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AN OVERVIEW OF BACTERIOPHAGE THERAPY OVER ANTIBIOTICS; AS AN ALTERNATIVE FOR CONTROLLING BACTERIAL INFECTIONS

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ABSTRACT: Bacteriophages are particular infective agents of diverse bacteria. They are separated into different groups according to their life cycle. The lytic phages kill their host cells; this property can be applied for the selective elimination of pathogenic bacteria. The first bacteriophage treatment was described one hundred years ago, and phage therapy had been extensively used until the Second World War. Upon the appearance of antibiotics, the medical application of phages retrograded in most parts of the world. In the last decades, owing to the costs of development of new antibiotics and the rapid emergence of multidrug-resistant bacteria, this old approach was revitalized and phage-based treatment was legalized from the middle of the last decade. Here, they summarize the current knowledge on phage therapy, its advantages and potential drawbacks. The current status of phage therapy against food-borne, animal and human pathogens is also presented. Among these, special focus is set on phages E. Amy of *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes*. Phage cocktails against *Listeria monocytogenes* and *E. amylovora* have already been commercialized.

INTRODUCTION:

1. Bacteriophage: Bacteriophages are bacterial viruses that contaminate bacterial cells with high specificity, and in the case of lytic phages, they disrupt and lyse their host cells, resulting in cell death [1]. Bacteriophages (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery.

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The term is commonly used in its shortened form, phage. Interestingly, Bacteriophages are much smaller than bacteria; they destroy bacterial cell [2]. Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestines of animals [3, 4].

One of the densest natural sources for phages and other viruses is seawater. They have been used for over 60 years as an alternative to antibiotics, however, this much controversial area of research [3]. Bacteriophages or "phages" are viruses of prokaryotes. At least 5,360 tailed and 179 cubics, filamentous and pleomorphic bacterial viruses have been examined in the electron microscope since the

introduction of negative staining in 1959. Since at least 100 novel bacterial viruses are described every year 1, the approximate number of viruses under consideration is over 6,000. Numerically, this makes bacteriophages the largest virus group has known [5]. Phages are presently classified in a hierarchical and holistic system with one order and 10 families.

Over 96% of phages are tailed and contain dsDNA. The seven families of cubic, filamentous and pleomorphic phages are small and well defined. They contain ds or ssDNA or RNA [6]. The most important developments are reclassifications of the *Podoviridae* and *Myoviridae* families of tailed phages. Bacteriophages (phages) have found use as natural antimicrobials that can be used in controlling bacterial pathogens in foods and food processing environments [7]. In contrast to cells that grow from an increase in the number of their components and reproduce by division, viruses are assembled from pre-made components. Viruses are nucleic acid molecules surrounded by a protective coating. They are not capable of generating energy and reproduce inside of cells. The nucleic acid inside the coating called the phage genome in a bacteriophage encodes most of the gene products needed for making more phage [8].

The phage genome can be made of either double- or single-stranded DNA or RNA, depending on the bacteriophage in question. The genome can be circular or linear. The protective coating or capsid surrounding the phage genome is composed of phage-encoded proteins [9].

2. Discovery of Bacteriophages: Bacteriophages or phages are bacterial viruses that invade bacterial cells and in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. The history of bacteriophage discovery has been the subject of lengthy debates, including a controversy over claims for priority [10]. In 1896, the British bacteriologist Ernest Hankin reported antibacterial activity against *Vibrio cholerae*, which he observed in the Ganges and Jumna rivers in India. He suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics [11]. Two years later, Gamaleya, the Russian bacteriologist, observed a similar phenomenon while working with *Bacillus subtilis* from 1898 to 1918, others had similar observations of what is thought to be the bacteriophage phenomena. It was not until 1914, however, that another British bacteriologist, Frederick Twort, advanced the hypothesis by proposing that it may have been due to, among another promise, a virus. For various reasons, including financial difficulties, Twort did not pursue this finding. The discovery or rediscovery of bacteriophages by d'Herelle is frequently associated with an outbreak of severe hemorrhagic dysentery among French troops stationed at Maisons- Laffitte (on the outskirts of Paris) in July-August 1915, although d'Herelle first observed the bacteriophage phenomenon in 1910 while studying microbiologic means of controlling an epizootic of locusts in Mexico. Several soldiers were hospitalized and d'Herelle was assigned to conduct an investigation of the outbreak. During these studies, he made bacterium-free filtrates of the patients' fecal samples and mixed and incubated them with *Shigella* strains isolated from the patients.

A portion of the mixtures was inoculated into experimental animals (as part of d'Herelle's studies on developing a vaccine against bacterial dysentery) and a portion was spread on agar medium in order to observe the growth of the bacteria. It was on these agar cultures that d'Herelle observed the appearance of small, clear areas, which he initially called taches, then taches vierges and later, plaques [12].

