagnostics, disinfection, decontamination, and neutralization of biological toxins. The ability to self-duplicate, the specificity of action, the harmlessness, the high degree of desirable mutation, and availability obligate scientists to carefully study the relationship between humans, diseases, and treatment (phage), as well as insufficiently clear *in vivo* dynamics of phage, immune response, and activation of defense mechanisms. It is obvious that the main principle of nature is to find d answers in the same place where questions arise.

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OD BAKTERIOFAGA DO ANTIBIOTIKA I NAZAD

S A Ž E T A K

Evolucija je životu kao fenomenu, dodijelila bezbrojne oblike i mogućnosti opstanka i nastajanja. Danas su poznati gotovo svi mehanizmi odnosa čovjeka s vrha evolucijske ljestvice s mikroorganizmima koji su na njezinom početku. Očuvati zdravlje, odnosno život, nije više samo instinktivni odgovor na ugrozu, nego smišljeno stvaranje i korištenje znanja. U okolnostima velikih epidemija i ratova koji stvaraju veliku patnju, primijenjena su iskustva tih odnosa čovjek-bolest (uzročnik) pa tako bilježimo uporabu bakteriofaga u Poljskoj i Rusiji prije i za vrijeme Drugog svjetskog rata, a gotovo istovremeno uvedena je i antibiotska terapija. Budući da su bakteriofagi – virusi „pratili" evoluciju bakterija, mehanizam njihovog djelovanja počiva na prokariotskoj stanici, pa time jesu neopasni za eukariotsku stanicu ljudskog parenhima. Bilo je dakle potrebito samo vratiti ta iskustva u današnje vrijeme kada smo se uvjerili da bakterije imaju na izboru neiscrpan vlastiti genski i fenotipski rezistencijski mehanizam. Antibiotici i dalje predstavljaju temelj očuvanja zdravlja, ali sada u kombiniranom djelovanju sa specifičnim virusima – bakteriofagima koje možemo proizvesti i primijeniti u okolnostima multirezistencije, ali i za pripremu novih farmakoloških pripravaka. Dakle, okrenimo se tim novim znanjima i mogućnostima „sve do novog modela antibiotik-bakteriofag".