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Review Article

Bacteriophage Therapy as an Alternative to Antibiotics: A Dead End or a Solution?

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Abstract

Antibiotic resistance has been around for years and could lead to a serious crisis in the near future. If the problem of antibiotic resistance is not solved, antimicrobial resistance is predicted to increase, killing 10 million people annually by 2050 (more than the number of cancer deaths), and costing the global economy approximately $100 trillion USD, because of this, it will need the rapid development of alternative therapies. This issue prompted scientists to find a solution; the use of bacteriophages as an alternative to antibiotics is one of these tactics. In this review article, I will first focus on bacteriophages from various aspects and then, by analyzing the available information, I will try to answer the following questions:

1. Given the meager standard clinical data and characteristics of bacteriophages, is bacteriophage therapy a safe and reliable method?
2. Given the short time left before the antibiotic resistance crisis, is it cost-effective to invest in bacteriophage?
3. Are bacteriophages a double-edged sword? (Besides being used to treat bacterial diseases in the future, do bacteriophages have the potential to become a human virus in the future?)

Keywords: Bacteriophage Therapy; Bacteriophage Resistance; Antibiotic Resistance; Biofilm; Challenges of the Pharmaceutical Industry; Microbiome

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Introduction

One of the major problems in today's world is the resistance of bacteria to chemical antibiotics, which has increased the death rate from infections worldwide. The World Health Organization (WHO) declared in 2017 that an examination of antibacterial clinical development, including tuberculosis, reveals a significant dearth of new antibiotics to tackle the growing issue of antimicrobial resistance. The majority of medications used in clinical settings today are modifications of existing classes of antibiotics and only provide short-term solutions. The report suggested very few potential treatments for those antibiotic-resistant infections that the WHO has identified as the most significant health threat, including drug-resistant tuberculosis, which kills around 250,000 people a year.

In 2019, the United Nations (UN), international agencies, and experts issued a landmark report calling for coordinated, ambitious, and immediate action to avert a potentially catastrophic crisis in drug resistance. Drug-resistant diseases might result in 10 million fatalities yearly by 2050 if nothing is done, as well as economic damage comparable to the 2008–2009 global financial crisis, according to the UN Ad Hoc Inter-Agency Coordination Group on Antimicrobial Resistance, which published the report. Antimicrobial resistance may cause up to 24 million people to live in extreme poverty by 2030. Drug-resistant illnesses claim the lives of at least 700,000 people annually, including 230,000 victims of multidrug-resistant tuberculosis. Common diseases, including respiratory tract infections, sexually transmitted infections, and urinary tract infections, are increasingly incurable; life-saving medical procedures are becoming much riskier and our food systems are increasingly insecure. As essential medications lose their effectiveness, the globe is already experiencing negative health and economic effects. Without investment from countries of all income categories, future generations will face the catastrophic consequences of unchecked antimicrobial resistance. For this reason, scientists are looking for an alternative to chemical antibiotics, and one solution could be the use of bacteriophages.

Of course, phage therapy was introduced in the early 20th century, but when chemical antibiotics were proven in the 1940s, phage therapy research stopped in Western countries. However, countries such as Poland, Georgia, and Russia continued to study (1-3).

Although phages have many advantages as an alternative to chemical antibiotics, they also have many problems that must be considered.

What are bacteriophages (BPs)?

Bacteriophages are bacterial viruses that do not infect eukaryotic cells. Lysogenic bacteriophages coexist with their host by inserting themselves into the bacterial genome, while lytic bacteriophages replicate inside their hosts and destroy them (the type best suited for therapeutic use), then release many new bacteriophages capable of infecting more bacteria (4). Bacteriophages have a narrow host range. They are specific not only in terms of killing a particular species, but often target only a subset of strains within a given species (5, 6). As a result, they cause less damage to non-targeted normal, and often beneficial, gastrointestinal tract microflora (7, 8), while antibiotics have more destructive effects (9).