D'Herelle's findings were presented during the September 1917 meeting of the Academy of Sciences, and they were subsequently published in the meeting's proceedings. In the lab, when he spread some cultures on agar, he observed round zones without growth, which he called plaques, and asserted they were caused by viral parasites. Six years later, he proposed the name "bacteriophage," or bacterium-eater [13].

In 1917, d'Herelle began testing his phage in human patients. Under the clinical supervision of Professor Victor-Henri Hutinel at the Hospital des Enfants-Malades in Paris, he demonstrated the safety of his phages by ingesting them. The next day, he demonstrated their efficacy by administering them to a 12-year-old boy with

severe dysentery. The patient's symptoms ceased after a single treatment and he made a complete recovery. Dr. d'Herelle's anti-dysentery phage was then administered to three additional patients, all of whom began to recover within 24 h of treatment. In 1923, two physicians from Baylor University's College of Medicine reported successful results from one of their phage therapy trials conducted in the United States and concluded that "the bacteriophage holds enormous possibilities as a new weapon for fighting infectious disease" [14].

3. Major Discoveries with Phages: The early history of bacteriophages might suggest that their study would immediately be applied to the treatment of diseases caused by bacteria. This was not the case; instead, antibiotic therapy became the mainstay of treatment for bacterial diseases. However, the importance of the bacteriophage in the advancement of biological science cannot be overstated. Bacteriophages were intensively studied in the decades after their discovery and came to play a leading role in the advancement of the basic science of microbiology and the new biology of molecular genetics.

In the 1940s and onward, it was the laboratory study of phage biology that directly yielded major insights into bacterial genetics, molecular biology and the exact manner in which viruses reproduce and spread. These discoveries include:

- Mutations arise in the absence of selection [15.]
- Genetic transduction [16].
- DNA is genetic material [17].
- Restriction and modification [18].
- Acquisition and loss of genes from genomes [19].
- Molecular basis of DNA recombination [20].
- Gene regulation [21].

TABLE 1: CLASSIFICATION OF DIFFERENT BACTERIOPHAGES

Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	Myoviridae	Non enveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Non enveloped, no contractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Non enveloped, no contractile tail (short)	Linear dsDNA	T7 phage, T3 phage, P22, P37
Ligamenvirales	Lipothrixviridae	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
Unassigned	Ampullaviridae	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	No enveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	No enveloped, rod-shaped	Circular dsDNA	
	Corticoviridae	No enveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	No enveloped, lemon-shaped	Circular dsDNA	
	Globuloviridae	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	No enveloped, ovoid	Circular dsDNA	
	Inoviridae	No enveloped, filamentous	Circular ssDNA	
	Leviviridae	No enveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	No enveloped, isometric	Circular ssDNA	Φ × 174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	No enveloped, isometric	Linear dsDNA	

4. History of Bacteriophages: The first characterization of bacteriophages (phages) dates back to 1917 to the work of Felix d'Herelle [22]. Earlier, Ernest Hankin, Nikolay Gamaleya, and Frederick Twort were recognized (in 1896, 1898, and 1915, respectively) for their independent observations of the bactericidal effects of these bacterial viruses. Throughout the 1920s, d'Herelle published extensive work on phage biology and was accredited for helping establish the International Bacteriophage Institute in Tbilisi, Georgia in 1923 [10].

Bacteriophages initially appeared to offer great potential as frontline therapeutics against infectious disease in the pre-antibiotic era and were employed in many countries up until World War II [22]. 13 Microbiologists subsequently began to include the idea of phages into their world view, with phage therapy almost straight away coming to play a central role in the development of the field. Indeed, one can voluntarily trace the sequence of phage biology as starting with an early, passionate period during which claims were excessive and frequently unrealistic, while at the same time little was understood of the viral nature of phages or their strengths and limitations (the early 1920s into the 1930s).

An important exception to these concerns, most closely associated with the concept of phage therapy as practiced during these early years and as formulated in impressive detail, is the work of Felix d'Herelle (see "France," below). This time of excessive expectations was followed by a period of declining enthusiasm for phage therapy in much of the western world, subsequent displacement of its use after World War II by antibiotics and a shift in focus to using phages as model genetic systems (Eaton MD, Bayne-Jones S., 1934-I). This second stage started with the quite critical 1934 Eaton-Bayne-Jones report 14-16 reviewing the available literature on phage therapy 3 and continued through the late 1940s.

At the same time, the development of phage therapy and its active application continued to increase within the Soviet Union and Eastern Europe, where it was well supported until the fall of the Soviet Union (Eaton MD, Bayne-Jones S., 1934-II). In the West, the golden age of phage-based development of molecular biology involved intense work with just a few phages infecting one a virulent lab host (*E. coli* B) rather than a broad exploration of phages targeting a range of key pathogens (Eaton MD, Bayne-Jones S., 1934-III). Subsequently, phage therapy was "rediscovered" by the English-language literature starting with the work of Smith and Huggins in the 1980s.

