Bacteriophages, a Solution to the Destruction of Antibiotic-Resistant Bacteria

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Abstract

Bacteriophage is the generic name given to microorganisms that can destroy bacteria. The name (which comes from the Latin bacteria and the Greek φᾰγεῖν phagein - "to eat", "to devour") was introduced by the Canadian bacteriologist Félix d'Herelle in 1917, the year he discovered a virus possessing such characteristics. Also called bacteria-eating viruses, bacteriophages represent a group of viruses with a destructive effect on bacteria (lytic effect), being widespread in nature, in all living environments (water, soil, air). Discovered during the First World War and developed in the 1920s and 1930s, phage therapy is currently making a comeback and enjoying a renewed interest in countries such as the USA, Belgium and France, in parallel with the development of antibiotic resistance, in the face of the increasing resistance of bacteria to antibiotics, a challenge for the whole planet. Today, phage therapy has begun to be used to disinfect food. Also, before surgery, in some hospitals, bacteriophages are sprayed in the operating rooms to reduce the risk of infection. Through their mechanism of action, bacteriophages can become a kind of additional immune system for organisms, and phage therapy, a therapy to be studied and perspective for maintaining human and animal health.

Key words: antibiotic resistance, bacteriophages, lytic effect, phage therapy

1. Introduction

Bacteriophage is the generic name given to microorganisms that can destroy bacteria. The name (which comes from the Latin *bacteria* and the Greek *φᾰγεῖν* phagein - "to eat", "to devour") was introduced by the Canadian bacteriologist Félix d'Herelle. In 1917, the French scientist Félix d'Hérelle discovered viruses that damage bacteria, which he called bacteriophages or phages [1, 2]. In 1915, he was asked by the government at the time to study a dysentery epidemic. After studies carried out on samples collected from the sick, he understood that he had discovered a virus that destroyed bacteria and caused healing, which he called then bacteriophage. In September 1917, just two years after isolating the first bacteriophage from dysentery stool, d'Hérelle presented a short note to the Academy of Sciences outlining the main characteristics of the bacteriophage, as well as the theory of cure and its implications for the treatment of infectious diseases [3]. The first patient to be cured of dysentery by phage therapy would be in August 1919. The attribution of the discovery of bacteriophages is often disputed between Frederick Twort and Félix d'Hérelle. However, Twort's publication in The Lancet in 1915 was very different from Félix d'Hérelle's in 1917. In the same year that bacteriophages were discovered, i.e. 1919, the French researcher Jules Bordet received the Nobel Prize for studying the development of