Symbiosis of bacteriophages in the human body

Phages are abundant in the intestinal tract and are frequently seen in urine, saliva, and ascitic fluid (10-12). Metagenomic analysis advances have also produced evidence that suggests phages may circulate in the blood in addition to being present in oropharyngeal and urine samples (10, 13-15). The greatest number and diversity of phages are present in the gut; enteric phages can translocate from the gut to blood, tissue, and lymph and mediate immunomodulatory functions (10, 16). Phages that are able to penetrate epithelial cell layers, propagate throughout the body, and affect the immune system are included in intracorporeal phageoma (10, 17, 18). Approximately 31 billion phages undergo transcytosis from the intestine daily, contributing to the circulation of bacteriophages in the human body (10, 17).

Bacteriophages in the environment

The most prevalent type of life on Earth is the bacteriophage, which is ten times more prevalent than bacteria (8, 19, 20). Bacteriophages can

tolerate adverse conditions (8). In fact, they can exist in every setting where bacteria flourishes; for example, in the Sahara, hot springs, the North Sea, and polar inland waters (8, 21-25). We can also detect them in ground and surface water, soil, food, sewage, and sludge (8, 26-30). Thanks to this, we can easily access them.

Effect of bacteriophage on biofilm

Biofilms are organized heterogeneous groupings of microbial cells that are enclosed in a self-created matrix. According to estimates, biofilms include up to 80% of all bacterial and archaeal cells, additionally, the microbial community is shielded from environmental stresses through biofilm formation (31). Since biofilms are a major form of microbial life, it is important to understand their biology and functions, especially since controlling the development of biofilms is crucial in industrial, infrastructure, and medicinal settings (31). About 80% of bacterial infections are caused by biofilms (31, 32), which are frequently very challenging to treat because of specific protective mechanisms that the biofilm provides (31, 33).

Biofilms reduce the effect of antibiotics on bacteria (35, 34) because the slow growth and greatly reduced metabolic activity of persistent bacteria in a biofilm can prevent the action of many antibiotics (4); moreover, due to the complex architecture of the biofilm, the biofilm can be considered an "innate tolerance" because it provides the bacterial cells with a protective environment. The extracellular matrix organizes a mechanical barrier that restricts the diffusion of antibiotics in the biofilm and their accessibility to microorganisms in response to combinations of the host's immune system. Electrostatic charges or certain matrix elements bind and trap antibacterial compounds. In addition, the antibiotic may not be able to reach its effective concentration in the deeper levels of the bacterial community due to the high viscosity of the polymer matrix. As a result, after antimicrobial treatment, bacteria in the biofilm's outer layers perish, while microorganisms in its deeper layers have time to respond (35, 36). This period of time ought to be long enough to allow bacteria exposed to antimicrobial agents to adapt progressively physiologically (secretion of inactivating enzymes, expression of resistance genes, etc.). For example, it has been shown that eDNA and alginate in the P. aeruginosa biofilm matrix could bind aminoglycosides and play a role in the tolerance of sessile bacteria to tobramycin (35, 37).

Because of the impermeability of the biofilm matrix, biofilms have often been assumed to be resistant to bacteriophages. Bacteriophages are significantly tiny than their bacterial hosts despite being much larger than chemical antibiotics, and many of them may infect bacteria in biofilms (4). In fact, phages contain enzymes that can degrade the biofilm matrix (biofilm-degrading enzymes), and then, by entering and multiplying in bacterial cells, they can decompose the bacteria and destroy biofilms (9, 3, 38, 39). The use of bacteriophage therapy in treating clinical biofilm infections is supported by multiple preclinical animal studies (39-48). According to these researches, biofilm is reduced when bacteriophages are applied topically to the location of biofilm infections (39-48). Additionally, these results demonstrate that the decrease of biofilms on hardware is not considerably reduced in the absence of local bacteriophage therapy (39, 44).

Therefore, bacteriophages have more destructive effects on biofilms than antibiotics; however, it seems doub140140141 140tful that a single phage could completely eradicate a sophisticated mature biofilm, but combined phage therapy with antibiotics and cocktails could be a potential strategy (40, 49-52).