This western phage therapy new beginning gained momentum only in the 1990s, however, as access was increasingly gained to the rich trove of Soviet and Polish work [23].

The field finally began maturing from those heady "wild west" days of the 1990s starting approximately in the year 2000, a progression that was coupled with an explosion of genomics and broad ecology-based phage research, with this latest era of phage therapy research as well as application continuing to this day [23].

Over the rest of this section, we provide an outline of phage therapy development in different parts of the world with special emphasis on France, which we cover over three sections; this extensive and well-documented French work has been largely ignored in previous reviews, presumably due to the language barrier [7]. Due to the widespread problem of antibiotic resistance coupled with the paucity of new antibacterial drugs, interest has been renewed in exploiting bacteriophages as a realistic option for treating antibiotic-resistant bacterial infections and for the control of problematic bacteria in many other areas including food [1].

5. Sources of Bacteriophages: Phages are found in almost all environments on Earth, ranging from soil, sediments, water (both river and seawater) and in/on living or dead plants/animals. Essentially, phages can be isolated from almost any material that will support bacterial growth.

The estimated global phage population size is extraordinarily high. For instance, it is estimated that aquatic environments have a total phage population above 10 [31]. Many terrestrial ecosystems have been shown to harbor 10^7 phages per gram of soil, and sewage is known to contain in the range of 10^8-10^{10} phage per milliliter [24].

6. Composition of Bacteriophages: Although different bacteriophages may contain different materials they all contain nucleic acid and protein. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acids of phages often contain unusual or modified bases, which protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection.

Simple phages may have only 3-5 genes while complex phages may have over 100 genes (Mayer-textbook). Certain phages are known to have single-stranded DNA as their nucleic acid.

The number of different kinds of protein and the amount of each kind of protein in the phage particle will vary depending upon the phage. The simplest phages have many copies of only one or two different proteins, while more complex phages may have many different kinds.

The proteins function in infection and to protect the nucleic acid from nucleases in the environment. Phages are also commonly employed in gene cloning, especially those exhibiting lytic and lysogenic cycles (Davidson, Michael W- textbook).

7. Structure of Bacteriophages: Bacteriophage comes in many different sizes and shapes. The basic structural features of bacteriophages are (which depicts the phage called T4).

Size: T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.

Head or Capsid: All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides); others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.

Tail: Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4, the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail, the more complex phages like T4 have a base plate and one or more tail fibers attached to it.

The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances, other structures are involved in the binding of the phage particle to the bacterium [25].

8. Infection of Host Cells:

Adsorption: The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers and it is reversible [26].

The tail fibers attach to specific receptors on the bacterial cell, and the host specificity of the phage is usually determined by the type of tail fibers that a phage has. The nature of the bacterial receptor varies for different bacteria [27]. Examples include proteins on the outer surface of the bacterium, LPS, pili, and lipoprotein. These receptors are on the bacteria for other purposes and phages have evolved to use these receptors for infection [28].

Irreversible Attachment: The attachment of the phage to the bacterium via the tail fibers is a weak one and is reversible. Irreversible binding of phage to a bacterium is mediated by one or more of the components of the base plate. Phages lacking base plates have other ways of becoming tightly bound to the bacterial cell [29].

Sheath Contraction: The irreversible binding of the phage to the bacterium results in the contraction of the sheath (for those phages which have a sheath) and the hollow tail fiber is pushed through the bacterial envelope. Phages that don't have contractile sheaths use other mechanisms to get the phage particle through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope [30].

Nucleic Acid Injection: When the phage has gotten through the bacterial envelope the nucleic acid from the head passes through the hollow tail and enters the bacterial cell. Usually, the only phage component that enters the cell is the nucleic acid. The remainder of the phage remains on the outside of the bacterium.

There are some exceptions to this rule. This is different from animal cell viruses in which most of the virus particle usually gets into the cell. This difference is probably due to the inability of bacteria to engulf materials [31] **Fig. 1** and **2**.

8.1 Lytic Cycle: Lytic or virulent phages are phages that can only multiply on bacteria and kill the cell by lysis at the end of the life cycle. Soon after the nucleic acid is injected, the phage cycle is said to be in an eclipse period. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell [32].

