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The Review on Bacteriophage Therapy

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ABSTRACT

The emergence of multiple drug-resistant bacteria has prompted interest in alternatives to conventional antimicrobials. One of the possible replacement options for antibiotics is the use of bacteriophages as antimicrobial agents. Phage therapy is an important alternative to antibiotics in the current era of drug-resistant pathogens. Bacteriophages have played an important role in the expansion of molecular biology and have been used as antibacterial agents since 1966. The seemingly inexorable spread of antibiotic-resistance genes among microbial pathogens now threatens the long-term viability of our current antimicrobial therapy to treat severe bacterial infections such as sepsis. Antibiotic resistance is reaching a crisis situation in some bacterial pathogens where few therapeutic alternatives remain and pan-resistant strains are becoming more prevalent. Phages as bactericidal agents have been employed for 90 years as a means of treating bacterial infections in humans as well as other species, a process known as phage therapy. In this review, we explore both the early historical and more modern use of phages to treat human infections. We discuss in particular the little-reviewed French early work, along with the Polish, US, Georgian, and Russian historical experiences.

Keywords: Phase therapy, Pathogens, Bacteriophase, Genes.

INTRODUCTION

Phage therapy involves the targeted application of bacteriophages that, upon encounter with specific pathogenic bacteria, can infect and kill them. As typically practiced, phages then lyse those bacteria, releasing virion progeny that can continue the cycle, including migrating to other sites of infection anywhere in the body. The actual phage-mediated bacterial killing, however, occurs well prior to the lysis stepe.g., such as in the first minutes of infection for a phage such as a phage as the phage converts the cell into a factory for making new phages. Phages are unique among antibacterial agents in their ability to increase their numbers when in the presence of bacterial targets. Of similar importance, phages only minimally impact non-target bacteria or body tissues. A more complete list of advantages associated with phage therapy, relative, particularly to chemical antibacterials, is presented in this issue and elsewhere. Here we review the potential for phages to treat bacterial infections afflicting humans. Other therapeutic applications, such as in veterinary medicine, have been reviewed in reference and will be also covered in future issues of this journal. Other reviews focusing on various aspects of human phage therapy are also available. This time of excessive expectations were 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. This second stage started with the quite critical 1934 Eaton-Bayne-Jones report reviewing the available literature on phage therapy 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. In the West, the golden age of phage-based development of molecular biology involved intense work with just a few phages infecting one avirulent lab host (E. coli B) rather than broad exploration of phages targeting a range of key pathogens.

BACKGROUND

Bacteriophages are a group of viruses widely distributed in nature whose life cycle is strictly associated with the bacterial cell. They are known as bacterial parasites because they lack the cell structure and enzyme systems necessary for food uptake, protein synthesis or construction of new particles, and as incomplete organisms can only replicate in a live cell. Bacteriophages were discovered by Twort (1915) as unidentified molecules that inhibit bacterial growth, but in 1917 D'Herelle was the first to isolate and characterize phages, and he also developed the first phage therapy against fowl typhoid induced by Salmonella Gallinarum in chickens [1]. Positive results of the use of bacteriophages in fighting bacterial infections have contributed to the development of research on the potential use of viruses that destroy bacteria in treatment of diseases in both human and animals [2, 3].

History of Bacteriophage:

The history of phage therapy could be separated into four timespans, according to Summers et al. [4]

Early Enthusiasm

Bacteriophages were discovered by English microbiologist Twort in 1915 [1] but ''the bacteriophage phenomenon'' era began after publication in 1917 by a French-Canadian microbiologist Felix d'Herelle. During his investigations, he observed ''invisible microbes'' in filtrates of stool from patients suffering from dysentery that was ''antagonistic'' to bacteria. He surmised that this filterable virus, ''ultra viruses,'' was a cofactor of bacterial infection. However, he proved that phage titers increased in disease progression and peaked during recovery. After those successes, d'Herelle branched out his investigations on humans. At first, he had tested the safety of phage suspension on himself, his co-workers, and family, then on patients suffering from ''bacillary dysentery'' and cholera (since 1919). After that, phages were applied as a therapy to wound recovery. Another experiment that focused on the healing value of phages investigated Salmonella GalliNarum as an infectious agent of avian kyphosis (published in 1926). This test also confirmed phage protection, as well as it did against other species, like Pasteurella multocida (bovine hemorrhagicsepticemia, published in the same year). Nevertheless, the first publication about phage therapy described the work of Bruynoghe and Maison. Their results were published in 1921 [4,5]

How Do Bacteriophages Kill Bacteria?

