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Bacteriophages: The possible solution to treat infections caused by pathogenic bacteria

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Bacteriophages to treat AMR bacteria

Abstract

Bacteriophages have been used to treat bacterial infections in animals and humans since their discovery in 1915, due to their unique ability to infect their specific bacterial hosts, without affecting other bacterial populations. The research carried out in this field throughout the twentieth century, largely in Georgia, part of USSR and Poland, led to the establishment of phage therapy protocols. However, the discovery of penicillin and sulphonamide antibiotics in the Western World during the 1930's was a setback in the advancement of phage therapy. The misuse of antibiotics reduced their efficacy in controlling pathogens and led to an increase in the number of antibiotic resistant bacteria. Bacteriophages have become a topic of interest as an alternative to antibiotics with the emergence of multidrug-resistant bacteria, which are a threat to public health. Recent studies have indicated that bacteriophages can be used indirectly to detect pathogenic bacteria or directly as biocontrol agents. Moreover, they can be used to develop new molecules for clinical applications, vaccine production, drug design and in the nanomedicine field via phage display.

Keywords: Bacteriophages, Antibiotics, Biocontrol, Infection, Pathogenesis

Introduction

Bacteriophages are small viruses that have the ability to infect bacteria. They have a huge influence on our environment as they play a vital role in maintaining its microbial balance. Phages are ubiquitous; they can be found in all the natural habitats, including aquatic and terrestrial systems, in which their bacterial hosts are present. Over 6000 different phages have been identified and described morphologically (Ackermann and Prangishvili 2012). They can be classified based on their morphology, genetic content, host, habitat, or life cycle. Phages exhibit different life cycles within its bacterial host: virulent and temperate. However, all phages are comprised of a nucleic acid genome (whether DNA or RNA) encased within a capsid. Upon infection, virulent phages take over the host's metabolic activities, directing the bacterial molecular machinery into synthesizing more phage particles. The host cell is lysed once the viral progeny is released, hence the term "virulent phages". Temperate phages, those initiating a lysogenic life cycle, often integrate their genome with that of their host maintaining a quiescent stage (prophage). The prophage is vertically transferred with the bacterial genome as the host cell reproduces until the lytic cycle is induced (Adams 1959; Lwoff 1953; Siringan et al. 2014; Weinbauer 2004). This life cycle is the one of the main reasons behind genetic diversity in bacteria.

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Discovery and Early History

In 1896, Ernst Hankin reported the presence of antibacterial activity against *Vibrio cholera* in the waters of the Ganges and Jumna Rivers in India (Hankin 1896). A similar occurrence was described, two years later, by a Russian bacteriologist while working with *Bacillus subtilis* (Samsygina and Boni 1984). It was not until 1915 that Frederick Twort hypothesized that a virus could be the reason behind this antibacterial activity. In his attempts to culture *Vaccinia* virus on cell-free agar media, the British bacteriologist noted the growth of micrococci "glassy" colonies (Twort 1915). When he examined those colonies under the microscope he noticed granules of degenerated bacteria and from there he formulated his hypothesis. However, Twort did not pursue his findings mainly due to financial constraints. A French-Canadian microbiologist, Felix d'Herelle continued Twort's research in the field of bacteriophages. Though he claims that he observed the "bacteriophage phenomenon" while studying microbiological approaches limiting the spread of an epizootic of locusts in Mexico (Duckworth 1976), it was during this investigation that d'Herelle witnessed clear zones around bacterial colonies on agar media, which he later called plaques. Shortly after his discovery (1919), he used phages therapeutically to treat dysentery under the supervision of Professor Victor-Henri Hutinel at the Hôpital des Enfants-Malades in Paris. Prior to the administration of the phage preparation to the patients d'Herelle confirmed its safety through self-administration. The phage preparation proved its efficacy after the treatment of four patients. However, these findings were not published until 1931, which gave the chance to Richard Bruynoghe and Joseph Masin to report the first application of phages in treating human infections (Bruynoghe and Maisin 1921). They used bacteriophages to treat staphylococcal skin disease. Following these important discoveries, microbiologists began to use phages in therapeutic aspects whether in animals or humans. Work in the field of phage therapy began to grow rapidly. D'Herelle established his own laboratory, which produced the first commercial phage cocktails. Scientists were experimenting with phages on various infections; a study on 21 patients with typhoid fever reported a drop in mortality rate from 15.6% to 4.8% with bacteriophage treatment and a reduction of 43.2% in complications. There were reports of successful bacteriophage treatment trials, summarized in Table 1, with cases of septicemia, urinary tract infections, surgical infections, skin infections, peritonitis, otolaryngology infections, in addition to *Shigella* and *Salmonella* related colitis (Abedon et al. 2011). The phage therapy boom had spread to the United States, even renowned pharmaceutical companies, like Eli Lilly, Abbott Labs and E. R. Squibb, began producing therapeutic phage cocktails. The enthusiasm for phage therapy subsided after the emergence of sulfonamide antibiotics and