Effect of phages on antibiotic resistance in bacteria

Hospitals and healthcare systems are likely not the only locations with high concentrations of bacteria, viruses, and resistance genes in the tiny spaces that are frequently regarded as hotspots for the spread of antibiotic resistance. Recently, wastewater treatment plants (WTPs) have been major players that have addressed the spread of resistance genes (53-57) and several reviews have focused specifically on the effect of bacteriophages in this environment (53, 58-61).

Through chromosomal mutations or by genetic material obtained from other bacteria or the environment via horizontal gene transfer, bacteria can obtain antibiotic resistance. This second process

is primarily regulated through mobile genetic elements like transposons, plasmids, or bacteriophages, which play a crucial role in the evolution and ecology of bacterial communities by regulating the intra- and inter-species exchange of genetic information (62, 63). While these mobile genetic components can be transferred through transformation or transduction, conjugation is thought to be the most effective method for exchanging genetic information across bacteria (62, 64). The process of conjugation is the horizontal gene transfer method that is most frequently mentioned, and likely the most effective way to transfer a transposon or plasmid between bacteria is through direct contact (53, 65, 66). Scientists first identified "R-factor" (resistance) and "F-factor" (sex pili) in the early 1960s (53, 67, 68), bacteria with the R-factor are resistant and can propagate resistance when the F-factor is present (53, 68). In hospitals and communities, substantial research has been done on antibiotic resistance genes (ARGs) and the role of these elements in antibiotic resistance, which are acquired and frequently transmitted through conjugation via conjugative plasmids and transposons (62, 69, 70). Recent discoveries based on cutting-edge genomic technology show that bacteriophages play a more significant role than previously thought in these regions for ARG mobilization (62).

Although phages can kill bacteria, while they act as prophages, they can change their pathogenicity and give them virulence (53, 71). In fact, phages can themselves carry virulence genes and is a phenomenon known as transduction, non-phage DNA can be packaged into phage capsids and injected into other bacteria. Because bacteriophages have a dual function (being capable of increasing bacterial pathogenicity and also killing bacteria), they have developed into a two-edged sword that makes it get challenging to utilize these effective bacteria killers without the capability to encourage bacterial genetic exchange (53). In fact, phage particles can transfer a genetic trait from a donor bacterial cell to a recipient cell through generic or specialized transduction, which is how phages operate as vectors for genetic exchange (61). In generalized transduction, either lytic or lysogenic (mild) phages are used to transfer any portion of the donor genome into the recipient cell, whereas in specialized transduction, mild phages are used exclusively to transfer multiple specific donor genes into the recipient cell (61). Particles produced by a specialized transducing phage carry both chromosomal and phage DNA and only contain particular portions of the bacterial chromosome close to the prophage attachment point. Through a mechanism called as lysogenic, some temperate phages can also cause the infected host's phenotypic to alter; through these mechanisms and due to the capacity of phages to transmit genetic material between bacteria, they have the potential to play a significant role in the ecology and evolution of bacterial species (61, 62, 72, 73).

Bacterial resistance to bacteriophages

Antibiotic resistance does not mean phage resistance; because phage infection and lysis don't depend on how antibiotics work to kill bacteria (50, 74).

Bacteria develop various defense mechanisms to avoid phage predation; for example, preventing phage attachment, digestion of phage nucleic acids, and the development of abortive infection or clustering of regularly spaced short palindromic repeats (CRISPR)/CRISPR-associated systems to survive the attack (7, 75). Phage resistance is still one of the main problems limiting its administration, for this reason, multiple studies report the use of phage cocktails to counteract phage resistance's development, although they do not necessarily eradicate it (7, 76, 77); in fact, the use of phage cocktails can reduce the rate of bacterial resistance to bacteriophages. Of course, bacteria can also become long-term resistant to phage cocktails (75). Therefore, it is necessary to isolate and characterize phages, which can be a hard and time-consuming process to complete ad hoc (78); in fact, due to the resistance of bacteria to phages and the narrow range of phage hosts, we have to create phage banks. It may be necessary for phage banks to regularly screen phages against prevalent infecting strains and generate them in batches so that they are always available (5). In addition, the phage would have to be treated to ensure a lengthy shelf life (usually 12 months), for instance, it would be formulated in excipients and spray-dried or lyophilized to ensure precise

dosing during therapy (5).