The increasing number of antibiotic-resistant bacterial strains is a serious problem in contemporary medicine. What is important is the fact that these bacteria do not implicate resistance to phage lysis mechanisms. Lytic bacteriophages are able to kill antibiotic-resistant bacteria at the end of the phage infection cycle. Most of them utilize two-component lysis systems to destroy a bacterial cell wall in order to release progeny virions. Thus, the development of phage therapy is a potential way to improve the treatment of bacterial infections. The course of a replication cycle is the criterion to divide bacteriophages into lytic and lysogenic ones. The release of lytic phage progeny from infected cells requires bacterial lysis. Scientific studies on phage lytic mechanisms contribute to the development of

phage therapy. Some lytic bacteriophages use single proteins, admins, to inhibit the synthesis of peptidoglycan [6]. However, most of them utilize two groups of proteins to kill the host cell. The first ones, holins, synergize with the second ones, endolysins, to cause lysis. Together, they make up the holin–lysin systems [7,8]. Holins are involved in the host cell lysis-triggering process. Their role is to perforate the host cytoplasmic membrane and thus cooperate with endolysins by giving them an access to bacterial peptidoglycan. Therefore, holins determine the time of bacterial lysis. Acting at a precise time point, they control bacterial murein accessibility for phage endolysins and thus they synchronize the activity of the holin–lysin system with the late-phase events of the phage replication cycle [9]. The primary structure of holins is not well conserved in evolution. However, differences in the amino acid sequences among holins are not reflected by their function. Every holin has at least one hydrophobic transmembrane domain (TMD) as well as a C-terminal hydrophilic domain that carries a high electric charge. There are three classes of holins. Class I includes proteins that have more than 95 amino acid residues in length.

Application of phages in biocontrol and therapeutic design:

Phage therapies are also an effective tool in eliminating bacterial infections in various species of animals. Bacteriophages have also proven successful in treating diseases in poultry. One of the objectives of phage therapy in animals is to assess the suitability of bacterial viruses for the control of pathogens having an important influence on animal productivity and health. Phages used in treatment have been effective in preventing infections and in the treatment of colibacteriosis in poultry [10]. Positive results, with a high success rate in eliminating pathogens, have also been obtained in combating infections induced by various Salmonella serotypes in gamefowl, such as Enteritidis and Typhimurium [11-14], as well as campy bacteriosis in poultry, particularly infections induced by Campylobacter jejuni and C. coli [15]. The effectiveness of phage therapy has also been confirmed in infections of broiler chickens by anaerobic Clostridium perfringens during the course of necrotic enteritis [16].

The nature of bacteriophages and bacteriophage infections:

Bacteriophages are obligate parasites of bacteria, using the resources of the bacterial cell to replicate. They are typically highly specific, often being restricted to particular strains within a single bacterial species. However, some bacteriophages have a relatively broad host range, infecting multiple species within a genus, and can even infect members of other genera closely related to their normal host. In some cases, this reflects the uncertain nature of taxonomy, and in others, the presence of common receptors. Bacteriophages will multiply when (and only when) their specific bacterial host is present, allowing the use of extremely low input doses when treating infection. More than 90% of bacteriophages have large, double-stranded DNA genomes located in heads with icosahedral symmetry, with tails of varying lengths. They belong to three major morphological groups of bacteriophages. These are the Myoviridae (with long, rigid, contractile tails), the Siphoviridae (with long, flexible, noncontractile tails), and the Podoviridae (with short, noncontractile tails). The morphology and genome type of the remaining bacteriophage families is highly variable, and they may have DNA or RNA genomes. One notable group with single-stranded DNA genomes (Inoviridae) appear as long filaments (Maniloff 2006). The outcome of infection of the bacterial cell may vary. Some bacteriophages undergo a lytic infectious cycle, with infection resulting in rapid lysis and death of the cell within a very short time.[17] Typically, this will result in the release of hundreds of new, infectious virus particles within minutes or