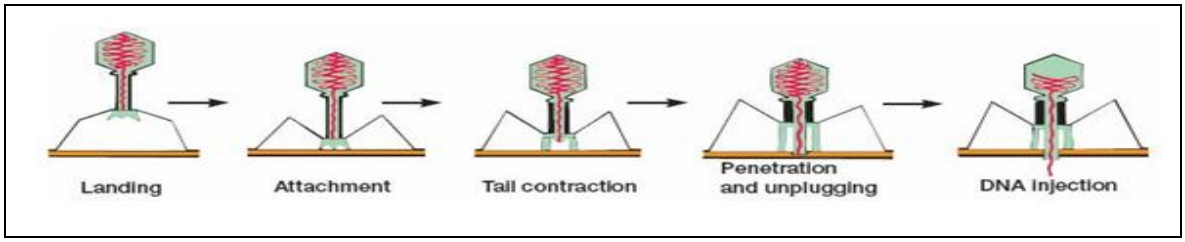

FIG. 1: BACTERIOPHAGE INFECTION TO THE HOST CELL

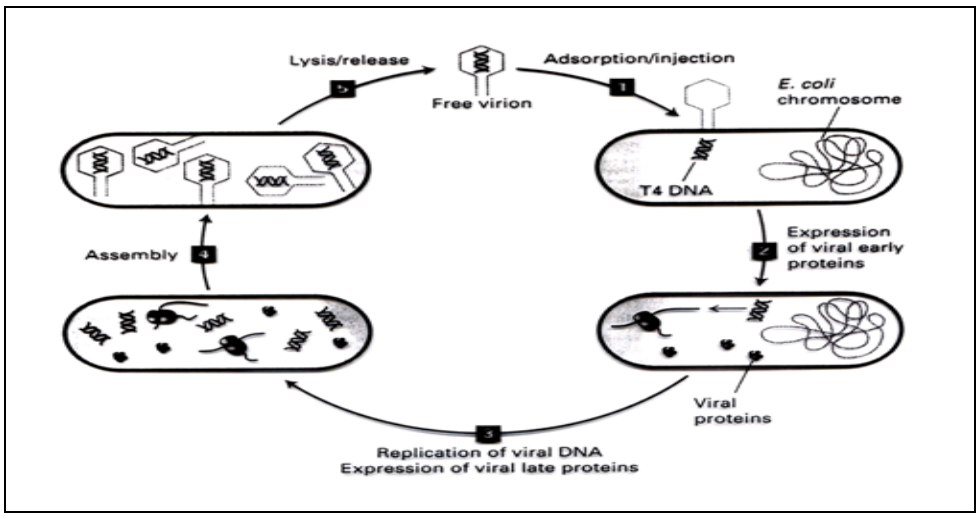

FIG. 2: LIFE CYCLE OF BACTERIOPHAGE

A. Eclipse Period: During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA and proteins are made.

There is an orderly expression of phage directed macromolecular synthesis, just as one sees in animal virus infections. Early mRNA's code for early proteins which are needed for phage DNA synthesis and for shutting off host DNA, RNA and protein biosynthesis. In some cases, the early proteins actually degrade the host chromosome. After phage DNA is made late m-RNA and late proteins are made. The late proteins are the structural proteins that comprise the phage as well as the proteins needed for lysis of the bacterial cell [33].

B. Intracellular Accumulation Phase: In this phase, the nucleic acid and structural proteins that have been made are assembled and infectious phage particles accumulate within the cell.

C. Lysis and Release Phase: After a while, the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage is released into the medium. The number of particles released per infected bacteria may be as high as 1000.

8.2 Lysogenic or Temperate Phage: Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a quiescent state in the cell. In this quiescent state, most of the phage genes are not transcribed; the phage genome exists in a repressed state. The phage DNA in this repressed state is called a prophage [34] because it is not a phage but it has the potential to produce phage. In most cases, the phage DNA integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. The cell harboring a prophage is not adversely affected by the presence of the prophage and the lysogenic state may persist indefinitely. The cell harboring a prophage is termed a lysogen [35].

8.2.1 Events Leading to Lysogeny-the Prototype Phage: Lambda

a. **Circularization of the Phage Chromosome:** Lambda DNA is a double-stranded linear molecule with small single-stranded regions at the 5' ends.

These single-stranded ends are complementary (cohesive ends) so that they can base pair and produce a circular molecule. In the cell, the free ends of the circle can be ligated to form a covalently closed circle [36].

b. Site-specific Recombination: A recombination event, catalyzed by a phage coded enzyme, occurs between a particular site on the circularized phage DNA and a particular site on the host chromosome. The result is the integration of the phage DNA into the host chromosome [37].

c. Repression of the Phage Genome: A phage coded protein, called a repressor, is made which binds to a particular site on the phage DNA, called the operator, and shuts off transcription of most phage genes except the repressor gene. The result is a stable repressed phage genome which is integrated into the host chromosome. Each temperate phage will only repress its own DNA and not that from other phages so that repression is very specific [38].

9. Classification: Bacteriophages occur abundantly in the biosphere, with different virions, genomes, and lifestyles. Phages are classified by the international committee on taxonomy of viruses (ICTV) according to morphology and nucleic acid. Nineteen families are currently recognized by the ICTV that infect bacteria and archaea of these, only two families have RNA genomes and only five families are enveloped of the viral families with DNA genomes, only two have single-stranded genomes.

Eight of the viral families with DNA genomes have circular genomes, while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only and one (Tectiviridae) infects both bacteria and archaea.