Bacteriophage (BP), the microbiome and the immune system

Despite the fact that phages are found in the environment, in and on the human body, and are members of microbial communities (50, 79, 80), since bacteriophages and the products they produce are non-self-antigens, the immune system is capable of recognizing them and responding in a way that theoretically reduces the benefits of bacteriophage therapy (77). Dąbrowska et al. (81) found that in humans, particular antibodies could be found in more than 80% of the participants enrolled, even though none had undergone phage therapy, in their study of the antigenicity of E. coli T4 BP head surface proteins (77, 82). In general, phages are thought to be naturally nontoxic (50, 74, 83). However, there is proof that phages have non-specific immunomodulatory properties (50, 84), furthermore evidence for phagocytosis activation and anti-inflammatory properties (50, 85). According to Roach et al., neutrophils are necessary for phage therapy against P. aeruginosa to be effective (50, 86). Furthermore, an in vivo study recently suggested that increased amounts of intestinal phages (administered via drinking water in mice) can stimulate nonspecific and phage-specific immunity (50, 87). Depending on the method of administration, the human immune system can identify phages as foreign antigens and manufacture phage-neutralizing antibodies (3, 50, 88); therefore, it is essential to use highly pure phage preparations, at least for parenteral use, to reduce the potential for side effects brought on by impurities (76, 50).

A higher titer than their normally occurring levels is needed for phage therapy. In addition, there is a potential possibility that administering high phage titers to patients could trigger life-threatening immunological reactions like anaphylaxis (50, 89).

Because the interactions between the human immune system, bacteriophages, and bacteria play crucial roles in sickness and health, bacteriophages are a crucial component of our connection with bacteria (90, 17). The bulk of phages in the body are produced by the gut microbiome, which can create up to 10^8 virus-like particles per milliliter of fecal filtrates (91, 90). Although significant interindividual variability is a norm, research on the phageome indicates that individual phage populations are very stable over time (90, 92, 93). In fact, it has been proposed that phages probably help the microbiome remain stable and resilient by fostering microbial variety and acting as a storehouse of advantageous genetic material (90, 94). Perhaps, as a result, successful fecal transplant outcomes for Clostridium difficile colitis have been linked to bacteriophage transmission (90, 95). On the other hand, there are studies that inflammatory bowel disease (96), diabetes (97), and other illnesses (98) may affect the relative quantity, diversity, and makeup of phages (90). It is uncertain, nevertheless, whether phages actually cause these illnesses or merely reflect changes in the bacterial community (90).

Phages play recognized, crucial functions in bacterial pathogenicity, microbial ecology, and the genetic evolution of bacterial populations (90, 99, 100). Phage genetic components serve as virulence factors that allow bacteria to invade and colonize their mammalian hosts. Antibiotic resistance, adhesion, epithelial invasion, and biofilm formation are all increased by bacterial expression of phage-encoded proteins, but neutrophil phagocytosis is inhibited. In addition, a barrier against the invasion of non-host bacteria may be created by phages accumulating in the mucosal layer. Finally, substantial titers of phages from sites of entry (gut, lung, genitourinary system) may be transferred into the circulation and eventually spread throughout the body by transcytosis of phage particles and apical-basal transport (90). Lysogenic phages produce proteins that allow their bacterial hosts to assault the tissue barriers that serve as the body's first line of defense against bacterial pathogens. For instance, the temperate phage Φctx, which lives as a parasite on Vibrio cholerae, produces the cholera toxin (90, 101). The majority of these phage-encoded virulence genes are controlled by transcription factors that are expressed on the chromosome (90, 102-104). For instance, the phage-encoded virus of Mycoplasma arthritidis and the λ-encoded boron of E. coli both reside on the noncoding strand in relation to the lytic phage genes (90, 105).