penicillin, as well as the Eaton-Bayne-Jones, highly critical, report that questioned the precision and consistency behind phage therapy protocols (Abedon et al. 2011; Wittebole et al. 2014). A few countries, such as Poland, Georgia and Russia, continued investigations in the field of phage therapy and to this day still treat bacterial infections using bacteriophages. In spite of this, papers documenting such studies are not readily available internationally, due to the use of non-English language. Bacteriophages are a heterogeneous group of viruses in terms of phenotype, genotype, and host range. Only virulent phages can be used as a biocontrol agent and are considered safe since they do not transfer toxin and antibiotic resistant genes from one bacterial host to another. Accordingly, they are considered nontoxic and their products have already been approved as food additives, as antimicrobials, by the regulatory agencies (Anonymous 2006).

The Rise of Antibiotic Resistance and Phage Revitalization

Since their discovery by Alexander Fleming in 1928, antibiotics have been successfully used to treat bacterial infections in humans and animals, as well as in food production. However, the effectiveness of antibiotics is challenged by the increasing number of antibiotic-resistant bacteria (Campos et al. 2015; WHO 2014). Most of the available antibiotics, including β-lactams, are becoming less effective and in some cases resistance rates exceed 98% (Akinkunmi et al. 2015). For example, *E. coli* O104:H4 was found to be resistant to at least 14 different antibiotics (Verstraete et al. 2013) and about 90 % of *Salmonella* isolates were found to be resistant to one or more antibiotics tested (Dias de Oliveira et al., 2005; Liang et al. 2015; Wang et al. 2014). Some *Salmonella* isolates from poultry were found to be resistant to 14 different antibiotics (Adesiji et al. 2014; Zhang et al. 2014). Moreover, 95% of nosocomial infections are caused by resistant staphylococci (CDC 2009). Hospital and community-acquired Methicillin-resistant *Staphylococcus aureus* (MRSA) were found to be resistant to many classes of antibiotics, including the fourth-generation fluoroquinolones, and can cause systemic infections with a mortality rate of 50% (Kollef and Micek 2005; Rubinstein et al. 2008; Roberts et al. 2013; Chang et al. 2015). Other pathogenic bacteria such as *Pseudomonas*, *Campylobacter*, and *Listeria* show similar trends of antibiotic resistance (Komba et al. 2015; Obaidat et al. 2015; Ozbey and Tasdemi 2014; Pobiega et al. 2014). Expectedly, the incidence of resistance among many medically important bacteria has increased over time. For instance, the rate of ciprofloxacin resistance in clinical *E. coli* isolates has increased from 4.3% to 16.7% between 1998 and 2013 in Southeast Austria. Such resistance is attributed to the misuse of antibiotics and will ultimately increase the cost of treatment, prolong the

illness, and increase the rate of mortality (Bhowmick et al. 2011; Fortini et al. 2011; Pavlickova et al. 2015; Van et al. 2007). Unfortunately, more than half a million people worldwide die each year from antibiotic resistant bacterial infections (Davies et al. 2013). About 25,000 people die annually in Europe (ECDC 2010) and 23,000 people die in USA due to untreatable bacterial infections (CDC 2013). The estimated annual cost of treating infections caused by antibiotic-resistant bacteria in Europe is about 1.5 billion euros (World Health Organization, 2014), while in Canada it is about $200 million (Conly 2002), and up to $77.7 billion in USA (Scharff 2012).

The interest in phage therapy has been revived in Western countries now that the number of antibiotic-resistant bacteria is rapidly growing, especially after the US National Institute of Allergy and Infectious Diseases listed phage therapy as one of seven strategies to fight antibiotic resistance. Bacteriophage therapy could be one of the best alternative treatments to control bacterial infections in humans and animals, as well as reduce food contamination (Summers 2001). In severe infectious diseases, a combination of bacteriophages and antibiotics is administered rather than monotherapy, to maximize the efficacy of the treatment (Kutateladze and Adamia 2010).

Advantages of Phage Therapy

Phage therapy has many advantages that make it an attractive alternative to antibiotics. Firstly, bacteriophages are very specific to their hosts, unlike antibiotics which have a much wider spectrum are likely to cause dysbiosis, secondary infections and other side effects. Since phages infect only bacterial cells and have no effect on mammalian cells there is no risk of toxicity to the host. Moreover, phages are prevalent in nature making the isolation and selection of new phages a relatively rapid process in contrast to development of antibiotics, which takes millions of dollars as well as years and years of research to develop an effective antibiotic drug (Golkar et al. 2014). Thus the development stage of a phage therapy is relatively inexpensive compared to that of antibiotics. Development of resistance, a major problem for antibiotics as discussed in the previous section, is a less significant issue for phage therapy. Although bacteria may develop resistance to a particular phage specific to them, there is always a range of different phages with the same target range. Also, a high frequency of mutation allows phages to co-evolve with their hosts, with strong evolutionary pressure to overcome any acquired resistance. One of the reasons why antibiotics are not always effective is that they are metabolized and excreted from the body without reaching the site of infection. Phages have the advantage that they will only replicate in the presence of their host bacteria and are widely spread throughout the body after systemic administration, thus reaching the site of infection.