10. Bacterial Host Specificity: The bacterial host range of phage is generally narrower than that found in the antibiotics that have been selected for clinical applications. Most phage is specific for one species of bacteria and many are only able to lyse specific strains within a species. Host specificity is generally found at the strain level, species-level or more rarely, at the genus level. This specificity allows for directed targeting of dangerous bacteria using phages. The concept of fighting pathogens with their bacteriophages or using phages directly in foods has been around for many years [39].

This limited host range can be advantageous, in principle, as phage therapy results in less harm to the normal body flora and ecology than commonly used antibiotics, which often disrupt the normal gastrointestinal flora and result in opportunistic secondary infections by organisms such as clostridium difficile.

The potential clinical disadvantages associated with the narrow host range of most phage strains is addressed through the development of a large collection of well-characterized phage for a broad range of pathogens and methods to rapidly determine which of the phage strains in the collection will be effective for any given infection [40].

11. Advantages of Bacteriophage Therapy:

- For every type of bacteria known in nature, there is at least one complementary bacteriophage that specifically infects a single bacterial species. So, bacteriophage therapy is possible in all bacterial infections.
- If a suitable bacteriophage is introduced onto an infected wound, it will continue to increase in numbers as long as there are bacteria to infect and destroy. However, as soon as all the bacteria have been destroyed, the action of the phage will cease and the dormant phage particles will disperse harmlessly.
- Because phages are so specific to the bacteria they infect, they will not harm other beneficial bacteria present in the intestine and other parts of the body and will not affect the microbial ecosystem in the body. There is no chance of superinfection with other bacteria. The bacterial imbalance caused by treatment with many antibiotics can lead to serious secondary infections involving relatively resistant bacteria, often extending hospitalization time, expense and mortality. This will not occur with specific bacteriophage therapy.
- Some people are allergic to antibiotics so phage therapy could be a useful alternative for

these patients. No patient has ever been known to suffer an allergic reaction to bacteriophages. That may be because phages are omnipresent living organisms on earth, found in soil, water, plants, and humans.

- Phage therapies can be administered to patients in different ways, which include pills, injections, enemas, nasal sprays, ointments, *etc*.

- Each phage infects a specific bacteria or range of bacteria. A person in the hospital, where bacterial infections abound, can be treated with a range of phages targeted at several types of bacteria. They can be given a cocktail of phage types to attack one type of bacteria or they can be given a combination of phage and antibiotic treatment.

- Phages are considered safe for therapeutic use. No major side effects have been described so far. Only a very few side effects have been reported in the patients undergoing phage therapy. This might be related to the extensive liberation of endotoxin from dead bacteria as the phages were destroying the bacteria most effectively. This type of reaction can also happen when antibiotics are used.

- Since the selection of active phages is a natural process, evolutionary arguments support the idea that active phage can be selected against every resistant bacterium, by an ever ongoing process of natural selection.

- Production is simple and relatively inexpensive. So the treatment costs of bacterial infections will be reduced. This facilitates their potential applications to underserved populations [40].

12. Advantage of Phage Therapy over Antibiotics: Phage therapy can be very effective in certain conditions and has some unique advantages over antibiotics. On reflection of these studies, perhaps it would be wise to reconsider and rediscover phage therapy.

1. Bacteriophages are very specific to their hosts, so this minimizes the chance of secondary infections, but antibiotics do target both pathogens and normal flora of patients, which can cause secondary infections or sometimes superinfections.
2. Bacteria also develop resistance to phages, but it is incomparably easier to develop new phage than a new antibiotic.
3. Bacteriophages replicate at the site of infection where they are most needed to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection.
4. Phages have a special advantage for localized use because they penetrate deeper as long as the infection is present, rather than decrease rapidly in a concentration below the surface like antibiotics.
5. No side effects have been reported during or after phage application, but resistant bacteria, allergies (sometimes even fatal anaphylactic reaction), and secondary infections are the common side effects of antibiotics treatment.
6. Bacteriophages are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, may cost millions of dollars for clinical trials and may also not be very cost-effective.
7. Moreover, although bacteria can become resistant to phages, phage resistance is not nearly as worrisome as drug resistance. Like bacteria, phages mutate and therefore can evolve to counter phage-resistant bacteria.
8. Furthermore, the development of phage resistance can be forestalled altogether if phages are used in cocktails (preparations containing multiple types of phages) and/or in conjunction with antibiotics. In fact, phage therapy and antibiotic therapy, when co-applied, are synergistic [14, 40].

13. Bacteriophages:

Novel Therapeutic Agents: Bacteriophages are viruses that can infect Bacteria. Phages are able to infect more than 150 bacterial genera, including aerobes and anaerobes, exospores and endospore formers, cyanobacteria, spirochetes, mycoplasmas and chlamydias [41]. Structurally, they consist of a nucleic acid genome enclosed within a protein or

lipoprotein coat and like all viruses are absolute parasites, inert particles outside their hosts, deprived of their metabolism. Inside their hosts, phages are able to replicate using the host cell as a factory to produce new phages particles identical to its ascendant, leading to cell lysis and consequent death of the host [42].