Immunoglobulin (Ig)-like domains on the capsid proteins of the E. coli T4 phage interact with mu-

cins and surface glycoproteins on epithelial cells (90, 106). There is a huge range of phage families with similar protein domains to the Ig superfamily, suggesting that other phages may be significantly enriched in mucosal layers (90, 107, 108). It has been proposed that mucosal binding makes some bacteria more susceptible to phage-mediated lysis (90, 109) and facilitates more effective phage diffusion in the mucus layer (90, 110). Thus, mucosal tissue contains phages that can act as a non-host-derived, strain-specific, ubiquitous barrier against bacterial invasion (90, 106, 109). However, results in murine models of colitis have been found to be influenced by the intestinal virome, and phage impacts on innate immunity may play a role. According to Yang et al. (111), a combination of antiviral medications made mice with dextran sulfate sodium (DSS)-induced colitis worse, whereas Toll-like receptor (TLR) 3 and TLR7 detecting gut-resident viruses were a protective mechanism involving the production of interferon (IFN). This study does not focus precisely on the function of phages, despite the fact that they make up a significant portion of the intestinal virome (90). According to Gogokhia et al. (87), oral treatment of a phage cocktail aggravated DSS colitis in a way that was TLR9-dependent. The function of phages in intestinal homeostasis requires further study (90).

The tissues and peripheral circulation both contain a large number of phages (17, 18, 90). Lytic phage therapy studies provide a thorough description of the pharmacokinetics of some of these phages. Independent of administration manner, circulating phages follow a temporal and spatial pattern of clearance. The lifespan of circulating phages spans almost several days, with the greatest decline (>99%) within the first hour (112, 90, 113). Phages are immunologically well tolerated in peripheral tissues and the blood (90). The spleen and liver have the greatest and most persistent phage titers, indicating that these organs serve as the primary organs for phage particle clearance after phages have circulated in most major organs (114, 115, 90). Studies on macrophages (Kupffer cells) in the spleen and liver show that these cells phagocytose phages quickly and effectively (116, 90). In comparison to the spleen, the liver typically has significantly lower levels of active phage persistence (90, 117, 118). While most circulating phages are eventually cleared by phagocytes, various eukaryotic cells can also internalize phages through the uptake of the bacteria-harboring prophage, nonspecific uptake, and receptor-mediated endocytosis (90, 119); phages are found in lysosomes, Golgi, cytoplasm, nucleus, and endosomal vesicles after internalization (120), where they are degraded (121, 90). However, as recently proposed for Mycobacteria abscessus infection, intracellular phages maintain some bioactivity against intracellular bacterial pathogens (122, 90). In addition, according to research on phage DNA vaccines, phages can enter the nucleus along with cellular vesicles and create both RNA and protein (123, 90). In a recent review, the subject of intracellular phages is thoroughly discussed (124, 90). A range of cell surface and intracellular pattern recognition receptors (PRRs) are to be used to determine the cellular uptake and transit positions of phages (125, 90). Pathways including induction of IFN responses, single-stranded DNA (ssDNA) sensing, and double-stranded DNA (dsDNA) sensing are most frequently involved (126, 90). For example, phages can induce the expression of antiviral and proinflammatory cytokines through TLR9, the endosomal PRR, and the adapter protein MyD88 (87, 90, 127-129). Also, recent studies on the sensing of phages in the gut have shown that TLR9 has a role in triggering inflammatory reactions to phages. A substantial increase in IFN-γ-producing CD4 + T cells was seen after taking an oral cocktail of E. coli-tailed phages, which was triggered by DC sensing of phage DNA via TLR9 (90, 87). TLR9's role in phage responses, however, may be complicated (90).