hours, a process that can be repeated as long as their bacterial host is present in sufficient numbers to support replication (Harper and Kutter, 2008). Many bacteriophages are strictly lytic (sometimes referred to as virulent) and are unable to produce any other kind of infection. Others, known as temperate bacteriophages, may infect cells but then become dormant in the latent state known as lysogeny and are replicated along with the host cell chromosome and are subsequently transmitted to each daughter cell following cell division. However, these dormant prophages may be activated into lytic infection by specific stimuli, such as DNA damage.[18] For some bacteriophages, host chromosomal DNA may be packaged into bacteriophage particles during bacteriophage replication instead of the bacteriophage genome. This can result in high levels of horizontal gene transfer within the bacterial population, a process known as transduction. In contrast to the lytic and lysogenic cycles of bacteriophage infection, the filamentous bacteriophages typically cause persistent infection of bacterial cells that does not kill the host but results in continued excretion of viral particles (Harper and Kutter 2008).[19]

Use of bacteriophages in food and agriculture:

There is considerable literature from the early days of the 20th century relating to the treatment of bacterial infections of animals (rather than the use of animals as models of human infection as described earlier) but much of this has generated very variable results. Early studies by d'Herelle suggest that avian typhoid (Salmonella enterica serovar Gallinarum) was susceptible to bacteriophage treatment although this was not repeatable by Pyle with a bacteriophage that was effective in vitro. Topley et al. were not successful with the treatment of murine typhoid caused by Salm. enterica serovar Typhimurium. Variable results were also obtained with attempts to treat staphylococcal and Yersinia pestis infections of rabbits and mice. The situation from a review of early work was confusing and potentially disappointing (Sulakvelidze and Barrow 2005).[20] The topic was taken up again in the early 1980s by Smith who was able to protect against E. coli infections. This was performed for septicaemia in mice and also for gastroenteritis in neonatal calves, pigs and lambs and involved strictly controlled experiments (Smith and Huggins 1982, 1983; Smith et al. 1987). The results showed therapeutic benefit and also that prophylaxis was possible even by treating animal bedding alone. These experiments were extended by his group with success when applied against E. coli septicaemia in poultry and calves (Barrow et al. 1998).[21] Use of bacteriophages against food-borne bacterial pathogens has been reviewed by Garcia et al. (2008), but showed variable success. Treatment of systemic salmonellosis in poultry was unsuccessful unless bacteriophages were administered immediately following the bacteria. Reduction in intestinal colonization, aimed to reduce entry of this organism into the human food chain, was also not successful (Berchieri et al. 1991). However, in 2010, the Korean CheilJedang Corporation introduced BioTector, a bacteriophage product intended to reduce Salmonella levels in poultry. [22] Connerton et al. were successful in reducing numbers of Campylobacter jejuni in the chicken gut by a factor of between 1 and 4 logs by bacteriophage administration immediately prior to slaughter (Loc Carrillo et al. 2005). To avoid the development of bacteriophage resistance in production facilities, it is important that animals are treated after being removed from the production sites shortly before slaughter so that recycling is impossible. However, it has been found that bacteriophage-resistant strains of Campylobacter recovered after bacteriophage infection are less able to colonize new birds (Loc Carrillo et al. 2005). Bacteriophages have also been found to be effective in experimental vancomycin-resistant Ent. faecium infection of mice and against Lactococcus garviae infection of yellowtail fish (Nakai and