As a result of their bacterial parasitism, phages can be found wherever bacteria exist and have already colonized every conceivable habitat. Phages are an extremely diversified group, and it has been estimated that ten phage particles exist for each bacterial cell. This fact accounts for an estimated size of the global phage population to be approximately 10^{31} particles making phages the most abundant living entities on earth.

Their presence in the biosphere is especially predominant in the oceans presenting an excess of 10^7 to 10^8 phage particles per milliliter in the coastal sea and in non-polluted water and comparably high numbers in other sources like sewage and feces, soil, sediments, deep thermal vents and in natural bodies of water [43]. In the absence of available hosts to infect, and as long as they are not damaged by external agents, phages can usually maintain their infective ability for decades [40].

14. Phage Therapy Versus Chemotherapy: Phage therapy presents many potential advantages over the use of antibiotics which are intrinsic to the nature of phages.

Phages are highly specific and very effective in lysing the target pathogen, preventing dysbiosis, that is, without disturbing the normal flora and thus reducing the likelihood of super-infection and other complications of normal-flora reduction that can often result following treatment with chemical antibacterials. This high specificity means that the diagnosis of the bacteria involved in the infection is required before therapy is employed [33, 37].

The specificity of phages also enables their use in the control of pathogenic bacteria in foods since they will not harm useful bacteria, like starter cultures.

Moreover, phages do not affect eukaryotic cells or cause adverse side effects as revealed through their extensive clinical use in the former Soviet Union. Furthermore, phages are equally effective against multidrug-resistant pathogenic bacteria. It was also found that phages can rapidly distribute throughout the body reaching most organs including the prostate gland, bones and brain that are usually not readily accessible to drugs and then multiply in the presence of their hosts [44].

The self-replicating nature of phages reduces the need for multiple doses to treat infectious diseases since they will replicate in their pathogenic host increasing their concentration throughout treatment leading to higher efficacy. This also implies that phages will be present and persist at a higher concentration where their hosts are present, which is where they are more needed in the place of infection. Reciprocally, where and if the target organism is not present, the phages will not replicate and will be removed from the system showing the other side of the self-replicating nature of phages, their self-limiting feature [45].

As it happens with antibiotics, bacteria also develop resistance to phages. The latter usually occurs through loss or modification of cell surface molecules (capsules, OMPs, LPS, pili, flagella) that the phage uses as receptors. Since some of these also function as virulence determinants their loss may in consequence dramatically decrease the virulence of the bacterium or reduce its competitiveness.

A good example is that of Smith (1987) that used phages against the K1 capsule antigen of *Escherichia coli* and verified that resistant K1 bacteria were far less virulent. Furthermore, different phages binding to the same bacteria may recognize different receptors and resistance to a specific phage does not result in resistance to all phages.

Phages are able to rapidly change in response to the appearance of phage-resistant mutants making them efficient in combating the emergence of newly arising bacterial threats [10, 37]. In addition, the isolation of a new phage able to infect the resistant bacteria can be easily accomplished. It is much cheaper, faster and easier to develop a new phage system than a new antibiotic which is a long and expensive process **Table 2** [45].

TABLE 2: COMPARISON OF THE PROPHYLACTIC AND/OR THERAPEUTIC USE OF PHAGES AND ANTIBIOTICS [10]

Bacteriophages	Antibiotics	Comments
Very specific (*i.e.*, usually affect only the targeted bacterial species); therefore, dysbiosis and chances of developing secondary infections are avoided	Antibiotics target both pathogenic microorganisms and normal microflora. This affects the microbial balance in the patient, which may lead to serious secondary infections	High specificity may be considered to be a disadvantage of phages because the disease-causing bacterium must be identified before phage therapy can be successfully initiated. Antibiotics have a higher probability of being effective than phages when the identity of the etiologic agent has not been determined.
Replicate at the site of infection and are thus available where they are most needed	They are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection.	The "exponential growth" of phages at the site of infection may require less frequent phage administration in order to achieve the optimal therapeutic effect
No serious side effects have been described	Multiple side effects, including intestinal disorders, allergies, and secondary infections (*e.g.*, yeast infections) have been reported	A few minor side effects reported (for therapeutic phages may have been due to the liberation of endotoxins from bacteria lysed *in-vivo* by the phages. Such effects also may be observed when antibiotics are used
Phage-resistant bacteria remain susceptible to other phages having a similar target range	Resistance to antibiotics is not limited to targeted Bacteria	Because of their more broad-spectrum activity, antibiotics select for many resistant bacteria species, not just for resistant mutants of the targeted bacteria.
Selecting new phages (*e.g.*, against phage-resistant bacteria) is a relatively rapid process that can frequently be accomplished in days or weeks	Developing a new antibiotic (*e.g.*, against antibiotic-resistant bacteria) is a time-consuming process and may take several years	Evolutionary arguments support the idea that active phages can be selected against every antibiotic-resistant or a phage-resistant bacterium by the ever-ongoing process of natural selection