Numerous contrasting examples of phages that either stimulate or suppress inflammatory responses can be found in the literature on phage-innate immunological interactions. For instance, the pseudomonal bacteriophage Pf inhibits phagocytosis and reduces cytokine production, which increases the risk of persistent wound infections (90, 130). Other phages are also involved in impaired phagocytosis (90, 131, 132). On the other hand, during bacteriophage therapy, bacteriophages show synergistic activity with neutrophils to remove bacterial infection (86, 90). In addition, in animal models of colitis, Escherichia coli bacteriophages activate DCs (dendritic cells) via

TLR9 to stimulate IFN-γ (IFN: interferon) production and Th1 (Th: T helper) bias, which exacerbates the disease (87, 90). These research results imply that the immunomodulatory properties of phages may be context- and possibly phage-dependent (90).

Against specific pathogens, adaptive immunity prepares a targeted defense. Phages can influence T cell (cellular immunity) and antibody (humoral immunity) responses, which has significant ramifications for phage therapy, interactions with the microbiome, and phage-displayed vaccines. APCs (antigen-presenting cells) present phage-derived peptides to naive T cells on MHC-II molecules. Naive antigen-MHC-specific CD4+ T cells are activated (IFN-γ release) and proliferated to Th cells. Subsequently, naive phage-specific B cells can be activated by phage-specific Th cells. Memory B cells, which can be reactivated upon phage exposure and begin the manufacture of additional antiphage antibodies, and plasmablasts, short-lived cells that circulate and produce high levels of phage-specific antibodies, are differentiated from activated B cells. Phages in tissues and circulation are bound to antiphage antibodies and inactivated (90).

Antiphage antibodies may play a role in controlling the biological activity of phages against microbiome. When T4 phages are ingested repeatedly, antiphage IgA is generated and restricts their biological activity (133, 90). The effectiveness of intravenous phage therapy is hypothesized to be adversely affected by antiphage adaptive immunity. Despite the paucity of studies on antiphage antibodies in humans, one study reveals that many people already have neutralizing antibodies to the phages used in phage therapy (134, 90). This may support the idea that many chronic infections are brought on by opportunistic pathogens that are normally a part of our commensal flora and that this presents a chance for exposure to their phages. Additionally, patients produce neutralizing antibodies during phage treatment (88, 90, 135). There are proposals to use artificial phages to prevent a severe immune system response, although some studies show otherwise; phages that have been changed to have modified capsid structures, such as via peptide display protocols, may be more immunogenic than their parent phages that have not undergone these changes (136, 137, 90) and might be removed from circulation more rapidly (138, 90). In addition, phages administered intravenously or at the site of the infection may be more immunogenic than those in the gastrointestinal tract (88, 90). Also, usually, phage vaccines made with modified T4 or other phages are pro-inflammatory (90, 139-141). There is evidence that indicates these phages induce mixed T helper (Th) 1 and Th2 responses as well as vigorous pro-inflammatory cytokines (90, 142, 143), which is compatible with a potent antibacterial response. However, since some research uses bacterial lysates as the immunogen, the level of endotoxin is not frequently measured in preparations of phage vaccines (144, 90). Thus, it is possible that bacterial contaminants increase these measures' immunogenicity. This indicates that an important factor in determining the immune response may be the purity of the respective phage preparation (145, 90).

The information presented here highlights how crucial bacteriophages are to human biology. Phages directly influence the immune system's response to bacteria in addition to having an indirect impact on human cells and tissues through interactions with their bacterial hosts. In other words, phages serve as a bridge between our bacterial and immune systems. The best way to understand how we interact with our microbiome is known probably as an interconnected network of bacteria, bacteriophages, and human cells. The stability of the entire network can be influenced by the three-kingdom interactions between these individual parts, for instance, potentially, phages limit bacterial over-proliferation and expansion, which would reduce inflammation at colonization sites. In contrast, phages likely encourage immunological tolerance to commensal colonization by direct and indirect modulation of host immunity. Our immune system's and metabolic health may be significantly affected if this equilibrium is upset by exogenous phage exposure, microbial dysbiosis, or immune dysregulation (90).

Discussion

Can bacteriophage therapy be a suitable alternative to antibiotics?