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Their miniscule size allows them to permeate areas that are impenetrable by drug molecules, for example, the blood brain barrier (Wittebole et al. 2014). Some phages are even capable of infiltrating and disrupting biofilms (Azeredo and Sutherland 2008). Once at the site of infection, the exponential growth of phages may allow a less frequent and lower dose of treatment than would be required from an antibiotic therapy (Sulakvelidze et al. 2001).

Disadvantages of Phage Therapy

Despite the apparent advantages of using phages as antimicrobial therapy, there have been quite a few setbacks. One of which is the lack of properly documented clinical research. There are no established protocols for the route of administration, dose, frequency and duration of the treatment. We have very limited knowledge regarding phage behavior *in vivo* (Sabouri Ghannad and Mohammadi 2012). It has been proposed that once inside the human body, the reticuloendothelial system may significantly reduce the numbers of phages to a low concentration reducing the possibility of fighting off the pathogen (Sulakvelidze et al. 2001). Furthermore, there is a possibility of the emergence of phage-neutralizing antibodies that will probably impede the phage's ability in combating the target bacterial pathogen. A study on 57 patients with bacterial infections, in Poland, documented this phenomenon, after parenteral administration of phages (Kucharewicz-Krukowska and Slopek 1987). Yet it is vague on whether this could occur during local and oral administration. Theoretically, the development of phage-neutralizing antibodies should not be a significant impediment to phage therapy since the adsorption and lysis kinetics of the phages should be more rapid than the production of antibodies by the host. Another concern is the efficacy of phages in tackling intracellular pathogens. Nonetheless, it has been reported that phages were successful in preventing salmonellosis (Lazriev et al. 1986) and *Salmonella* biofilm production (Garcia et al. 2017). The side effects of phage therapy in the long run, remain unknown. Moreover, the purity and stability of phage preparations are dubious without sufficient quality control data being available. Security and language barriers have hindered scientists from interpreting experimental procedures in Russian and Polish journals.

Naturally, phages are highly specific in their target host range, which may encompass members of a whole species or just a few strains within a species. This characteristic has both negative and positive aspects in that it is beneficial in terms of avoiding negative effects on the microbiome and a hindrance when it comes to detection and elimination of the target pathogen. It is time consuming to detect the causative agent of an infection that could result in a worsening of the patients' condition. Using phage cocktails, which contain a number of different phages that cover a wide range of potential strains, is one to overcome this issue. Phages used for phage therapy should be composed of

only virulent phages, not temperate ones, to avoid the horizontal transfer of pathogenicity traits (Brouwer et al. 2013). Understanding the exact mode of action of the many different types of phages is not a simple matter as phages may behave differently under *in vivo* conditions than they do *in vitro*. A randomized trial on children with acute bacterial diarrhea was carried out in Bangladesh showing that orally administered phages failed to amplify in the intestine, causing no change in the occurrence of diarrhea episodes (Sarker et al. 2016). More phage therapy trials as well as pharmokinetic studies should be done in order to elucidate the mechanism of phage action *in vivo.*

Just as bacteria may become resistant to antibiotics they may also become resistant to phages through a number of mechanisms. These include: modification of the phage surface receptors on the bacterial cell, integration of the phage's genome within that of the bacteria, and loss of the genes specific for replication or assembly of the phage (Sabouri Ghannad and Mohammadi 2012). However the phages' receptors are generally structures that are essential for the bacteria to survive and compete, so there is strong evolutionary pressure for the phages to co-evolve with bacteria. Short-term resistance in clinical applications may be overcome by using phage cocktails that target different receptors.

Another crucial limitation is the ability of phages to transfer antibiotic resistance genes that have been acquired from AMR bacteria. Metagenomic studies indicate that gene transfer among bacterial populations via transduction is occurring at high frequencies. This may be due to the vast prevalence of phages in direct contact with their bacterial hosts in all kinds of environments (Kenzaka et al. 2010). Polyvalent phages facilitate the transfer of genetic materials, including resistance genes, among bacteria of different taxa. Theoretically, this increases the probability of genetic exchange between pathogenic bacteria and bacteria of the microbiome escalating the spread of antibiotic resistance (Mazaheri Nezhad Fard et al. 2011; Muniesa et al. 2013; van den Bogaard and Stobberingh 2000). Exhaustive studies should be executed prior to phage selection for therapeutic application to avoid harmful gene transfer. Such challenges are the reason why scientists face difficulties in regulation approval of phage-based therapeutic applications.