TABLE 3: SOME OF THE PROBLEMS WITH EARLY THERAPEUTIC PHAGE RESEARCH AND THE WAYS THEY HAVE BEEN ADDRESSED IN MORE RECENT STUDIES OR CAN BE ADDRESSED IN THE FUTURE [10]

Problem	Comments	Solution and/or required approach
Narrow host range of phages	Because of the high specificity of phages, many negative results may have been obtained because of the failure to select phages lytic for the targeted bacterial species.	Determine the phage susceptibility of the etiological agent before using phages therapeutically; use polyvalent phage cocktails which lyse the majority of strains of the etiologic agent.
Insufficient purity of phage preparations	Early therapeutic phages were in crude lysates of host bacteria, and they contained numerous contaminants (including endotoxins) that may have counteracted the effect of phages.	Ion-exchange chromatography, high-speed centrifugation, and other modern purification techniques should be used to obtain phage preparations of high purity.
Poor stability and/or viability of phage preparations	Some commercial phage preparations were supplemented with mercurials or oxidizing agents or were heat-treated to ensure bacterial sterility. Many of these treatments also may have inactivated the phages, resulting in ineffective phage preparations.	Advanced purification techniques can be used to purify phages and to ensure that they are bacterium free. The viability and titer of phages should be determined before using them therapeutically.
Lack of understanding of the heterogeneity and mode of action of phages (*i.e.*, lytic *vs.* lysogenic phages)	Failure to differentiate between lytic and lysogenic phages may have resulted in some investigators using lysogenic phages, which are much less effective than lytic phages	Carefully select for lytic phages. This is also critical for avoiding the possible horizontal transfer of bacterial toxin, antibiotic resistance, *etc.*, genes by lysogenic phages
Exaggerated claims of effectiveness of commercial phage preparations	One example of this would be the preparation called *Enterophagos*, which was marketed as being effective against herpes infections, urticaria, and eczema - conditions against which phages could not possibly be effective	Phage preparations should be accompanied by specific, scientifically supported information about their efficacy against specific bacterial pathogens, their possible side-effects, etc.
Failure to establish scientific proof of efficacy of phage treatment	Most clinical studies using therapeutic phages were conducted without placebo controls; also, when placebo controls were used, data were evaluated in a subjective manner questioned by many peers.	Carefully controlled, double-blinded placebo studies with highly purified, lytic phages should be conducted and results must be evaluated based on both clinical observations and scrupulous laboratory analysis.

15. Problems Associated With Bacteriophage Therapy:

- Because of the high specificity of phages, the disease-causing bacterium has to be identified before the administration of phage therapy. One phage kills only a specific subgroup of bacteria. One species of bacteria may contain many subgroups. But one antibiotic may kill many different species and subgroups of bacteria simultaneously. So, a physician would need to make a specific diagnosis before prescribing a phage treatment.
- Absences of bacteriophage action efficacy in certain cases were reported. It may be due to insufficient diagnostics and incorrect choice of the method for the implementation of a specific phage.
- The gastric acidity should be neutralized prior to oral phage administration.
- Bacteriophage with a lytic lifecycle within a well-defined *in-vitro* environment does not ensure that the bacteriophage will always remain lytic under normal physiological conditions found in a body. It may change to adapt lysogenic cycle in some circumstances.
- Bacteriophages are viruses and in general, viruses tend to swap genes with each other and other organisms with which they come into contact. So, there is a chance of the spread of antibiotic resistance in bacteria.
- Many doctors are scared to give live bacteriophage to the patients.

16. Phage Therapy: Phage therapy or viral phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phages were discovered to be antibacterial agents and were used in Georgia and the United States during the 1920s and 1930s for treating bacterial infections. Frederick Twort promoted the use of phages as antibacterial agents soon after their discovery, but antibiotics, upon their discovery, proved more practical. Research on phage therapy was largely discontinued in the West, but phage therapy has been used since the 1940s in the former Soviet Union as an alternative to antibiotics for treating bacterial infections. They had widespread use, including the treatment of soldiers in the Red Army. However, they were abandoned for general use in the West for several reasons:

- Medical trials were carried out, but a basic lack of understanding of phages made these invalid.
- Phage therapy was seen as untrustworthy because many of the trials were conducted on totally unrelated diseases such as allergies and viral infections.
- Antibiotics were discovered and marketed widely. They were easier to make, store and prescribe.
- Former Soviet research continued, but publications were mainly in Russian or Georgian languages and were unavailable internationally for many years.
- Clinical trials evaluating the antibacterial efficacy of bacteriophage preparations were conducted without proper controls and were methodologically incomplete preventing the formulation of important conclusions

Their use has continued since the end of the Cold War in Georgia and elsewhere in Central and Eastern Europe. Globalyz Biotech is an international joint venture that commercializes bacteriophage treatment and its various applications across the globe. The company has successfully used bacteriophages in administering phage therapy to patients suffering from bacterial infections, including *Staphylo-coccus* (including MRSA), *Streptococcus*, *Pseudom onas*, *Salmonella*, skin and soft tissue, gastrointestinal, respiratory and orthopaedic infections. In 1923, the Eliava Institute was opened in Tbilisi, Georgia, to research this new science and put it into practice.