Although there have been many clinical trials with phage therapy in Eastern Europe, Russia, and Georgia, we have not yet reached the point

where we can safely replace antibiotics with phage therapy as an effective and safe method. Because not only are these clinical data not standard but there are also conflicting data on some aspects of phage therapy (146). Additionally, due to the narrow standard clinical data available in the Western world, regulatory agencies have not yet established standard treatment guidelines for phage therapy (1).

conclusion

Even though about a hundred years have passed since the emergence of phage as a treatment method, there are complex and important questions and challenges; also, the viral nature of bacteriophages (although they are bacterial viruses, not eukaryotic viruses) and their effective involvement in maintaining the balance of the body's microbiome and promoting bacterial evolution has contributed to these complexities and problems.

Here I would like to address the various challenges of using bacteriophages as therapy in terms of the properties of bacteriophages, the clinical use of bacteriophages, and the commercial production of bacteriophages as medicine:

1. Nature and properties of bacteriophages:

Bacteriophages are viral in nature as well as due to the interaction they have with our body, they are able to enter human cells through various mechanisms that could sometimes cause inflammatory reactions; indeed, during the occurrence of internalization, phages can flee lysosomal degradation, which may open doors for trans-kingdom genetic exchange or stimulation of cellular immunity (147-149). The presence of homologs of fragments that belong to different genes in phages and eukaryotic cells is one of the prerequisites for internalization. There is considerable evidence that indicates DNA sequences linked to genes found in bacteriophages of the Microviridae family are present in eukaryotic cells as well as a variety of prokaryotic organisms (150, 120). In addition, bidirectional DNA transfer can be promoted by the presence of bacteriophages in obligate intracellular bacterial parasites of eukaryotes (151, 120). This could have potentially harmful ramifications, especially given the wider usage of phage therapy (120).

According to recent studies, bacteriophages can interact with eukaryotic cells in a way that significantly alters the way that tissues, organs, and systems in mammals, including humans, work (120). If the human body is viewed as an ecological ecosystem, bacteriophages can be found in blood, urine, or cerebrospinal fluid in addition to the gastrointestinal tract (because of the intestinal bacteria present there), which may theoretically offer significant dangers (152). Also, different species of bacteriophage and human cell matrix molecules such as fibronectin, gelatin, and heparin interact with each other (153); consequently, these types of addictions are critical because they can directly cause changes in tissue and organ function (120). In addition, in various studies, bacteriophages of the body microbiome are out of balance in some diseases, which may indicate the importance of body phage balance and its influence on human health and disease.

Assuming that antibiotics will completely lose their effectiveness in the future, given that antibiotics are used in a wide variety of fields such as medicine, agriculture, aquaculture, and veterinary medicine, by employing bacteriophage instead of antibiotic, we would have to wait for bacteriophages so that have greater interactions with bacteria, humans, and animals, which is not in the balance that bacteriophages have with environment, specially bacteriophages are designed as bio preservatives in the food and as tools to detect pathogenic bacteria throughout the food chains (154).

The result is a disruption of this balance and many mutations in bacteriophages, which likely disrupt the balance of the natural microbiome of humans and animals, consequently, this outcome leads to endanger human health.

There are many viruses, although they were not human viruses that became deadly human viruses due to their mutations; in particular, although bacteriophages are inherently nontoxic, they are used in vaccines because of their similarity to mammalian viruses and their potential to stimulate the immune system (155). Therefore, by causing large mutations in bacteriophages, these viruses would be able to become human viruses (156-158).

Since bacteriophages are in two forms of lytic and lysogenic, they can enter human cells, and

have access to different parts of body, they would be very dangerous if they became human viruses.

