

Bacteriophages or “bacteria eaters” alternative to antibiotics, vaccine preparation and treatment of severe infectious diseases

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ABSTRACT

Bacteriophages are viruses with a simple structure that attack and kill bacteria, but like other more complex relatives they are perfect parasites that survive by infecting a host, in their case bacteria. Bacteriophages are among the most common and diverse organisms in the biosphere, ubiquitous where there are also bacteria. Without being harmful to macroorganisms, bacteriophages can be found in ocean and sea water, soils, plants, animals, people, wastewater and food of animal origin contaminated by bacteria. It is believed that there are over 1,031 bacteriophages on earth. Currently, the expansion and intensification of the use of antibiotics has led to the increase of antibiotic resistance at the global level, and the focus of treatments on intestinal microecology has determined the intensification of research on its role in human health.

Keywords: phage and bacterial lysis, antibiotics and resistance, gut microbiota, phage display and vaccines

INTRODUCTION

Bacteriophages were discovered independently by Frederick W. Twort in Great Britain (1915) and Felix d'Hérelle in France (1915), who, from the beginning, intuited and tried their use in the treatment of bubonic plague and cholera without notable results. Further research was done in Georgia and the United States in the 1920s and 1930s, but phage therapy for bacterial infections was unsuccessful and, after the discovery of antibiotics in the 1940s, was virtually abandoned [1].

The discovery of penicillin by Alexander Fleming in 1940 ushered in the “era of antibiotics” which experienced an unprecedented development that led to effective control of most bacterial infectious diseases [2]. But the expansion of antibiotic use globally, while increasing their accessibility has exceeded medical indication, the lack in some cases of a clinical and laboratory diagnosis that differentiates

between a viral and a bacterial disease, self-medication of patients and the use of antibiotics by livestock breeders and veterinarians has inevitably led to an alarming increase in simple and even multiple antibiotic resistance [3]. It has come to a situation where bacterial infections controlled in the past are no longer possible to treat with any antibiotics and infected people are exposed to fatal danger.

In this context, the WHO declared “antibiotic resistance” a major global public health emergency and developed a strategy transmitted to the countries of the world for taking urgent measures to control this serious threat. Among the recommended strategic measures are:

- reducing inadequate antibiotic prescriptions
- strict control of population accessibility to antibiotics
- improving laboratory tests to detect bacterial infections
- development of new antibiotics

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- decrease and strict control of antibiotic use in livestock farms and veterinary medicine
- control of nosocomial infections
- development of antibacterial agents such as bacteriophages [4]

To understand the role of bacteriophages in human health, it is important to know their structure, types, mode of infection of bacteria, survival cycles and gut microbiota in humans.

Phages are simple organisms that have a nucleic acid (DNA or RNA, single-stranded or double-stranded), surrounded by a protein capsid. There are three structural forms of phages: with icosahedral head (with 20 faces) and a tail, with icosahedral head without tail and filamentous shape [5,6].

Infection of bacteria has the following phases in cycles:

1. Virulent cycle (lytic)

- adsorption on the surface of the bacterium by phage encoded proteins that recognize specific receptors in the bacterial cell membrane, tail contraction, enzymatic lysis of peptidoglycan in the bacterium membrane;
- invasion is produced by injection of phage nucleic acid into the cytoplasm of the bacterial cell;
- replication of phage nucleic acid inserted into bacterial DNA - synthesis of phage proteins (head, tail);
- assembly of the phage particle;
- ripening;
- lysis of bacterial cell;
- release of new phage particles and infection of other bacterial cells [7].

2. Temperate cycle (lysogenic) has the same phases, but stops at integrating phage nucleic acid into the bacterial chromosome with which it replicates without lysing the cell. Tempered phages and lysogenesis were discovered by Mihai Ciucă and Jules Bordet in 1920. Lysogenic phages are largely found in the human intestine and can, under certain conditions, become lytic which can be used in phage antibacterial therapy [8,9].

3. Pseudolysogenic cycle: phage enters the cell but does not replicate, and phage nucleic acid does not integrate stably into the cell genome, but is conserved. This condition is due to unfavorable conditions for the growth of the host cell, which disappears when favorable growth conditions return [10].

4. Chronic infection: phage particles are produced over long periods of time, but without cell destruction [11].

Phage research in the laboratory has opened many perspectives in obtaining artificial proteins with antibody roles or developing therapies for in-

fectious but also non-infectious diseases and we will review some important achievements [12,13]:

The first phages studied were T1-T7, T-even, T4 and T6 phages have been used as models for multiplying viruses.

In 1943, Alfred Day Hershey, Salvador Luria, and Max Delbrück discovered bacterial resistance to T1 phage through spontaneous mutation (fluctuation test).