Park 2002). In Pseudomonas plecoglossicida infection of Ayu fish, feeding bacterio phage-treated feed pellets has been found to be effective, providing a practical approach for the treatment of large numbers of animals (Park and Nakai 2003). In agriculture, bacteriophages have also been applied against plant infections including Erwinia amylovora infection of apple blossom (Schnabel and Jones 2001), and Ralstonia solanacearum and Xanthomonas campestris infection causing bacterial wilt and spot, respectively, in tomatoes (Fox 2000). Bacteriophages can also be applied in combination with other crop protection technologies (Obradovic et al. 2004). The successes in this research area lead to the development of a commercial biocontrol product (Agriphage) produced by the US company Omnilytics. This comprises tailored bacteriophage preparations and has been used primarily to treat tomato and pepper spot, but has shown to be successful for the treatment of a wide range of diseases affecting other crops. The product is marketed throughout North and South Amer ica and has more recently been licensed for use in Asia. In 2006, Agri-Phage was recognized by the Organic Materials Review Institute (OMRI) as being compatible with organic food production.[23-28]

Bacteriophages for control pathogens:

The most common bacteria inducing foodborne infections in humans include bacteria of the genera Salmonella and Campylobacter and E. coli. According to the 2015 EFSA report on resistance to antibacterial agents in selected zoonotic bacteria (Salmonella and Campylobacter), indicator bacteria (E. coli and Enterococcus spp.), and other bacteria isolated from poultry and from food, a considerable percentage of the isolates posing a threat to humans and animals are resistant to available antibiotics, partially as a result of their widespread use in the treatment of disease in humans and animals. The use of bacteriophages to eliminate pathogens seems quite promising, especially as they are present in every ecosystem and number 1031, which is more than 10 times the number of characterized bacteria [11, 19, 20]. The effectiveness and safety of phage therapy in comparison to antibiotics is partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype or strain. This mechanism of action does not cause the destruction of the commensal intestinal flora. Self-replication of bacteriophages takes place during treatment, which eliminates the need to apply them repeatedly. Another advantage of phages is that they cannot bind to and replicate in eukaryotic cells, which causes a decrease in their titer, correlated with a marked reduction in the number of pathogenic bacteria inducing a given infection in the organism. An equally important advantage is that phages are not toxic, because most of them are composed mainly of proteins and nucleic acids [21]. Despite its numerous advantages, the use of phage therapy is substantially limited, partly because single bacteriophages cannot be used to combat broad-spectrum infections. In many cases complex identification and characterization of the aetiological agent is necessary. Moreover, not all bacterial viruses meet the criteria for use in treatment, particularly lysogenic phages, which encode genes of bacterial toxins and thereby transform harmless bacteria into pathogenic ones. They can also be involved in transferring drug-resistance genes among bacteria. Another adverse phenomenon in phage therapy is that phages can be cleared by the reticuloendothelial system, reducing their half-life in the organism and limiting the effectiveness of treatment [29-35].

Clinical use of phage Short overview:

Among all scientists more than one century ago who described lytic effects in bacterial colonies, which could have been caused by an agent called bacteriophage today, the Franco-Canadian Felix d`Herelle, in