2. Clinical use of bacteriophages:

For the clinical use of a drug, we need to have extensive information about the drug, such as the most effective and best way to administer the drug (injection, oral administration, etc.), the side effects and efficacy of the drug, etc. most clinical studies of bacteriophages involve animals rather than humans; and standard and reliable studies of bacteriophage therapy in humans are few. Additionally, most human clinical trials have investigated topical administration of bacteriophages in burns rather than injection and oral administration. However, suppose we get the bacteriophages into the bloodstream by any means so that they reach different parts of the body; if we put all of the following together like a puzzle:

• Most bacterial diseases are caused by the presence of biofilms

• Maintaining a balance between the human immune system, bacteria, and bacteriophages of the human body is very important for human health

• Bacteriophages interact with our body, but they are ultimately antigens and the body can release antibodies against them, so according to several studies, antibodies to natural bacteriophages are naturally present in the blood of most people

• We need to use high doses of bacteriophages to treat the patient because the antibodies that are released can reduce the therapeutic effect of the bacteriophages.

• To destroy the biofilm structure, we need to use high doses of bacteriophages rather than individual bacteria

We can state that in bacteriophage therapy, even if we use high purity bacteriophage, this treatment can cause inflammation, overstimulate the immune system and endanger the patient's health. Another problem is the host restriction in bacteriophages (of course, host restriction also has the advantage of causing less damage to the gut bacteria), which forces us to spend a lot of time and money on just one patient to identify the bacteria causing the infections; in particular, biofilms can be formed by several types of bacteria.

3. Production of commercial bacteriophages as medicine:

Before manufacturing a drug, each pharmaceutical company evaluates the profits and losses of manufacturing the drug and decides whether or not to manufacture the drug.

Although bacteriophages are abundant in nature. However, due to the limitation of the bacteriophage host, we must create a bacteriophage bank; examining each bacteriophage in nature infects which species of bacteria are costly, time-consuming, and challenging (6, 78, 159). The bacteriophage bank must be screened periodically to identify bacteriophage-resistant bacteria; indeed, the phage host range can be altered by individual mutations (73, 160). In other words, the host range of bacteriophages is not a stable feature of each bacteriophage species. It can evolve and may indicate unexpected flexibility (6). Because measured host ranges depend on the method employed, determining the host range of a particular phage can be challenging (6, 161); moreover, host range methods can give spurious and inaccurate results, and testing on clinical isolates is preferred over testing on laboratory host strains for applications like phage therapy (6). The effective host range as seen in the patient may not be entirely indicated by in vitro host range testing; therefore, despite the apparent easy access to phages in many settings, it may be difficult to find a phage that is against a specific host, just as temperate phages are typically viewed as undesirable for phage therapy (159). Therefore, it is crucial to avoid using isolation hosts that could be prompted to release mild phages as enriched strains (159).

The high price of clinical trials is another issue. Nonprofit research facilities and clinics won't be able to shoulder the financial responsibility for the regulatory regime that was initially created by pharmaceutical companies for drug development (2), and it takes a long time to gain approval from regulatory agencies (50).

Another challenge is that some bacterial pathogens can be different combinations of phages to treat the same bacterial disease in different geographical areas (9).

For bacteriophage therapy to be successful, apart from the route of drug administration (injection, oral, etc.), the purity or titer or dose of the phage

is very important (73), and it is difficult to ensure conditions for this purity to remain stable at a commercial level (162). For this reason, it is likely as easy as the storage and use of antibiotics, pharmaceutical companies would not be able to stock bacteriophages on a commercial scale and easily sell them widely. The purity of phage medicinal products is not only an important safety factor but also has an impact on phage consistency; also, purification of bacteriophages on an industrial scale is challenging (163).

Perhaps these contexts are why most pharmaceutical companies are reluctant to invest heavily in bacteriophage therapy, even with government incentives.

Finally, for the following reasons:

• Little standard clinical information

• Lack of extensive cooperation of large pharmaceutical companies

• Lack of sufficient information about how bacteriophages interact with parts of our body, especially the immune system

• Lack of information about the relationship of bacteriophages to the body's microbiome and its influence on human health and disease

And many other challenges and questions, and since antibiotic resistance has been started for many years and we are approaching an antibiotic resistance crisis, it is better to look for an alternative way to deal with this crisis.

Conflict of interest

There is no conflict of interest.

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