Bacteriophage Applications

The development of bacteriophage applications in food and animals to reduce pathogens have increased during the last few years because of concerns over the rise in antibiotic resistance as described above. Bacteriophages may be used in combination with disinfectants or engineered to produce biofilm-degrading enzymes to kill biofilm-producing bacteria (Lu and Collins 2007; Tait et al. 2002). Phages are easy to prepare, easy to apply and are

harmless to plants, animals, and humans as stated by experiments using phages to target *E. coli* in human volunteers in Switzerland (Bruttin and Brussow 2005). The first bacteriophage application, after its discovery in 1917, was to treat bacterial dysentery (d'Herelle 1917). Since then applications included the treatment of infections in: stomatology, pediatrics, dermatology, otolaryngology, gastroenterology, ophthalmology, gynecology, surgery, urology and pulmonology in the republics of the former Soviet Union during the 1960s and 1970s, even when antibiotics were still effective (Chanishvili 2012; Kutateladze and Adamia 2010). The first steps in developing a phage-based biocontrol application involve testing different virulent phages against the target pathogenic bacteria *in vitro* (Gill and Hayman, 2010). The effectiveness of phages to reduce bacterial numbers *in vitro* depends on many factors, such as the ratio of phages administered to each bacterial cell (MOI), the method of administration, and the timing of administration (Huff et al. 2003; Ryan et al. 2011). In 2001, a cocktail of bacteriophages targeting different pathogenic bacteria including *E. coli* and staphylococci was used to treat wound infections, ear infections, gastrointestinal infections, and in surgery (Sulakvelidze et al. 2001) and the success of phage treatment is host immune dependent (Roach et al. 2017). Later on, bacteriophages were produced commercially by integrating a cocktail of phages with a biodegradable polymer and an antibiotic (ciprofloxacin) to give the best effect as a dressing against multidrug resistant bacteria, like *S. aureus.* The commercial name of this product is 'PhagoBioDerm' (Jikia et al. 2005; Markoishvili et al. 2002). Consideration should be given to the potential release of endotoxin caused by bacteriophage-induced lysis, which may stimulate an inflammatory response. However, an endotoxin removal kit that can be used for clinical trials has been developed to overcome this potential problem (Matsuda et al. 2005; Merabishvili et al. 2009). Furthermore, Hagens and Blasi (2003) engineered filamentous phages that could be toxic to bacteria but do not cause cell lysis, thus reducing the chance of endotoxin release. The technology of synthetic biology could be used to improve the effectiveness of phage therapy. For example, nonlytic phages can be genetically engineered to deliver a specific DNA sequence that encodes bactericidal proteins to bacteria (Hagens et al. 2003; Westwater et al. 2003). Alternative antimicrobial agents, such as programmable RNA-guided nucleases (RGNs) through the modification of the spacers in the CRISPR locus can pose a selective pressure on specific genes damaging the target DNA sequence in target strains with RGNs and causing cell death in antibiotic resistance bacteria (Bikard et al. 2014; Citorik et al. 2014b; Gomaa et al. 2014). ΦRGN*eae* treated enterohemorrhagic *E. coli* O157:H7 (EHEC) showed a 20-fold reduction in viable counts in comparison with phage-free bacterial cells and when the phage was administrated to *Galleria mellonella* larvae infected with EHEC, the

survival rate of the larvae was improved significantly ($P < 0.001$) in comparison with untreated samples (Citorik et al. 2014b). Another RGN, one for Cas9 virulence and antibiotic was used successfully to target MRSA in an infected mouse skin model using phagemid-delivered CRISPR system (Bikard et al. 2014). In general, the RGNs can be cytotoxic if they target the bacterial chromosome or cause plasmid loss if the plasmid in the absence of toxin-antitoxin system is targeted, depending on the delivery efficiency of RGNs to the bacterial host. Engineered phages can be applied both *in vivo* and *in situ* to deliver RGNs that can target many genetic signatures of antibiotic resistance genes found in bacterial populations simultaneously via transduction (Citorik et al. 2014b). Moreover, bacteriophages can be engineered to express a biofilm-degrading enzyme that can target and lyse biofilm-producing bacteria that contain extracellular polymeric substances enabling it to highly resist antibiotics (Lu and Collins 2007) or it could be inspired with gold nanoparticles to reduce up to 80% of biofilm formation (Ahiwale et al. 2017). It can also be applied as a disinfectant and sanitizer to kill antibiotic resistant bacteria without affecting antibiotic sensitive ones (Yosef et al. 2015).