In 1952, Alfred Day Hershey (Nobel Prize in 1969) and Martha Chase used T2 phage to demonstrate that only phage nucleic acids are necessary for replication in bacteria.

Lambda, Mu and M13 phages are used in recombinant DNA technology, and pX174 phage was fully sequenced by Frederick Sanger et al. (1977).

In 1980, George P. Smith developed a technology called phage display, which allowed the generation of artificial proteins. These proteins were produced by fusing foreign DNA fragments or engineered into the phage III gene. The phage III gene encodes a protein expressed on the surface of the phage virion. Thus, fusion proteins of gene III taken up by phages were displayed on the surfaces of virion particles. Subsequently, researchers could employ the generated antibodies to specifically identify the foreign protein fragment, enabling the purification of fusion phage cultures. This approach effectively amplifies the foreign gene sequence for subsequent investigation.

Research has also discovered the mechanisms of action of phages in anti-infective therapy, but also the role of bacteriophages in the immunity of the human body [14].

During the phage lytic infection cycle, phage-encoded binding proteins recognize and attach to receptors on the bacterial surface, such as fimbriae, flagella, porins, or specific receptor proteins on efflux pumps. The phage subsequently adheres and delivers its genomic material into the bacterial host, initiating viral replication within the cytoplasm. Following assembly into new phage particles, the cycle culminates in bacterial lysis, releasing progeny phage to infect other susceptible bacteria [15].

Phage therapy has the capacity to eliminate target bacteria but also exerts selective pressure favoring bacterial virulence or antibiotic resistance through bacterial mutations aimed at evading phage attack. Additionally, phage therapy can modulate immune responses within the gut. The interaction between phages and their hosts is heavily influenced by the intestinal mucosa. Phage communities establish contact with mucosal barriers, eliciting phage-mediated innate immune responses. Phage-mediated lysis contributes to the generation of pathogen-associated molecular patterns (PAMPs), which can translocate and activate immune responses when intestinal permeability is compromised [15-17].

An important role in homeostasis and immunity of the human body is played by the intestinal microbiota, which is increasingly researched. The intestinal microbiota comprises a complex ecosystem primarily consisting of organisms such as viruses, bacteria, fungi, and protozoa. The interactions and homeostasis maintained among these microorganisms play a crucial role in human health. Among the various microorganisms, bacteria and viruses are the most abundant within the intestinal microbiota, with bacteriophages (phages) representing the predominant type of viruses. Phages play a significant role in regulating the composition and dynamics of the intestinal microbiota [18-21].

The expansion and intensification of antibiotic use has led to the growth of antibiotic resistance globally, and the focus of treatments on gut microecology has led to increased research on its role in human health. The total bacterial population in the human digestive tract exceeds 10^{14} , with the gut microbiota of healthy individuals predominantly composed of *Firmicutes*, *Bacteroides*, *Proteobacteria* and *Actinobacteria*, which account for over 90% of the total number of gut bacteria [17,22].

Phages are the predominant constituents of intestinal viruses, with fecal filtrates containing as many as 10^8 billion virus-like particles (VLPs) per milliliter. Lysogenic phages are prevalent in the human intestine. Current research indicates that the most common intestinal phages worldwide belong to the crass-like phages. In healthy individuals from diverse geographic regions, studies have identified 23 core phages (present in >50% of individuals) and 132 common phages (present in 20-50% of individuals) across the population. The association between phages and human health can be elucidated through research on intestinal phageome, enabling exploration of their roles in disease diagnosis and treatment [17,23].

THE RELATIONSHIP BETWEEN PHAGES AND DISEASES. CLINICAL APPLICATION OF PHAGES

Phages and infectious diseases

At the outset of the twentieth century, phage preparations demonstrated efficacy in treating bacillary dysentery and cholera. Subsequently, extensive research has been dedicated to exploring phage therapy for various challenging infectious diseases [24,26].

In Poland, a research institute utilized phage therapy to treat 1,307 patients with multidrug-resistant bacterial infections, resulting in clinical improvement or cure in 85.9% of cases. Fecal filtrates derived from healthy human feces, containing extracted phages, have shown effectiveness in treating refractory *Clostridium difficile* infection. Further-

more, most phage preparations entering clinical trials target infections caused by multidrug-resistant (MDR) bacteria. Current phage preparations are mainly concentrated on *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [17,27-29].

We will list below the application of phage therapy in bacterial infections in different locations of the human body:

1. Skin and soft tissue infections

- severe burns are bacterial complicated with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and multidrug-resistant *Staphylococcus aureus*, have been treated with oral bacteriophages.
- dystrophic ulcers in the lower limbs from diabetes superinfected with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, have been successfully treated with dressings soaked in beech solutions.
- chronic acne, atopic dermatitis, psoriasis and eczema superinfected with multidrug-resistant *Staphylococcus aureus* have been treated by topical application of phages [17,29-32].