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immunity against infections using antibodies. He demonstrated that antibodies are what induce cell lysis of bacteria. Jules Bordet rejected the idea that phages could have a therapeutic role, arguing that they did not exist. Félix D'Hérelle did not realize the impact of his discovery because at the time the resolution of microscopes was insufficient to visualize phages. He explained that something in the body's biological nature was digesting the bacteria. It was only 10 years after the demonstration of the therapeutic effect of phages, in 1928, that penicillin was discovered by a Scottish researcher named Alexander Fleming [4, 5]. During the same period, the theory about phages and how they could induce cell lysis of bacteria caused a heated dispute among doctors. It was only much later that d'Hérelle's theory was confirmed, as soon as the visualization of bacteriophages became possible with the help of the electron microscope. But because of the war, scientific progress slowed down. Western countries preferred the use of antibiotics in favour of phage therapy, despite its proven efficacy, because they were easier to understand, to produce in large quantities, and these antibiotics represented a universally valid solution to a wide spectrum of infections [6]. So, in the Western world, the bacteriophage was then replaced by antibiotics, but is still used to identify bacterial strains and, more and more, for its ability to hijack the DNA of the bacteria it attacks: its ability to recombine them, a real tool of genetic engineering [7]. Bacteriophage is also being explored again in the context of increasing bacterial resistance to antibiotics. These types of viruses are less familiar than the flu virus, the Influenza virus, or the Ebola virus. These viruses are different from human, animal or plant viruses, being known as bacteriophages (simply called phages), loosely translated "those that eat bacteria" [8, 9]. As their name suggests, they attack bacteria and destroy them or cause them to self-destruct. Starting from here, a form of therapy using bacteriophages - phageotherapy - was developed. Bacteriophages are far more numerous than pathogenic human viruses or those infecting any other mammal on earth. The reason is related to the fact that there are more hosts for phages than for viruses that infect our own cells. Bacteriophages are made up of nucleic acid (DNA or RNA), protein shell (capsid), and three main morphological forms of phages are known: icosahedral, cylindrical and complex (icosahedral head and helical tail) [10, 11]. Although bacteriophages are everywhere (land, oceans, ice, air), scientists first discovered them inside the human body [12, 13]. Felix d'Herelle, one of the first to talk about bacteriophages, noticed them while treating French soldiers in the First World War. He filtered the stool of soldiers suffering from dysentery and isolated microscopic particles that could destroy the bacteria that cause dysentery [14, 15]. Felix d'Herelle's idea seemed far-fetched to most scientists until around the 1930s, when the microscope had developed enough to allow attacks on bacteria to be observed. Félix d'Herelle spent the rest of his life trying to turn phages into a medical weapon used against bacterial infections. Fagiotherapy continued to attract curious researchers for decades after his death. In 1930, Stalin brought fagiotherapy to the Soviet Union in Tbilisi, Georgia to treat communist troops for dysentery. Today, in Tbilisi, there is a phageotherapy center known all over the world, where patients arrive with diseases that antibiotics cannot solve. Georgian doctors treat a wide variety of acute and chronic infections, antibiotic-resistant infections. Treatment is individualized: doctors identify pathogenic bacteria and then create bacteriophages capable of destroying them. Lately, the world of bacteria inside us – the so-called microbiome – has become the subject of more and more scientific research [16]. Those who have studied the microbiome have focused their efforts on mapping their diversity: more than 100 trillion bacteria live in our body, especially in the colon. Many cells with an immune role are located in the intestinal walls, and a breach in the intestinal wall can be very dangerous: inflammation can occur and, in addition, an environment conductive to the multiplication of pathogenic bacteria is created. To prevent such a disaster, our body tries to keep the microbiome under control, with the thick mucus layer playing an important role. The body produces approximately one liter of mucus per day, and within this mucus are bacteriophages. About five bacteriophages for each bacteria were found in the saliva, around the gums. As long as the mucus layer is dense and there is nutritious material food for bacteria on its surface, it is difficult for pathogens to penetrate. The intestine has the ability to defend itself, by secreting molecules that destroy microbes that get too close and try to pass through the intestinal wall [17]. But this way of defending does not always have the expected results. There are pathogens that have evolved, using the body's

defending capacity in their own interest, as happened with *Salmonella*. In the gut, *Salmonella* competes with other bacteria and often wins the battle with immune cells. Focused attention on the relationship between body cells and bacteria loses sight of the third important player in this ecosystem: the bacteriophage [18, 19]. Bacteriophages multiply in the colon attacking bacteria. Bacteriophages tend to specialize on certain species of bacteria. To invade a microbe host, they act like a burglar breaking into a locked house. Proteins on the surface of phages attach to host cell proteins, creating a passage through which the phages can transmit their genes. This close relationship between beeches and their victims turns them into partners. When a species of bacteria multiplies a lot, it creates many opportunities for phages to multiply as well. Today, phagiotherapy has started to be used for food disinfection [20-22]. Also, before surgery, in some hospitals, bacteriophages are sprayed in the operating rooms to reduce the risk of infection. Since they destroy pathogenic bacteria, the question arose: can bacteriophages also be used where antibiotics, so widely used in bacterial infections, do not give results? The answer could be the following: although they have a more limited applicability than antibiotics, bacteriophages still have some advantages. Among these advantages, the following can be mentioned:

- bacteriophages do not produce adverse effects because they only attack the target bacteria, while antibiotics attack both pathogenic and sanogenic bacteria;
- follow prolonged antibiotic therapy, an imbalance occurs in the intestinal microflora, with an increased risk of subsequent infections resistance to antibiotics, while bacteriophages can destroy antibiotic-resistant bacteria;
- bacteria also develop resistance in the case of phages, but it only takes a few weeks to create bacteriophages capable of destroying a bacterium that has changed its strain, while creating an antibiotic for a bacterium with a different strain can take years;
- unlike antibiotics that are metabolized by the body and eliminated to a certain extent from the body, the bacteriophages act at the level of the infection until it is removed. Through their mechanism of action, bacteriophages become a real additional immune system for the body, and phage therapy (phagotherapy) becomes a therapy to be studied and a perspective for maintaining health [23-25].

2. Classification of bacteriophages

Bacteriophages are microorganisms, in fact genetic units that have no cellular structure or metabolism of their own, being able to reproduce only in the host cell. It can be stated that they are obligate intracellular parasites of bacterial cells. They can infect all bacteria, but usually a particular phage infects only bacteria of a particular genus, species or strain, which is why we can conclude that phages show infection specificity, dependent on the presence of receptors for a particular phage on the surface of the host bacterial cell. In 1967, Bradley proposed the classification of bacteriophage into six morphological groups [3, 10, 26]. But the only organization authorized for the taxonomy of viruses proposed the classification of bacteriophages according to the nature of their nucleic acid, morphology and physicochemical properties of their virion particles, today being classified into 9 orders, 48 families with 32 subfamilies and 317 genera. Bacteriophages with tails account for about 96% of those discovered (Table 1). Bacteriophages are found in the state of extracellular virions and when a bacterium is infected they can cause two types of infection: lytic and lysogenic or non-lytic. Lytic infection is driven by virulent phages, and the end of the multiplication cycle the lysed bacteria will release the newly formed phages. Lysogenic infection is caused by temperate phages that infect bacteria without killing them. In the latter case, either a complete multiplication cycle is induced that will lyse the bacterium, or, most frequently, after the introduction of the DNA into the host cell, it integrates into the bacterial chromosome, specific recombination (lysogenation) forming a prophage, or another situation, the DNA remains in the cytoplasm of the bacterium as a plasmid. The bacteria, in the last two cases, does not die and multiplying in turn will replicate the viral genome together with its own genome, a situation in which the bacterium is called lysogenic, having the possibility of transmitting to its descendants the ability to produce phages in the absence of infection. Cultures with lysogenic bacteria they have the property of inducing the lytic cycle under

the action of some mutagens, they can acquire immunity against the virulent phage homologous to the hosted prophage and they can also induce the phenomenon of phage conversion, i.e. the change in the cell phenotype determined by the prophage genes. Bacteriophages have high host specificity, high resistance in the natural systems where they are found, and the ability to reproduce rapidly in suitable hosts. In nature, there are apparently 10 times more bacteriophages than bacterial cells, but they are also important components of the human microbiome. Natural bacteriophages have been stored for about 40 years in huge "libraries" of genetic informations, medical sciences, biotechnologies and genetic engineering intend to artificially create viruses capable of destroying infectious bacteria such as cholera or the bacilli that cause nosocomial infections in hospitals.

Table 1. Morphological classification of bacteriophages

Type of bacteriophage	Class	Family	Species	Morphology
Tailed bacteriophages	Caudovirales	Myoviridae	T4, P1	icosahedral head, contractile tails, double-stranded DNA
		Siphoviridae	λ, L. C2 *	icosahedral head, long tails, and non-contractile, double-stranded DNA
		Podoviridae	T7, P2	icosaedral head, short tails, double- stranded DNA
Polyhedral or cubic bacteriophages		Microviridae	φX174	icosaedral head, single stranded DNA
		Corticoviridae	PM2	non-enveloped, double-stranded DNA
		Tectiviridae	PRD1	non-enveloped, flexible lipid vesicles, pseudotail, double-stranded DNA
		Leviviridae	MS2 *	non-enveloped, single-stranded RNA
		Cystoviridae	P. ϕ6 *	covered icosahedral head, double- stranded RNA
Filamentous bacteriopfages		Inoviridae	M 13	non-enveloped, long filament, or short straight rods, single-stranded
		Lipothrixviridae	TTV1	enveloped, rod shaped, double- stranded DNA
		Rudiviridae	SIRV1	encovered straight stems, double- stranded DNA
Pleomorphic bacteriophages		Plasmaviridae	MVL2	enveloped, without capsids, double- stranded DNA
		Fusellovirida	SSV1	enveloped, conical capsid with short end, double stranded DNA
		Guttaviridae	SNDV	drop-like shape, double-stranded DNA
		Bicaudavirid	ATV	lemon shape-like, with long tails, double-stranded DNA
		Ampullavirida	ABV	in the shape of glass phial, double- stranded DNA
		Globuloviridae	PSV	spherical shape, enveloped, double- stranded DNA