Non-Clinical Applications

Besides the various clinical applications, bacteriophage treatments have been applied to all levels of food production For example, phages have been used in the veterinary treatment of food producing animals to improve food safety by reducing pathogens in live animals and fish (Pereira et al. 2011). Phages have also been applied on meat as well as fruits (Leverentz et al. 2001) and vegetables (Viazis et al. 2011) to control pathogens. They have successfully controlled bacterial infections caused by different kinds of bacteria, like *Salmonella* (Andreatti Filho et al. 2007), *Pseudomonas aeruginosa* (McVay et al. 2007), *Staphylococcus aureus* (Wills et al. 2005), *Clostridium difficile* (Ramesh et al. 1999), *Escherichia coli* (Huff et al. 2002), and *Campylobacter* (Loc Carrillo et al. 2005) in large animals and poultry. They significantly reduced *Salmonella enterica* colonization and horizontal transmission (Lim et al. 2012). Furthermore, bacteriophages have been used as a technique for detecting pathogenic bacteria in food and clinical samples (Kuhn 2007; Pearson et al. 1996), decontaminating surfaces and food, and as a nanostructured material (Hyman 2012; Lee et al. 2009).

Examples of Applications of Bacteriophage as a Biocontrol Agent in Food

Infection with *E. coli* O157:H7 often causes abdominal cramps and acute hemorrhagic diarrhea. This bacterium is usually acquired from undercooked beef or from direct contact with infected animals (Belongia et al. 1991). As this

is such a serious food borne illness it is not surprising that research has focused on the efficacy of phages to reduce the numbers of *E. coli* O157:H7 and the results were very promising (Bach et al. 2003; Kutter et al. 2011; Raya et al. 2006). A cocktail of bacteriophages was orally administrated to treat *E. coli* O157:H7 in the gastrointestinal tracts of mice and it significantly reduced colonization by the pathogen (Tanji et al. 2005). The phage treatment also decreased the numbers of *E. coli* O157:H7 significantly on meat surfaces (El-Shibiny et al., 2017; O'Flynn et al. 2004).

Salmonella Typhimurium is a Gram-negative human pathogen that causes non-typhoid salmonellosis. This pathogen is frequently transmitted via contaminated food or water (Kingsley and Baumler 2002; Santos et al. 2003). The annual number of non-typhoid salmonellosis cases in USA alone was approximately 1,200,000 cases in 2013 and about 100,000 of those cases involved drug-resistant *Salmonella*, according to the Centers for Disease Control and Prevention (CDC 2013). Similarly, *Salmonella* is considered a big cause of food-borne disease in Europe (EFSA 2012) with over 80,000 confirmed human cases reported in 2013 (EFSA 2015). The economic and health burdens of this pathogen have made it an obvious choice for the use of phage biocontrol applications. Bacteriophage applications have been shown to successfully reduce the numbers of *S.* Typhimurium in chocolate milk and in turkey deli meat by 5 log (Guenther et al. 2012). It has also been shown to reduce the survival rate of *Salmonella* in cheddar cheese made from raw and pasteurized milk (Modi et al. 2001), chicken frankfurters (Whichard et al. 2003), chicken skin (Pao et al. 2004), pig skin, chicken breasts (Spricigo et al. 2013), energy drinks, whole and skimmed milk, apple juice (Zinno et al. 2014), alfalfa seeds (Kocharunchitt et al. 2009), and sprouts (Ye et al. 2010).

Campylobacteriosis is another common bacterial disease and constitutes a serious problem worldwide. Two species, *Campylobacter coli* and *C. jejuni,* live in the intestinal tract of most avian species and cause the majority of human infections. The disease usually is associated with the consumption of undercooked poultry, particularly chicken (Shane 2000). Approximately 17,000 cases of *Campylobacter* infections per year have been recorded in the USA alone and most of them were antibiotic resistant isolates (Barza and Travers 2002). A large proportion of commercial broiler chickens are colonized by campylobacters leading to high numbers of the pathogen on finished poultry products. Given that, mathematical modeling (Rosenquist et al. 2003) has predicted that a relatively small decrease in numbers colonizing birds would significantly reduce the number of human cases. Bacteriophage treatment of chickens is an application showing a great deal of interest. Experiments that showed significant reductions in *Campylobacter* numbers in the cecal contents of experimental birds were first reported in 2005 (Loc

Carrillo et al. 2005; Wagenaar et al. 2005). Further studies were carried out that confirmed the promising potential of this application (Carvalho et al. 2010; El-Shibiny et al. 2007; El-Shibiny et al. 2009; Fischer et al. 2013). Bacteriophages successfully diminished the numbers of *C. jejuni* in cooked and raw beef by 2 $\log_{10}$ (Bigwood et al. 2008) as well as on poultry meat surfaces (Atterbury et al. 2003; Goode et al. 2003).