1917, forwarded the most conclusive description of this multiplying agent. From the beginning, he forced the use of phage for therapeutic measures to kill pathogenic bacteria. In the twenties and thirties of the twentieth century, when he helped to establish the bacteriophage institute in Tbilisi, Georgia, he even wrote a book "Bacteriophage and the phenomenon of recovery" in the Russian language [36] This institute is a leading institution in the production and use of therapeutic phage. However, it has been argued that only a few systematic studies were performed in Tbilisi and the clinical trials did not comply with internationally approved standards. This appraisal might have not considered those facts as military secrecy in the Soviet Union, loss of documentation, and language barriers. Therefore, the Tbilisi colleagues published conclusive overviews demonstrating the methods and results of their studies [37,38]. Until World War II, phage preparations were produced and used in Europe, Asia, and America [39]. One has to envision that, at this time, nobody had beheld these virus particles. By use of the newly developed electron microscope, Helmut Ruska, a medical doctor at the Charité Medical School in Berlin, visualized phage and phage-induced lysis of bacteria for the first time. Production of phage preparations for therapeutic use continued also after World War II but ceased during the next decennia because of the "triumphal procession" of antibiotics. Instead, phage became momentous experimental models contributing to the rapid development of molecular biology [40]. Scientific milestones such as the heredity role of DNA, the uncovering of the genetic code, the discovery of restriction-modification systems, and CRISPR Cas in bacteria or the capability of viruses to integrate into the host genome are due to basic research on phage. Phage preparation in France stopped as late as in the 1990s. This decision appears something paradoxical since this was the time of the beginning "antibiotics crisis". However, French pharmaceutical legislation responding to the AIDS crisis forbade any quantities of 'viruses' in medicinal products [41]. From the discovery of bacteriophage at the beginning of the twentieth century till nowadays, they were applied to cure bacterial infections in the former Soviet Union and in Poland but were more or less ignored in Western medicine [42]. Numerous excellent overviews have been published on the history demonstrating the efficiency of therapy is limited and studies did not demonstrate credible efficacy in all cases. For major controlled studies, see references [43]. Older studies have been summarized by Abedon et al.y and the clinical use of phage therapy [44]. Until today, the number of controlled clinical studies to demonstrate the efficiency of therapy is limited and studies did not demonstrate credible efficacy in all cases. For major controlled studies, see references. Older studies have been summarized by Abedon et al.

Transduction ability:

Therapeutic phage should not have the ability to transduce bacterial genes, which could include virulence, toxin, and antibiotics resistance genes. Therefore, potential therapeutic phages should be investigated for their general ability to perform transduction. Bothering genes being transduced could be originate from bacteria in the organism of the patient or from cells used for phage production in the laboratory. Therefore, bacterial strains for phage production should undergo control to show the absence of those genes. However, especially for some target bacteria, it may not be possible to find production strains that are both functional and plausibly clean [45]. In generalized transduction, any fragment of the bacterial genome can be packaged into a phage capsid and transferred to another bacterial cell. This process can occur with members of both virulent and temperate phages. The ability of generalized transduction appears to depend on the genome packaging mechanism of phage. Phages with packaging initiation from non- or low-specific DNA sequences are considered to accept also foreign genetic

material during maturation. However, some phages, such as classical T3 and T7 [46], require specific sequences in their genomes to initiate DNA packaging; therefore, they should be unable to attach and insert foreign genetic material. Moreover, phages that degrade the DNA of the infected cell and use it as building blocks for their own genomes should be considered as non-transducers [47]. Generalized transducing phages can be predicted by microbiological techniques (testing the ability to transfer easily selectable markers between bacterial strains) or by PCR-based techniques (testing the presence of bacterial DNA in purified phage particles). In addition to the use of sequencing and bioinformatic prediction programs, one can remember classical phage techniques to assess the transduction ability of phage [48]. In specialized transduction, induction of a prophage can lead to additional packaging of neighboring bacterial genes into the phage particles. Therefore, specialized transduction can be only performed by temperate phage, which, however, should be excluded from therapeutic use. To predict the ability of a phage for special transduction, one can also search for motifs of integrase-encoding genes or attachment regions.

Phage engineering for pathogen treatment:

Phage engineering is an area of research that is attracting intense interest and has great potential to enhance phage antimicrobial activity for pathogen treatment. Several original papers and reviews present techniques successfully applied to engineer phage [49]. Companies in several countries already take advantage of the methods described in the following chapter. Sophisticated molecular biological techniques, developments in synthetic biology, and fast and low-cost DNA sequencing methods provide technical preconditions to optimize phage for their increased antimicrobialeffectiveness and efficiency [50]. Very recently, the first use of engineered phage to treat a severe disseminated Mycobacterium abscesses infection in a 15-year-old cystic fibrosis patient was reported. Lytic phage derivatives, which effectively killed the pathogen, were developed by Bacteriophage Recombineering of Electropo-rated DNA (BRED) to eliminate a phage repressor gene and by a genetic selection of host range mutants. Intravenous therapy using a three-phage cocktail, conducted for 32 weeks, was well tolerated and was associated with significantclinical improvement [51] To enhance the bactericidal effect, the phage can be altered either to enter the target cell or to damage the cell after entry more efficiently. To the first aim, Scholl et al. designed a T7 construct with the K1-5 endosialidase gene cloned downstream of the major capsid gene of T7 (T7endo). The endo sialidase was produced and released into the lysate after T7endo infection and lysis of the host cells. T7endo, in contrast to wild-type T7, allowed the phage to infect also capsule-producing E. coli cells [51]. Lu and Collins equipped phage T7 with gene dB from Actinobacillus actinomycetescontain that confers biofilm-degrading activity to T7 by Dispersion B [52]. The engineered T7 phage reduced bacterialbiofilm cell counts by two orders of magnitude when compared with wild-type T7. An alternative approach is to introduce genes into the phage genome, the products of which severely interfere with functional cellular networks. The same authors engineered the non-lytic filamentous phage M13mp18 to produce the Lex3 repressor of the SOS response [99]. The antibiotictreatment induces hydroxyl radical formation that leads to DNA, protein, and lipid damage and eventually to cell death. DNA damage in turn induces the SOS response resulting in DNA repair. By preventing the repair process, the engineered M13mp18 enhanced the antibiotic-induced killing of Escherichia coli in vivo and in vitro. In another study, phage T7 was engineered to express a lactonase enzyme with broad-range activity to disturb quo rum-sensing [53]. Most bacteria use this biochemical communication process to coordinate the behavior of individual bacterial cells in a group by small

molecules (acyl homoserine lactones, AHL). Quorum sensing is a pre-requisite for biofilm formation that displays an important pathogenicity marker. T7 lactonase produced by a recombinant T7 variant degrades essential small AHL autoinducer molecules and inhibited biofilm formation by a mixture of Escherichia coli and Pseudomonas aeruginosa [54]. A further route is to introduce "lethal genes" into bacteria that (i) restore bacterial susceptibility to antibiotics [55], (ii) reduce the bacterial population [56], or (iii) achieve endocytosis of engineered phage into eukaryotic cells to combat in travelregularly replicating pathogens [56]. Other studies showed how phage therapy becomes more effective.

The Risk of Emergence of Bacterial Resistant to Bacteriophages (BPs):

Emergence of bacterial resistance against BPs is potentially possible, as bacteria possess or can develop several mechanisms to prevent viral infections. Among these are hiding, change or loss of receptor, secretion of substances that prevent phage adhesion to the bacterial pathogen, activation of measures for blocking phage DNA injection into the cell and inhibition of phage replication and release.[57-60] Alteration or loss of receptor for membrane protein modifications has been demonstrated for E. coli S. aureus Bordetella bronchiseptica and Vibrio cholerae. Secretion of extracellular polymeric substances and glycoconjugates has been described for Pseudomonas spp. and Enterobacteriaceae respectively. Development of bacterial resistance to BPs can be reduced with the use of BP cocktails, with the administration of a higher initial BP inoculum or the association with antibiotics. If BPs kill pathogens faster than they can replicate, a high inoculum is associated with a lower risk of the development of BP-resistant bacteria. However, all these findings indicate that selection of a therapeutic BP must take into account the ability of each virus to induce bacterial resistance and the amount needed to avoid bacterial resistance development encapsulating.[61-65]

CONCLUSION

The use of BPs to overcome the problem of increasing microbial resistance to antibiotics is attractive, and some research data seem to indicate that it might be a rational measure. However, present knowledge seems insufficient to allow the use of BPs for this purpose. To date, properly designed clinical trials specifically planned to evaluate BP efficacy are very few and partially negative. Moreover, the problem of how to prepare the formulations for standardized and clinical use in bacterial control, how to avoid or limit the risk of emergence of bacterial resistance, and the transmission of genetic material is not completely solved problems. In addition, the mechanisms concerning coevolution between BP and bacteria are unknown. Further studies specifically devoted to solving these problems are needed before BPs can be used in humans.phage in liposomes delivered to treat intracellular bacteria.

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