*L. C2 : *Lactococcus* phage C2; * MS2 : similar with *Poliovirus;* *P. ϕ6: *Pseudomonas* ϕ6

Recently, these attempts have become more insistent and promising, under the pressure of the fact that some superbacteria have become immune to antibiotics. It is much more likely that, in fact, antibiotics, which have not been invested in for more than 40 years, have become completely ineffective. Bacteriophages can also be created in the laboratory as a result of genetic manipulations.

If bacteriophages resulting from genetic manipulation are used in this beneficial sense, as a means of fighting microbes much more effectively and much healthier for the patient and for nature than antibiotics, so much the better for mankind [25, 27]. However, antibiotics are allopathic drugs that do not attack the source of the disease and, above all, leave harmful traces in the human and animal body and nature, while bacteriophages converge directly on the cause of the infection and are totally harmless to the body and the environment.

3. Development of bacteriophage therapy

By 2050, 10 million people a year are expected to die from superbug infections, meaning one person will die every three seconds from these antibiotic-resistant bacteria, according to infectious disease epidemiologist Steffanie Strathdee, co-director of North America's first bacteriophage therapy center – the Center for Innovative Uses and Bacteriophage Therapy (IPATH). IPATH researchers have identified several bacteriophages that successfully attack the bacteria responsible for serious ear and eye infections. What is this little creature that can destroy a bacterium capable of resisting the most modern drugs ever produced? And more importantly, could bacteriophage treatment become the main player in the battle against the superbug crisis? These microscopic creatures have saved the lives of many patients who were about to die from superbug infections. Superbacteria are so resistant to antibiotics that regular treatments are no longer effective against them [28]. Bacteria such as *Clostridium difficile* and *Staphylococcus aureus*, which almost no medicine can help, cause infections for which antibiotics are often useless. Fortunately, researchers have also found a possible solution: one of nature's oldest predators. In Europe, more than 25 thousand people die every trials are currently underway to test the effectiveness of bacteriophages against urinary tract infections, chronic constipation, joint infections, diabetic foot ulcers, tonsillitis, and persistent, recurrent infections that occur in patients with cystic fibrosis [29]. Doctors at National Jewish Health, in the United States of America, treated a young cystic fibrosis patient with bacteriophages to make a life-saving lung transplant possible. Having tried unsuccessfully for year due to infections with multi-resistant bacteria. In the USA, more than 2.8 million superbug infections occur each year. Such infections are "an urgent global public health threat," according to 2019 statistics published by the US Centers for Disease Control and Prevention. Worldwide, the number of victims exceeds 700,000. These multidrug-resistant superbacteria can cause chronic infections for months to years and sometimes even decades. It's ridiculous how virulent some of these bacteria become over time. Bacteriophages can be collected from water or soil, and in laboratories they are processed and multiplied to be used in treatments. Every year, hundreds of Romanians are infected with multi-resistant bacteria that put their lives in danger. Since antibiotic treatments are not enough, doctors have turned their attention to bacteriophage therapies. In Bucharest, at the Matei Balș Institute, in the last 2 years 15 patients were cured thanks to these therapies [17]. Because multi-resistant bacteria are a global threat, researchers around the world are looking for solutions to cure dangerous infections. Doctors from China spoke in a debate at the European Parliament about the clinical studies they are doing in this regard. They look for alternatives where antibiotics no longer give results, and their attention is directed towards solutions of an organic nature such as mineral mud obtained from different types of soil, organic acid extracted from plants or probiotics, i.e. live bacteria found in different types of food [23]. Each set of bacteriophages is specifically designed to identify, attack and devour a specific type of bacteria. And even if they don't manage to complete the mission, i.e. destroy the bacteria, they will have to use all kinds of evasive methods in an attempt to survive, such as removing the outer membrane to prevent the viruses from entering and destroying the bacteria, which finally explodes into pieces of bacterial substance. Thus, the bacterium could lose its resistance to antibiotics, once again becoming vulnerable to conventional treatments.