Another pathogen that is a potential target for bacteriophage applications is *Listeria monocytogenes.* It is frequently found in milk products and processing environments (Kells and Gilmour 2004). Bacteriophages were found to be very effective in reducing the numbers of *L. monocytogenes* on melon, pear, apple slices and juices (Oliveira et al. 2014). Phages also reduced the numbers of *L. monocytogenes* in cheese and other dairy products (Carlton et al. 2005; Schellekens et al. 2007). The Food and Drug Administration (FDA) has approved the use of phage cocktails as a food additive, Generally Recognized As Safe (GRAS), to control *L. monocytogenes* on ready-to-eat food (Bren 2007; Monk et al. 2010). Other products produced by Omnilytics Company have been approved for treatment, combating crop pathogens such as *Xanthomonas*, *Pseudomonas* and *E. coli* (Balogh et al. 2010; Hagens and Loessner 2010).

Indirect Applications of Bacteriophage

The bacteriophage enzymes (lysins), are produced during the infection cycle and target the peptidoglycan layer of bacterial cells to release the new phage progeny from the cell. These enzymes can be purified and used as a therapeutic agent. However, those studied so far are only active against Gram-positive bacteria since lysins cannot penetrate the outer membrane of Gram-negative bacteria (Loessner 2005). Phage particles can be also engineered to carry vaccine antigens on their surfaces or they can be used as a vehicle to deliver DNA vaccines (Clark and March 2004). Such structural vaccines can be used to mimic Hepatitis E viral infections (Larralde and Petrik 2017). Bacteriophages have been used for many other applications, including the detection of pathogenic bacteria such as *Salmonella* and *E. coli* O157:H7 in food and clinical samples as well as to differentiate between viable but non-culturable (VBNC) and dead cells (Awais et al. 2006; Fernandes et al. 2013). Some bacteriophages have been approved by the FDA to detect human pathogenic bacteria, such as *Bacillus anthracis*, and *Staphylococcus aureus* (Schofield et al. 2012). Additionally, phage display technology has been used successfully for the production of antivenoms for animal toxin neutralization and antibody production, showing great promise for future diagnostic applications (Bahara et al. 2013; Gazarian et al. 2000). The peptides produced from phage display can be used in

drug design, as therapeutic and pathogens detection agents (Petrenko and Vodyanoy 2003). Phage display techniques can also be used in both nanotechnology (Hemminga et al. 2010) and nanomedicine fields (Souza et al. 2010).

Due to the variety and success of bacteriophage applications, many scientists agree that in the future, many phage-based techniques will be available for detecting, treating and preventing pathogenic bacteria in both medical and food industries (Hagens and Loessner 2010).

Future applications

Bacteriophages can be an excellent, alternative to antibiotics if proof of efficacy is obtained. The promising results of current research indicate great potential in improving food safety. Phages have been shown to reduce the number of pathogenic bacteria in poultry and large animals before slaughter and they could be added to the feed and drinking water for easy delivery to poultry and animals. They could also be added to the packaging materials of food by immobilization to extend the shelf life of food products and also as a sanitizer to disinfect the production line (Lone et al. 2016). Phages can be adsorbed to the surface of soya protein powder, whey protein and skim milk powder and dried under vacuum to be encapsulated. This enhances its stability for different applications in agriculture, veterinary medicine and human medicine (Murthy and Rainer 2008). Recently, an immobilized cocktail of *E. coli* and *L. monocytogenes* phages on cellulose membranes was used to control the growth of their hosts on experimental meat and the results were assuring (Anany et al. 2011).

As for clinical applications, the results of current research are also hopeful. Studies from Eliava Institute and from Queen Astrid Military Hospital in Brussels showed the efficacy of phage therapy in curing bacterial infections, such as wound infections (Merabishvili et al. 2009; Weber-Dabrowska et al. 2003). The ongoing research aims at improving phage therapy to be effective in reducing the number of pathogenic bacteria accompanying the infection. The use of phage cocktails to treat *P. aeruginosa*-associated chronic otitis was successful in cutting down about 50% of *P. aeruginosa* in the treated ears of patients (Wright et al. 2009). These results are still considered insufficient and should be improved possibly by using phage cocktails (Skurnik and Strauch 2006). Phage sequencing and bioinformatics can be used to study the phage properties and select the most appropriate phages for personalized medicine.

Bacteriophages to treat AMR bacteria

Phages are extremely stable and can be stored for several months at room temperature. They can tolerate the acidity of stomach, making them useful for the treatment of intestinal colonization of pathogenic bacteria such as *E. coli*, *Salmonella*, *Campylobacter* and *Helicobacter*.