2. Oral infections - treatment for dental plaque, periodontitis, tooth decay and parodontitis caused by bacteria (*Actinobacillus*, *Fusobacterium nucleatum*, *Streptococcus mutans*) [17,33,34].

3. Gastrointestinal infections - phage treatment has been applied with good results in acute and chronic gastrointestinal infections caused by *Escherichia coli*, *Campylobacter jejuni*, *Salmonella* and *Shigella*, less often *Vibrio cholerae*, and *Clostridium difficile* [17,35,36].

4. Respiratory infections - application of phages in respiratory infections caused by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and multidrug-resistant *Staphylococcus aureus* was performed by inhalation or intravenously in animal models in which survival, and significant improvement of animal infections has been achieved [17,37,38].

5. Urinary tract infections - urinary tract infections are predominantly aerobic bacterial infections that originate in the gut in people with several underlying diseases, post-prostatectomy, kidney transplantation, or those with long-term urinary catheters. The bacteria more commonly involved are *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, bacteria often multidrug-resistant. Phages were applied orally or by impregnating catheters with phages [17,39-41].

6. Eye infections - application of phages in keratitis induced by *Pseudomonas aeruginosa*. It was performed on experimental animal models (mice). Instillation of eye drops with phages caused at 5

days the disappearance of corneal opacity and significant reduction of inflammation [17,42].

7. Ear infections. The clinical effect of some phage preparations in chronic otitis media in humans caused by antibiotic-resistant *Pseudomonas aeruginosa*, but also in animals (dogs) was evaluated, and the results consisted in significantly reducing the bacterial load and improving inflammatory phenomena and symptoms until disappearance [17,43-45].

8. Nasal infection

Phage therapy has been tested in chronic sinusitis caused by antibiotic-resistant *Staphylococcus aureus*. Intranasal application of a mixture of phages resulted in a decrease in the number of bacteria and a significant improvement in symptoms [17,46,47].

9. Sepsis/bacteremia

Sepsis refers to an infection caused by various pathogenic bacteria that invade the blood with severe evolution, septic metastases in various organs and the production of toxins in the blood (toxic-septic state). If the bacteria that invade the bloodstream are eliminated by the body's defense function and there are no obvious symptoms of toxemia, the condition will be called bacteremia. Phage therapy in sepsis/bacteremia was tested in animals by injecting lethal doses of *Escherichia coli* into mice & rats. After obtaining bacteremia at 10 minutes, 1 hour, 3 hours and 7 hours, specific phages were administered that deteriorated a survival of animals from 100% to 50% depending on the time of administration of phages.

10. Pneumonia caused by the new coronavirus

Phages can intervene in inducing an immune response to an infection due to advantages such as stability and reduced cost compared to a vaccine. Phage display technology (PDT) involves inserting the gene sequence of the exogenous polypeptide of interest into the gene encoding the phage shell protein. The modified phage protein can induce a robust immune response. Selected epitopes of Coronavirus protein S inserted into the shell protein of a vector phage induced a strong response, acting as a

vaccine produced with lower cost and greater stability [17,50-53].

PHAGE THERAPY IN NON-INFECTIOUS DISEASES

Numerous studies have demonstrated significant differences in the diversity and structure of intestinal phageome between healthy individuals and patients with chronic diseases. Comprehensive analysis of the intestinal phageome holds promise for uncovering novel insights into the pathogenic mechanisms underlying various diseases and identifying new diagnostic and therapeutic markers.

Intestinal phages play a pivotal role in the pathogenesis of inflammatory bowel disease, alcohol-dependent liver disease, diabetes, colorectal cancer, breast cancer, Parkinson's disease, and schizophrenia. They may serve not only as diagnostic biomarkers but also as targeted agents for bactericidal therapy [17,54-57].

CONCLUSIONS

Bacteriophage therapy is an extremely valuable alternative to antibiotic treatments of bacterial infections, but in the case of infections with multi-resistant bacteria will be the therapy of last resort to save lives. But it should not be forgotten that bacteriophages are modelers of the response immune to infectious diseases and can be used in obtaining safe vaccines and with robust immune response predominantly neutralizing. If we refer to research into the application of bacteriophages in non-infectious diseases, we will find favorable results in diseases by surprise considered without important therapeutic solutions at present. Analyzing some research results we will be tempted to consider bacteriophages as a “universal panacea”, but we believe that we will also witness other surprising discoveries, such as bacteriophages that are true “guardians” of people's health.

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