years to eliminate the *Mycobacterium abscessus* infection with different varieties of antibiotics, this was only achieved with the help of a bacteriophage and the transplant saved the patient's life. Mycobacteria are a common, widespread genus of bacteria that can cause tuberculosis, leprosy, and mycobacterial infections (NTM) [29, 30]. Dozens of candidates were tested, identifying two bacteriophages that effectively destroyed the mycobacteria infecting the patient's lungs.

Bacteriophages have been genetically modified to optimize their potential. Bacteriophages therapy has also been successfully applied in cases of severe diarrhoea caused by the bacteria *Escherichia coli, Vibrio sp., Shigella sp.*, in skin infections caused by streptococci and staphylococci. Bacteriophages are also used in the food industry, agriculture, animal husbandry and fish farming, which are capable of destroying bacteria dangerous to human and animal health, such as those belonging to the genera *Campylobacter, Listeria, Salmonella, Vibrio, Lactococcus, Erwinia, Xanthomonas*. Work is underway to test bacteriophages that could destroy bacteria involved in infectious diseases, sinusitis, laryngitis, stomatitis or surgical wound infections. Since the quality of drinking water has decreased in recent years due to the development of industry and population growth in certain areas, it was necessary to find solutions for the biological treatment of wastewater. Wastewater of different origin is discharged daily, being a great diversity of bacteria in these waters, with very high risks for the health of consumers. Dangerous pathogenic bacteria, such as *Acinetobacter baumanni*, have been identified in these sewage waters, which are then released into nature. A promising alternative is the treatment of wastewater with bacteriophages, which offers an ecological and Clinical cost-effective remedy for the elimination of microorganisms from wastewater [31]. Bacteriophages affect the host in two ways, either by altering the metabolic process, adaptation and survival potential of the hosts, or by directly destroying the host cells. Among the properties of bacteriophages that make them attractive as therapeutic agents or as biocontrol agents are the lysis of only their specific bacteria through specific receptors, the increase in number according to the density of pathogens, and the easy adaptation to environmental conditions. Bacteriophages also play a role in removing bacterial biofilms. The practical applications of bacteriophages can be in the therapeutic field, by treating and preventing infectious diseases, infections with different locations, caused by bacteria that already acquire resistance to antibiotics.

Another important application of bacteriophages is the field of diagnostics. In this case, phage-identification reactions are important when unknown bacterial strains can be identified using homologous phages (Otto method, Furt and others). Another practical application is represented by the lysotyping reaction or phagotyping, which allows the differentiation of some strains within the same species (subdivision of the species into lysotypes/phagovars). It is used for strains of *Staphylococcus aureus, Salmonella typhi*, etc. The lysotype is an epidemiological marker. Phages represent a study model for geneticists, temperate phages are used in genetic engineering (they can ensure the transfer of genetic material through transduction). Good clinical results have been achieved by administering specific phages together with antibiotics to which the bacteria causing the infection have been shown to be sensitive, but clinical trials are still needed to demonstrate the effectiveness of these combinations. Future studies will need to specify which antibiotic and phages can act synergistically, at what concentrations, and at what optimal time when they should be inoculated into affected subjects. Testing of the synergy between phages and antibiotics was carried out on a wide variety of antibiotic animal models, both in vivo and in vitro, on chickens, mice and rats in order to find out and evaluate the application potential of the two in different branches of human and veterinary medicine [27]. Treatments in the clinic have often focused primarily on effectively treating the patient and less on providing evidence of phage-mediated therapeutic efficacy. Some authors consider it difficult to accept that phage therapy in combination with antibiotics in which the bacteria involved in the etiology of the disease would represent evidence of the effectiveness of phage therapy. They believe that the lack of effectiveness of antibiotic treatments for a long time solved by the involvement of phages, in the absence of new drugs administered additionally, we should not rush to draw conclusions [30-32]. They also believe that whenever antibiotics are given in combination with phages, especially antibiotics to which a target etiology is sensitive, there is always the possibility that the entire success of the treatment is due to the use of that antibiotic [24, 31, 33].

4. Conclusions

Phage therapy is not new, but its use in humans and animals is not yet well understood either. Successful ongoing research may mean it may become more common. Bacteriophages which are

considered natural "antibiotics" may at some point be a good alternative treatment. It may be useful for other purposes, but more research is needed before its use is approved for humans.

To date, several bacteriophages have been identified that have successfully destroyed the bacteria responsible for serious human infections. Bacteriophages can destroy bacteria capable of resisting the most modern drugs ever produced. In the future, it is important that bacteriophages treatment could become the main factors in the battle against the superbug crisis. These microorganisms have saved the lives of many patients who were about to die from superbug infections.

Through their mechanism of action, bacteriophages can become a kind of additional immune system for organisms, and phage therapy, a therapy to study and perspective for maintaining human and animal health.

Currently, therapeutic uses of bacteriophages are limited, in part because each species of phage seeks out and destroys only one bacterial species, and the current arsenal of known therapeutically useful phages is relatively small. As a result, phage therapy testing is currently limited to experimental treatments and only if all other viable alternatives fail.

In order to be able to use them in therapy, the question arose of the need to significantly expand the range of useful phages, developed either from isolated strains, or by creating synthetic versions in the laboratory. An encouraging finding from the research was the lack of phage resistance, which supports the use of a single phage as treatment, although if several suitable phages are available, it is suggested to rotate them to prevent neutralization of the phage to the patient's immune system.

The optimal administration of bacteriophages, following clinical trials, has been observed that whether given intravenously or in the form of aerosols, depends on the nature of the infection and whether the patient's immune system is compromised. If bacteriophages appear to be well tolerated without adverse effects, higher doses and longer treatment periods may be possible and even recommended. Treatment with bacteriophages is usually short-lived (two to three weeks) and significantly less expensive than antibiotics.

Unlike antibiotics that are metabolized by the body and eliminated to a certain extent from the body, bacteriophages act at the level of the infection until it is removed. Bacteriophages do not produce adverse effects because they only attack the target bacteria, while antibiotics attack both pathogenic and sanogenic bacteria.

Specialists claim that the problem is not to replace antibiotic therapy with phage therapy, but to associate them and draw attention to the approach, being very cautious regarding the potential large-scale impact of a possible phage therapy on the environment, arguing that there is a risk of changing the global environment of the chain of life.

The application of bacteriophages at water treatment plants significantly influences the capacity of the treatment process, therefore the quality of the wastewater, as well as the elimination of pathogens from biofilms and sludge. Fagiotherapy began to be used to disinfect food. Also, before surgery, in some hospitals, bacteriophages are sprayed in the operating rooms to reduce the risk of infection.

The development of phagotherapy is hindered by the lack of interest of large laboratories, because phagocytes are derived from nature and therefore "non-patentable". The laboratories have abandoned the center of interest, because the return on investment is considered too low. However, some companies have begun to take an interest in these bacteriophages, classified as drugs by the European Union since 2011. Today, no phage is yet approved as a human treatment, mainly because of the need for clinical trials that can take several years and have an expensive cost.

There are authors who believe that many other researches are still needed to concretely establish the effectiveness of bacteriophages in therapies, the clear synergistic associations with antibiotics, according to their concentrations, time and mode of administration.

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