The first regulated randomized, double-blind clinical trial was reported in the Journal of Wound Care in June 2009, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The study was approved by the FDA as a phase I clinical trial. Study results satisfactorily demonstrated the safety of the therapeutic application of bacteriophages, however it did not

show efficacy. The authors explain that the use of certain chemicals that are part of standard wound care (*e.g.* lactoferrin, silver) may have interfered with bacteriophage viability. Another regulated clinical trial in Western Europe (treatment of ear infections caused by *Pseudomonas aeruginosa*) was reported shortly after in the Journal Clinical Otolaryngology in August 2009.

The study concludes that bacteriophage preparations were safe and effective for treatment of chronic ear infections in humans. Additionally, there have been numerous animal and other experimental clinical trials evaluating the efficacy of bacteriophages for various diseases, such as infected burns and wounds and cystic fibrosis associated lung infections, among others. Meanwhile, Western scientists are developing engineered viruses to overcome antibiotic resistance and engineering the phage genes responsible for coding enzymes which degrade the biofilm matrix, phage structural proteins and also enzymes responsible for lysis of bacterial cell wall. The water within some rivers traditionally thought to have healing powers, including India's Ganges river, may provide sources of naturally-occurring viral candidates for phage therapy.

17. Bacteriophages as Therapeutic Agents: Mode of Action and Safety Profile:

Mode of Action: Despite the large number of publications on phage therapy, there are very few reports in which the pharmacokinetics of therapeutic phage preparations is delineated. The few publications available on the subject suggest that phages get into the bloodstream of laboratory animals (after a single oral dose) within 2 to 4 h and that they are found in the internal organs (liver, spleen, kidney, *etc*.) in approximately 10 h. Also, data concerning the persistence of administered phages indicate that phages can remain in the human body for relatively prolonged periods of time, *i.e.*, up to several days. However, additional research is needed in order to obtain rigorous pharmacological data concerning lytic phages, including full-scale toxicological studies, before lytic phages can be used therapeutically in the West. As for their bactericidal activity, therapeutic phages were assumed to kill their target bacteria by replicating inside and lysing the host cell (*i.e., via*. a lytic cycle). However, subsequent studies revealed that not all phages replicate similarly and that there are important differences in the replication cycles of lytic and lysogenic phages.

Furthermore, the recent delineation of the full sequence of the T4 phage (Gen Bank accession No. AF158101) and many years of elegant studies of the mechanism of T4 phage replication have shown that lysis of host bacteria by a lytic phage is a complex process consisting of a cascade of events involving several structural and regulatory genes. Since T4 phage is a typical lytic phage, it is possible that many therapeutic phages act via a similar cascade; however, it is also possible that some therapeutic phages have some unique yet unidentified genes or mechanisms responsible for their ability to effectively lyse their target bacteria. For example, a group of authors from the EIBMV identified and cloned an anti-*Salmonella* phage gene responsible, at least in part, for the phage's potent lethal activity against the *Salmonella enterica* serovar typhimurium host strains. In another study, a unique mechanism has been described for protecting phage DNA from the restriction-modification defenses of an *S. aureus* host strain. Further elucidation of these and similar mechanisms are likely to yield information useful for genetically engineering optimally effective therapeutic phage preparations.

CONCLUSION: As per the literature survey on the use of bacteriophages against bacterial infections, specifically those of multidrug-resistant bacteria, further confirm safe for the view of phage therapy as either an alternative or a supplement to antibiotics. Bacteriophages have some characteristics that make them potentially attractive therapeutic agents like.

It is effective against multidrug-resistant pathogenic bacteria because the mechanisms by which it induces bacteriolysins differ completely from antibiotics. Substituted microbiome does not occur because it has high specificity for target bacteria. It can respond rapidly to the appearance of phage-resistant mutants because the phages themselves are able to mutate.

The Cost of developing a phage system is cheaper than that of developing a new antibiotic and because phages or their products (*e.g.*, lysin, see

below) do not affect eukaryotic cells, side effects from phages per se are uncommon. In adding up, a large number of survey reports, some of which are re-evaluated in this review, propose that phages may be effective therapeutic agents in selected clinical situations.

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