Conclusion

The various phage applications presented here show that phages have many uses such as detection, typing, biocontrol of food-borne pathogens, and drug design. The optimization of phage numbers (MOI), time of infection, and the delivery method of phage are the most important factors to get the highest rate of bacterial reduction. By developing phage cocktails, it will be easier to treat antibiotic resistant bacteria including chronic infections to reduce human illnesses significantly. Furthermore, the displayed polypeptides can be used to design drugs to treat pathogenic bacteria or as a prophylactic measure through vaccines. The possibility of using phages in combination with antibiotics, vaccines and probiotics to reduce the numbers of foodborne and pathogenic bacteria may become the best choice in the future. However, more research is needed to gain the regulatory agencies' approval for commercial use.

Conflict of Interest

We disclose that we have no conflict of interest to declare.

This statement is to certify that the article is the authors' original work. We warrant that the article has not received prior publication and is not under consideration for publication elsewhere. This research has not been submitted for publication nor has it been published in whole or in part elsewhere.

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Table 1. The history of phage therapy studies

Reference	Year	Country	Pathogen	Disease	Description
D'Hérelle	1919	France	*Shigella*	Bacterial dysentery	Treatment of children suffering from severe dysentery using previously isolated phages
Brungnoghe and Maisin	1921	France	*Staphylococcus*	Carbunculosis and Furunculosis	The injection of phages near the base of the carbuncles and furuncles in 6 patients led to reduction in swelling, pain and fever.
D'Hérelle	1927	India	*Vibrio cholerae*	Cholera	This study was the first in using intravenous administration of bacteriophages by Asheshov in India.
Larkum	1929	USA	*Staphylococcus*	Chronic furunculosis	Subcutaneous treatment of 208 patients showed 78% with no recurrent infections.
Schultz	1929	USA	*Staphylococcus*	Septicemia	Remarkable success
D'Hérelle	1931	Egypt	*Yersinia pestis*	Bubonic plague	In 1927, d'Hérelle treated 4 cases of bubonic plague successfully by injecting bacteriophages in buboes.
Schless	1932	USA	*S. aureus*	Meningitis	Remarkable success
MacNeal and Frisbee	1936	USA	*Staphylococcus*	Staphylococcal bacteremia	Relatively successful treatment in 100 patients.
Sauvé	1936	France	*Staphylococcus*	Surgical infections	Cure of abscesses using polyvalent phages
Mikeladze et al.	1936	Georgia	*Salmonella* Typhi	Typhoid fever	Treatment of 21 patients resulted in a drop of 10.8% in mortality and 43.2% in complications.
Mikeladze et al.	1936	Georgia	*Salmonella* and *Shigella*	Acute colitis	All 43 patients with colitis were cured after treatment using "bacti-intesti-phage".
Tsulukidze	1936	Georgia	*Salmonella* Typhi	Peritonitis caused by intestinal perforations in typhoid fever	Mortality was reduced from 85% to 20-35%.
MacNeal et al.	1942	USA	*Staphylococcus*	Staphylococcal bacteremia	Very positive results in treatment of 500 patients.
Knouf et al.	1946	USA	*Salmonella*	Typhoid fever	The results were inconclusive; however, the positive results were astounding and encouraged them to continue the research.
Desranleau	1949	Canada	*Salmonella*	Typhoid fever	Several phage cocktails were used to treat 100 patients. The most successful one reduced the mortality rate from 20% to 2%.
Babalova et al.	1968	Russia	*Salmonella* and *Shigella*	Acute colitis	All 43 patients of colitis were cured after treatment using "bacti-intesti-phage".
Sakandelidze and Meipariani	1974	Russia	*Proteus, Staphylococcus*, and *Streptococcus*	Peritonitis, osteomyelitis, lung abscesses, and postsurgical wound infections	Subcutaneous or through surgical wounds administration of phages in 236 patients resistant to antibiotics with a success rate of 92%.
Pipiia et al.	1976	Russia		Abscessing pneumonia	A complex treatment was given to the patients including intensive antibacterial therapy, immunotherapy, bacteriophage, protein preparations,

					vitamin-therapy, and fresh blood transfusion.
Litvinova et al.	1978	Russia	*E. coli* and *Proteus*	Antibiotic-associated intestinal dysbiosis	A combination of phages and bifidobacteria were used to restore the intestinal microbiota in 500 infants.
Zhukov-Verezhnikov et al.	1978	Russia	*E. coli, Proteus, Staphylococcus*, and *Streptococcus*	Suppurative surgical infections	A comparison between commercial phage preparations and phages selected against bacterial strains isolated from patients was done. The selected phages were more effective in treating 60 patients.
Lang et al.	1979	France	*Enterobacter, Klebsiella, Proteus, Povidencia,* and *S. aureus*	Chronic orthopedic infections	7 cases of chronic orthopedic infections were successfully treated with phages.
Ioseliani et al.	1980	Russia	*E. coli, Proteus, Staphylococcus*, and *Streptococcus*	Lung and pleural infections	Treatment of 45 patients using the combination of phages and antibiotics.
Tolkacheva et al.	1981	Russia	*E. coli* and *Proteus*	Bacterial dysentery	A combination of phages and bifidobacteria were used to treat 59 immunosuppressed leukemia patients. The treatment was reported to be more effective than antibiotics.
Slopek et al.	1981-1986	Poland	*E. coli, Klebsiella, Pseudomonas, Salmonella, Shigella,* and *Staphylococcus*	Gastrointestinal tract, skin, head and neck infections	Phages were administered to over 1000 patients in a series of studies. The success rates varied between 91% and 96%.
Meladze et al.	1982	Russia	*Staphylococcus*	Lung and pleural infections	Full recovery was reported in 82% of the patients treated with phages as opposed to 64% of patients treated with antibiotics.
Anpilov and Prokudin	1984	Russia	*Shigella*	Bacterial dysentery	The double-blinded study showed a 10-fold lower incidence of dysentery in those treated with phages.
Martynova et al.	1984	Russia	*P. aeruginosa* and *S. aureus*		A prophylactic mouth wash was administered to patients with acute leukemia.
Kucharewicz-Krukowska and Slopek	1986	Poland	*E. coli, Klebsiella, Proteus, Pseudomonas,* and *Staphylococcus*	Bacterial monoinfections and polyinfections	The immunogenic effects of therapeutic phages were evaluated in 57 patients, showing an insignificant impact.
Weber-Dabrowska et al.	1986	Poland	*Staphylococcus* and several Gram negative bacteria	Suppurative infections	During therapy, phages seemed to infiltrate the blood circulation and urinary tract.
Cislo et al.	1987	Poland	*E. coli, Klebsiella, Proteus, Pseudomonas,* and *Staphylococcus*	Suppurative skin infections	A success rate of 74% in 31 patients with chronically infected skin ulcers was observed upon phage administration.
Kochetkova et al.	1988	Russia	*Pseudomonas* and *Staphylococcus*	Post-surgical infections	Therapeutic phages were administered on 65 of 131 cancer patients, while the others received antibiotics. Phage therapy was a success in 82% of the patients in comparison to 61% of success in using antibiotics.
Sakandelidze	1991	Russia	Enterococcus, *E.*	Infectious allergoses	1,380 patients with infectious allergosis were treated

			coli, P. aeruginosa, Proteus, Staphylococcus, and *Streptococcus*		using 3 different regimens: antibiotics, phages, and a mixture of both. The rates of success were 48%, 86% and 83% respectively.
Bogovazova et al.	1992	Russia	*K. ozaenae, K. pneumonia,* and *K. rhinoscleromatis*	Purulent inflammatory diseases	The administration of *Klebsiella* bacteriophages was successful in treating 109 patients with *Klebsiella* infections.
Miliutina and Vorotyntseva	1993	Russia	*Salmonella* and *Shigella*	Bacterial dysentery and salmonellosis	1646 children were successfully treated with phages and a combination of antibiotics and phages, where antibiotics alone were ineffective.
Kwarcinski et al.	1994	Poland	*E. coli*	Recurrent subphrenic abscess	A case of recurrent subphrenic abscess caused by an antibiotic resistant strain of *E. coli* was successfully treated with phages.
Perepanova et al.	1995	Russia	*E. coli, Proteus,* and *Staphylococcus*	Inflammatory urologenital diseases	Adapted phages were used to treat 46 patients. The treatment was a success in 92% of the patients while 84% showed bacterial clearance.
Stroj et al.	1999	Poland	*K. pneumonia*	Cerebrospinal meningitis	Oral administration of a phage preparation successfully cleared bacteria from cerebrospinal fluid in a newborn.
Lazareva et al.	2001	Russia	*Proteus, Staphylococcus*, and *Streptococcus*	Burn wounds	Pyophage treatment in patients with burn wounds reduced septic complications, had a 2-fold reduction of staphylococci and streptococci, 1.5-fold of *Proteus*, and full reduction of *E. coli*.
Markoishvili et al.	2002	Republic of Georgia	*E. coli, Proteus, Pseudomonas,* and *Staphylococcus*	Ulcers and wounds	PhageBioDerm was administered and showed a 70% rate of success.
Wright et al.	2009	UK	*Pseudomonas aeruginosa*	Chronic otitis	Bacteriophage significantly reduced the numbers of *Pseudomonas aeruginosa* in phage treated group in chronic otitis externa patients.
Fadlallah et al.	2015	France	*S. aureus*	Eye corneal abscess and interstitial keratitis	Bacteriophage eye-drops with successful results after 6 months.
Totté et al.	2017	Netherlands	*S. aureus*	Dermatoses	Successful treatment of chronic *S. aureus* with Endolysin Staphefekt phage.
Zhvania et al.	2017	Republic of Georgia	staphylococci	Netherton syndrome	Successful treatment of manifestations Netherton syndrome
Jennes et al.	2017	Belgium	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* septicaemia and acute kidney injury	Treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia