VIROLOGICA SINICA 2015, 30 (1): 11-18
DOI 10.1007/s12250-014-3543-6

REVIEW

Bacteriophage therapy against *Enterobacteriaceae*

Youqiang Xu[1,2], Yong Liu[1,2], Yang Liu[1,2], Jiangsen Pei[1,2], Su Yao[1,2], Chi Cheng[1,2]✉

1. China National Research Institute of Food and Fermentation Industries, Beijing 100015, China
2. China Center of Industrial Culture Collection, Beijing 100015, China

The *Enterobacteriaceae* are a class of gram-negative facultative anaerobic rods, which can cause a variety of diseases, such as bacteremia, septic arthritis, endocarditis, osteomyelitis, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections, intra-abdominal infections and ophthalmic infections, in humans, poultry, animals and fish. Disease caused by *Enterobacteriaceae* cause the deaths of millions of people every year, resulting in enormous economic loss. Drug treatment is a useful and efficient way to control *Enterobacteriaceae* infections. However, with the abuse of antibiotics, drug resistance has been found in growing number of *Enterobacteriaceae* infections and, as such, there is an urgent need to find new methods of control. Bacteriophage therapy is an efficient alternative to antibiotics as it employs a different antibacterial mechanism. This paper summarizes the history of bacteriophage therapy, its bacterial lytic mechanisms, and the studies that have focused on *Enterobacteriaceae* and bacteriophage therapy.

KEYWORDS bacteriophage therapy; *Enterobacteriaceae*; antibiotics; bacteriolytic mechanism

INTRODUCTION

The *Enterobacteriaceae* are a class of gram-negative bacteria. Within this family, all bacterial species have been implicated in bloodstream, cholangitis, peritonitis and other intra-abdominal infections (Paterson, 2006). Additionally, *Escherichia coli* often causes urinary tract infections; *Klebsiella pneumoniae* has been regarded as an important cause of pneumonia; *Salmonella enterica* produces gastroenteritis, and subsequently invasive infection in some patient; and *Shigella* strains could cause shigellosis, which often occurs in epidemic form and causes considerable morbidity and mortality (Donnenberg, 2002; Paterson, 2006; Phalipon and Sansonetti, 2007; Verma et al., 2010). Unfortunately, emerging drug resistance is a serious problem in handling *Enterobacteriaceae* infections, and threatens to create pan-resistant species (Falagas et al., 2010). There is a pressing need to find alternative ways to control *Enterobacteriaceae* infections, and one of the efficient methods is bacteriophage therapy.

Bacteriophage therapy has a long history, which originated in the early 20th century. Since the discovery of penicillin and other antibiotics, with their easy use and notable effects in treating microbial related diseases, little attention has been paid to bacteriophage therapy for nearly half a century (Burrowes et al., 2011). However, due to the abuse of antibiotics, a greater number of pathogenic bacteria becoming resistant to drugs, resulting in great threat to human health (Merril et al., 2003). In China, the situation is more serious. Susceptibility rates of clinically isolated *Enterobacteriaceae* declined by about 30% against the new generations of cephalosporins from 2002 to 2009; meanwhile, there was a rapid increase in the occurrence of extended-spectrum β-lactamases, especially for the *E. coli* strains (from 20.8% in 2002 to 64.9% in 2009) (Yang et al., 2010). Three reasons lead to this situation: the first was the abuse of antibiotics; the second was the lack of antibiotics with new antibacterial mechanisms; and the third was the investment atrophy for the development of new antibiotics (Sulakvelidze et al., 2001). More seriously, the rate of development of new antibiotics is slower than the rate of the appearance of antibiotic resistance; therefore, the prospect of new kinds

Received: 13 November 2014, Accepted: 7 January 2015

✉ Correspondence:
Phone: +86-10-53218306, E-mail: cheng100027@163.com
ORCID: 0000-0003-0534-7508

of drugs for clinical use is not optimistic. These realities have stimulated researchers in the study of bacteriophage therapy, which has met with great progress (Sulakvelidze et al., 2001).

HISTORY OF PHAGE THERAPY

The history of phage therapy started with phage discovery. Briefly, Hankin first reported the presence of an antibacterial activity against *Vibrio cholera* in 1896 (Hankin E, 1896). Two years later, Gamaleya discovered the same phenomenon when working with *Bacillus subtilis* (Samsygina and Boni, 1984; Sulakvelidze et al., 2001). Twort first reported the discovery of plaque in plates during the culture of *Staphylococcus aureus*, and explained it as a viral infection in 1915 (Twort, 1915). In 1917, d'Herelle proved the phages therapy (d'Herelle, 1917). He used phages to treat *Shigella* strains isolated from patients with dysentery after which small and clear areas appeared on the agar plates. He proposed that the phenomenon was due to parasitizing bacteria caused by virus infection. The name "bacteriophage" was also proposed by d'Herelle; it was derived from the words "bacteria" and "phagein" (d'Herelle, 1917; Summers, 1999). Another important contribution by d'Herelle was that, he firmly promoted the idea that phages were live viruses, but not "enzymes" as many researchers thought in his era. The research and application of bacteriophages was started thereafter. In 1919, a boy with dysentery in Paris was treated with d'Herelle's anti-dysentery phage and recovered within a few days. Soon after, three patients with bacterial dysentery were also recovered after being treated with d'Herelle's anti-dysentery phage, which confirmed the efficacy of phage therapy. Unfortunately these studies were not reported. The first report of phage therapy came in 1921 when Bruynoghe and Maisin used bacteriophages to treat staphylococcal skin disease (Bruynoghe and Maisin, 1921; Payne et al., 2000). In 1932, East European scientists identified the scientific dosage of phage based on abundant animal and human tests. Thereafter, several companies, such as the Parke-Davis Company and Eli Lilly and Company, began active commercial production of phages against various bacterial pathogens. In China, phage therapy was started in 1955 when Si et al. successfully used bacteriophages to treat *Shigella* dysenteriae (Si, 1955). However, in these early studies, the phages used had a narrow host range, low purity and instability, which limited the application of phage therapy (Qian et al., 2007).

THE BACTERIOLYTIC MECHANISM

Bacteriophage therapy is the therapeutic use of phages to split pathogenic bacteria. After adsorption to bacteria, phages start the process of bacteriolysis. According to the requirement of endolysin or not, host cell lysis mechanisms can be divided into two basic modes. One mode depends on the lysozymes produced by phages with dsDNA such as phages K and T4 to split bacteria; the other mode does not require the lysozymes such as phage MS2 with ssRNA and phage φX174 with ssDNA (Young, 1992). Hense, there are two basic types of bacterial lytic mechanisms.

Lysozyme independent lysis system

This type of bacteriophages lacks the genes encoding lysozymes, and they split host strains by synthesizing proteins to inhibit the host cell wall biosynthesis, leading to lysis of host cells during growth. *E. coli* ssRNA phage Qβ encodes a protein A_2 and binds to protein MurA, an enzyme that catalyzes the first step in cell wall synthesis, preventing catalysis by occluding phosphoenolpyruvate from accessing the active site (Reed et al., 2012). The ssDNA phage φX174 encodes a membrane protein E, which inhibits the enzyme activity of MraY and results in host cell lysis (Tanaka and Clemons, 2012). MraY catalyzes the first membrane-localized step for the synthesis of peptidoglycan precursor (Tanaka and Clemons, 2012). During this type of lysis, the host cell wall forms small lesions, and leaves the ghost or large cell debris at last (Young, 1992).

Lysozyme dependent lysis system

Other than the lysozyme independence of some bacteriophages, most bacteriophages have dsDNA encode lysozymes to crack the host cells. The dsDNA bacteriophages belong to the order *Caudovirales*, and account for about 95% of all the bacteriophages discovered (McAuliffe et al., 2007). This type of phage splits the host cells by expressing two types of lysozymes, holins and lysins.

Bacteriophage-encoded holins are a diverse group of membrane proteins. Holins control the transmembrane or activity of phage-encoded endolysins to degrade the host cell walls, and thereby initiate the bacteriolytic process as the "lysis clock" (Young and Bläsi, 1995). Based on structural difference, Young et al. classified phage holins into three groups, class I, II and III (Figure 1) (Young and Bläsi, 1995).

The typical class I holin is S105 coded by the *S* gene of phage λ, which contains 105 amino acids, and forms three transmembrane domains (TMDs) (Gründling et al., 2000). The zwitterionic and non-ionic detergents permit S105 to form oligomers, and the oligomers form ring-shaped structures (Savva et al., 2008). The ring has an external diameter of 23 nm, an inner diameter of 9 nm for the upper ring and 8 nm for the lower ring (Savva et al., 2008). The 8 nm inner diameter of the ring is large

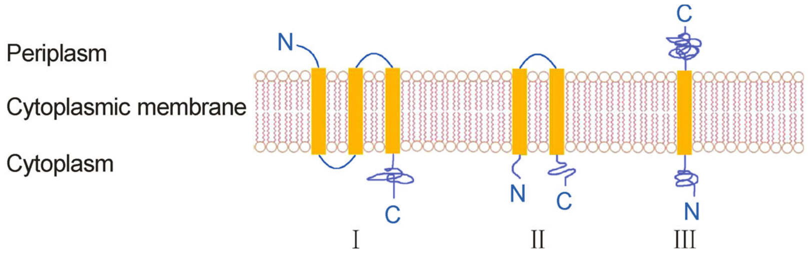

Figure 1. Topology structure of phage holins. Phage holins can be divided into three groups, classes I, II and III.

enough for the movement of endolysin R of phage λ to the periplasm where it attacks the host cell wall. Studies also show that the amnio acid mutation of S105 could affect the lysis time (Gründling et al., 2000).

The class II holins have two TMDs, which usually have a length of 65 to 95 amino acids (Shi et al., 2012). The S^{21} protein from lambdoid phage 21 is a holin of this type (Pang et al., 2009). In the early stage of cytoplasmic membrane damage, both the N- and the C-terminus of S^{21} are in the cytoplasm, and form small membrane holes that depolarize the membrane, thus class II holins are also considered as pinholins, as opposed to large-hole-forming holins like S105. The lysins of this type of phage are exported by the *sec* system of host cells and accumulate in the inactive form tethered to the membrane by an N-terminal SAR ("signal-anchor-release") sequence in the periplasm. The depolarization by S^{21} leads to the release of the lysin from the biolayer and splits cell wall. During this process, the holin triggers the depolarization of the cell membrane, and leads to the release of the SAR lysin from the bilayer; in this way it is thought to impose the timing on the lytic event (Pang et al., 2009; Xu et al., 2004).

The class III holin has only one TMD, which is highly hydrophilic. A representative holin is T protein from phage T4 (Ramanculov and Young, 2001). The T protein of bacteriophage T4, as other holins, has the ability to cause the formation of a lethal membrane lesion, and allows the phage lysin to target the cell wall. The N-terminus of T is in the cytoplasm and acts as membrane lesion; the C-terminus is in the periplasm and functions as the regulator. Moreover, T acts in the vegetative cycle like other holins at an accurate programmed time (Ramanculov and Young, 2001).

Lysins, also known as endolysins, muralysins, muramidases or virolysins, are highly evolved enzymes to digest the bacterial cell wall for the release of phage progeny during the final stage of bacteriophage lytic cycle (Fischetti, 2008). Functionally, four types of phage lysins can be classified: endo-β-*N*-acetylglucosaminidase, *N*-acetylmuramidase, endopeptidase and *N*-acetylmuramoyl-L-alanine amidase (Loessner, 2005; Pastagia et al., 2013). The targets of these lysins on host cell wall are shown in Figure 2. Due to the high effectiveness and specificity to drugs, the lysins have been applied as antibacterial agents (Fischetti, 2008). Lysins must perform two basic functions: substrate recognition and enzymic hydrolysis (Díaz et al., 1990). Generally, the N-terminal domain cleaves specific peptidoglycan bonds, such as endo-β-*N*-acetylglucosaminidase and *N*-acetylmuramidases which hydrolyse glycosidic bonds in the glycan strand, endopeptidases cleave the peptide bonds of the cross-bridge, and *N*-acetylmuramoyl-L-alanine amidases cleave the amide bond connecting the glycan moiety and the stem peptide (Pastagia et al., 2013).

We can see the difference between lysozyme independent and dependent lysis systems. Phages with lysozyme independent lysis system produce no holins or lysins. These phages encode proteins or peptides to inactivate the enzymes of synthesis pathways of the host cell wall, thus they function only on the cells in the growth stage; bacterial ghosts or large debris are left after cell lysis, and this mechanism often exists in the ssRNA or ssDNA phages. On the contrary, phages with lysozyme dependent lysis system use a combination of holins and lysins to split the host cells at any growth stage, the host cells are thoroughly lysed; this system is often found in dsDNA phages.

As an alternative to antibiotics, bacteriophages have a long history of treatment of a variety of bacterial diseases (Sulakvelidze et al., 2001). The following is a summarization of studies regarding phage therapy and *Enterobacteriaceae*.

BACTERIOPHAGE THERAPY TARGETED AGAINST *ENTEROBACTERIACEAE*

Escherichia coli

It is estimated by the World Health Organization that about 5 million children die each year as a consequence of acute diarrhea (Snyder and Merson, 1982). One third cases of childhood diarrhea in developing countries are caused by *E. coli* (Albert et al., 1995). Due to its mallea-

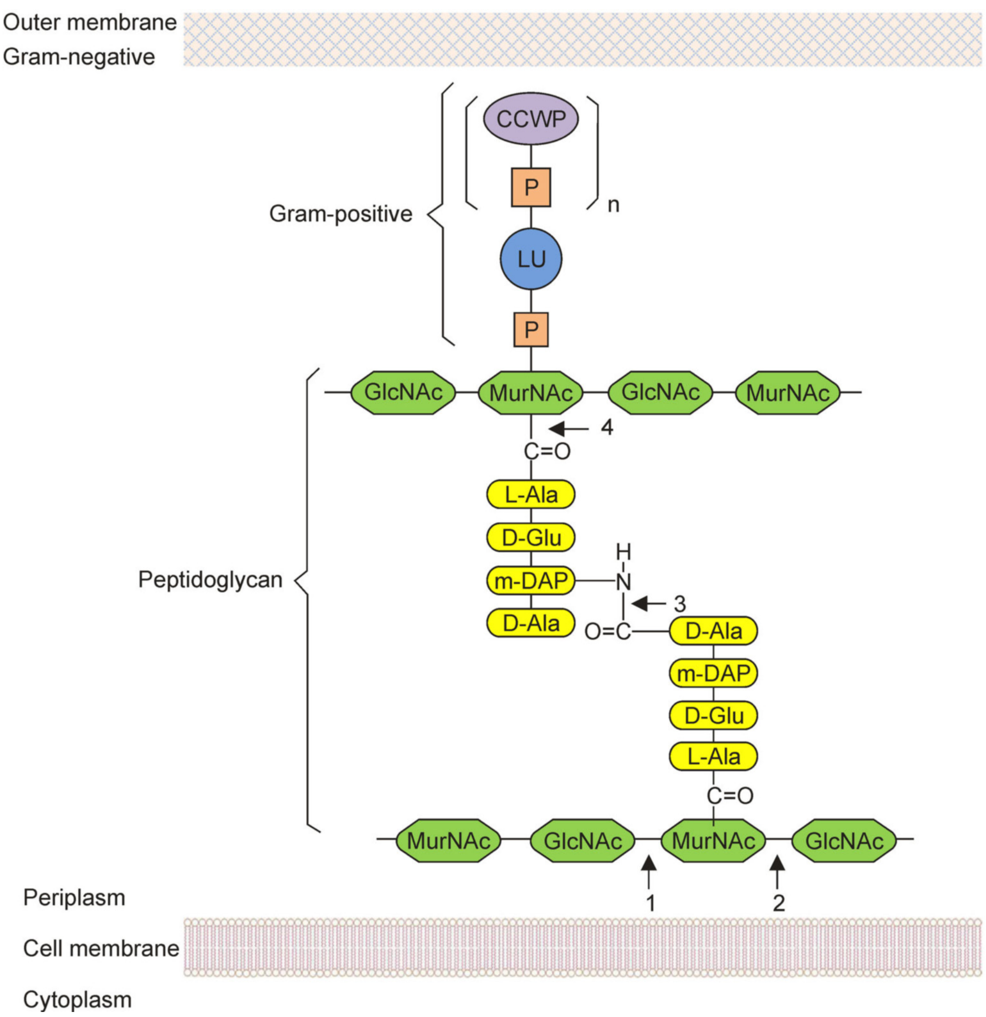

Figure 2. Lysins targeted on the peptidoglycan of bacterial cell wall (Adapted from Loessner, 2005). CCWP: carbohydrate cell wall polymer; P: phosphate group; LU: linkage unit; m-DAP: meso-diaminopimelic acid; GlcNAc: *N*-acetylglucosamine; MurNAc: *N*-acetylmuramic acid. 1: Endo-β-*N*-acetylglucosaminidase; 2: *N*-acetylmuramidase; 3: Endopeptidase; 4: *N*-acetylmuramoyl-L-alanine amidase.

ble genetic characteristic, *E. coli* has one of the widest spectra of disease of any bacterial species (Donnenberg, 2002). The *E. coli* O157 that recently emerged as a major food pathogen is a live example (Sharma et al., 2009). Furthermore, there is a lack of effective treatment for *E. coli* infections. Oral rehydration acts as the mainstay of treatment (Bhan et al., 1994). Countless lives have been saved by this simple and inexpensive measure. However, it does not affect the natural course of disease or the intrinsic activity of anti-bacteria. The use of antibiotics is of doubtful value since antibiotic resistance is widespread (Savarino et al., 2002).

Bacteriophages have been used to treat *E. coli* infections. Smith et al. used a mixture of phages to treat diarrhea in calves, piglets and lambs (Smith and Huggins, 1983). Similar results were achieved among these different animals; the diarrhea caused by the enteropathogenic *E. coli* strain was much reduced *in vivo*, and treatment had an ameliorating effect on the course of the disease. Recently, studies have also evaluated the safety of phage treatment through murine and human tests (Denou et al., 2009; Sarker et al., 2012). Denou et al. used a T4 coliphage to treat *E. coli* diarrhea by a combination of *in vitro* and *in vivo* tests (Denou et al., 2009). They selected phages based on genome sequencing and bioinformatic analysis, and found that the coliphage had a significant curative effect and no negative impact or anti-T4 antibodies were present after one month of treatment. Sarker et al. selected 9 phages without horizontal gene transfer and undesired genes from 99 T4-like coliphages based on genome sequencing to produce the cocktail, and gave it to 15 healthy adults from Bangladesh; no phage amplification or side effects were observed, which might indicate the safety of phage therapy (Sarker et al., 2012).

Salmonella enterica

S. enterica is the *Enterobacteriaceae* family bacteria which can cause human salmonellosis (Paterson, 2006). *S. enterica* can live in the gastrointestinal tracts of birds and mammals, which could reptile and survive for a long time in environments such as water, soil and kinds of foods (Capparelli et al., 2010). Ingestion of food with animal feces is the cause of most human cases of salmonellosis. Clinical manifestations of human salmonellosis have a wide range, from subclinical gastroenteritis to severe bacteremia and meningitis, as well as other forms of extraintestinal infections (Angulo et al., 2000). In fact, more than 2300 serovars of *S. enterica* are now known, and display great differences in virulence (Heithoff et al., 2008). Because of the widespread presence of antibiotic resistance among *S. enterica* isolates, the only available antibiotics are the fluoroquinolone and third-generation cephalosporins (Angulo et al., 2000), hence there is a need for antimicrobial alternatives other than antibiotics.

The phage was firstly exploited for classifying *S. enterica* bacteria by its specificity for bacterial species or serovars (Anderson et al., 1977). Recently, phages have been used for the control of *S. enterica* contamination (Atterbury et al., 2007; Leverentz et al., 2001; Wall et al., 2010). Leverentz et al. used *Salmonella*-specific lytic phages to reduce the colony numbers in experimentally contaminated fresh-cut melons and apples (Leverentz et al., 2001). They found that the phages could significantly reduce *Salmonella* populations of melons at different temperatures, but did not work well on apples due to the low pH, which might affect the phage activity. Atterbury et al. tried to reduce *Salmonella* colonization of broiler chickens through the use of host-specific phages (Atterbury et al., 2007). Three broad host range phages were selected from 232 *Salmonella* phages. Two of the three chosen phages showed an obvious reduction of *S. enterica* serotype enteritidis cecal colonization. Their studies also indicated the key factors in the successful phage-mediated control of salmonellas were appropriate phages and optimization of both the timing and method of phage delivery (Atterbury et al., 2007). Wall et al. used a phage cocktail to treat small and market-weight pigs, and the results showed that the group of pigs with the phage cocktail had a significantly reduced ileal *Salmonella* concentrations as well as cecal *Salmonella* concentrations (Wall et al., 2010).

Klebsiella pneumoniae

K. pneumoniae is a gram-negative opportunistic pathogen, which is often associated with pneumonia, urinary tract, bloodstream, and intra-abdominal infections (Verma et al., 2010). *K. pneumoniae* caused bacteremia usually leads to significant morbidity and mortality among the general population (Tsay et al., 2002). More seriously, due to the prevalence of multidrug-resistant *K. pneumoniae* strains, treatment of these infections becomes ever more difficult (Verma et al., 2009).

Virulent phages specific to *K. pneumoniae* cells have been studied to control the infection caused by *K. pneumoniae* (Verma et al., 2009; Chhibber et al., 2008). Malik et al. used bacteriophage KØ1 to treat third-degree burn wounds of mice administered with a fatal dose of *K. pneumonia* (Malik and Chhibber, 2009). After treatment, a substantial bacterial load decrease was noted in the blood, peritoneal lavage, and lung tissue of mice compared with those of the control groups. The decrease in microbial count was evident via the subcutaneous or intraperitoneal bacteriophage therapy. Gu et al. established a “step-by-step” approach to take advantage of the occurrence of phage-resistant bacteria variants (Gu et al., 2012). A phage cocktail consisted of three phages was established for *K. pneumoniae* by this method. The phage cocktail significantly reduced the mutation frequency of *K. pneumoniae* compared with any single phage, and effectively rescued *K. pneumoniae* bacteremia. Besides, the minimal protective dose of the phage cocktail was significantly smaller than that of single monophage to protect bacteremic mice from lethal *K. pneumoniae* K7 infection. Hung et al. treated *K. pneumoniae*-induced liver infection by using an isolated phage φNK5 (Hung et al., 2011). The results indicated that a single dose of lower than 2×10^8 PFU phages was effective. The mice, via intraperitoneal or intragastric treatment, showed an elimination of *K. pneumoniae* from both blood and liver tissues compared with those of the control groups. This work suggested the low dose of φNK5 is an efficient therapeutic agent against *K. pneumoniae*-induced liver infection. Chhibber et al. used phage SS as the agent against an experimental model of *K. pneumoniae*-mediated lobar pneumonia in mice (Chhibber et al., 2008). A single intraperitoneal injection of 10^{10} PFU/mL phage administered immediately after intranasal challenge of 10^8 CFU/mL of *K. pneumoniae* B5055 was sufficient to rescue *K. pneumoniae*-mediated respiratory infections. A significant protection was observed in infected mice by administration of the phage preparation three hours prior to intranasal bacterial challenge. However, the phage treatment was ineffective even six-hour delay of phage administration following the induction of infection. Therefore, the results of this study suggested that the timing of initial phage therapy after initiation of infection significantly contributed to the success of treatment. Although few phage therapies on human *K. pneumonia* infection have been reported, the studies suggest that bacteriophages or bacteriophage cocktails have the potential to modulate the infection caused by *K. pneumonia*.

Shigella strains

Shigella is a kind of small, unencapsulated, non-motile gram-negative rod bacteria that caused shigellosis in humans. There are four species of *Shigella* pathogenic to human: *S. dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri* (Subekti et al., 2001). Shigellosis is a major public-health problem in many developing countries, and causes considerable morbidity and mortality (Niyogi 2005; Phalipon and Sansonetti, 2007). Shigellosis causes an estimated 120 million cases and 1.1 million deaths worldwide annually (Niyogi, 2005). *Shigella* contamination occurs mainly through the fecal-oral route, with fomites, food, water, insects or direct person to person contact, and the infectious dose is as little as 100 bacterial cells (Phalipon and Sansonetti, 2007). In addition, secondary transmission through environmental sources cannot be ignored (Levine and Levine, 1991).

Bacteriophage treatment of *Shigella* originated in 1917, while d'Herelle first used phages to split *Shigella* strains isolated from several soldier patients with hemorrhagic dysentery (d'Herelle, 1917; Summers, 1999). One of the most extensive studies was conducted to evaluate the utility of therapeutic phages for prophylaxis of bacterial dysentery during 1963 and 1964 in Tbilisi, Georgia (Babalova et al., 1968). In total, 30,769 children between 6 months to 7 years of age were included in the study. Of these children, 17,044 on one side of the street were given *Shigella* phages orally, and the remainder on the other side of the street were not treated with phages. The final results showed that the incidence of dysentery of the placebo group was 3.8-fold higher than that of the phage-treated group, which indicated the efficiency of phage therapy against *Shigella* strains.

Serratia marcescens

Neonates are commonly infeced with *S. marcescens*, especially immunocompromised neonates of low birth weight (Larson et al., 2005). Furthermore, *S. marcescens* has a specific affinity for the central nervous system, and meningoencephalitis or a brain abscess with this pathogen has a severe neurologic prognosis (Messerschmidt et al., 2004). Recently, there have been reports concerning multidrug-resistant strains of *S. marcescens* in pediatrics, which make it difficult to treat diseases caused by this bacterium with drug therapy (Maragakis et al., 2008).

In 1967, phages were used to infect *S. marcescens*. Iino et al. used a broad host range phage χ to split 20 of *S. marcescens* strains (Iino and Mitani, 1967). Their results showed that phage χ could only infect the strains with flagella, which indicated the receptor site of phages. Matsushita et al. isolated two phages, KSP90 and KSP100, from environmental water that were related to the T4-type phage and phiEco32 phage, respectively (Matsushita et al., 2009). They extensively studied the biological features, DNA features, virion proteins and phylogenic relationships of the two phages, and their work indicated the therapeutic potential of the phages to control *S. marcescens* infection. Denyes et al. sequenced the genome of *Serratia* bacteriophage η, and have a well known about the genome length and structure, as well as all the functional CDSs (Denyes et al., 2014). Whether it carries virulence genes or not is also clear. This kind of work is useful for the application of phages for therapy.

Other strains of *Enterobacteriaceae* family

Besides the above mentioned *Enterobacteriaceae*, studies of phages related to other *Enterobacteriaceae* such as *Edwardsiella* (Yasuike et al., 2013), *Proteus* (Lazareva et al., 2001), *Erwinia* (Born et al., 2011), and *Citrobacter* (Chaudhry, 2014) have also been reported recently. These works indicate the practicability of therapeutic candidates of bacteriophages.

CONCLUSION

Bacteriophage therapy is an effective way to control bacterial infections; it is superior to antibiotic treatment in the following aspects. The phages are capable of increasing in numbers specifically where hosts are located during the bacterial-killing process, and contribute to establishing the phage dose; in addition, the cost of agent production is relatively low (Abedon and Thomas-Abedon, 2010). Most phages have a specific host range, which makes them split the target pathogenic bacteria while leaving minimal disruption of normal flora (Gupta and Prasad, 2011). By contrast, many chemical antibiotics have broader spectrums of activity, which may be prone to inducing super infections (Carlton, 1999). Unlike antibiotics, which can be toxic, phages display little or no toxicity to the flora and environment (Bentley and Bennett, 2003).

Bacteriophage therapy also has limitations. One is the safety problem. All of the phages in a given cocktail will need to be appropriately characterized before they can be used in clinical treatment. Fortunately, the rapid improvement of genome sequencing technologies assures the safety of phages used in therapy approaches. Another problem is the narrow host range; however, this limitation can be counteracted by the use of recently developed and improved phage cocktails. The third limitation is the instability of the phage therapeutic agent; further study is needed to improve and prefect this factor. The forth problem is the phase-resistance developed by bacteria during their co-evolution with phages. Disputes focus on whether the same result will appear in the future, as bacteria will develop multi-phage resistance as the same as the abuse of antibiotics. This is an important problem and needs future studies.

ACKNOWLEDGMENTS

This work was supported by the National Infrastructure of Microbial Resources (NIMR2014-4).

COMPLIANCE WITH ETHICS GUIDELINES

All the authors declare that they have no competing interests. This article does not contain any studies with human or animals subjects performed by any of the authors.

REFERENCES

Abedon ST, Thomas-Abedon C. 2010. Phage therapy pharmacology. Curr Pharm Biotechnol, 11:28–47.

Albert MJ, Faruque SM, Faruque AS, Neogi PK, Ansaruzzaman M, Bhuiyan NA, Alam K, Akbar MS. 1995. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. J Clin Microbiol, 33:973–977.

Anderson ES, Ward L, DeSaxe M, de Sa JD. 1977. Bacteriophage-typing designations of *Salmonella* typhimurium. J Hyg (Lond), 78:297–300.

Angulo FJ, Johnson KR, Tauxe RV, Cohen ML. 2000. Significance and sources of antimicrobial-resistant nontyphoidal *Salmonella* infections in the United States. Microb Drug Resist, 6:77–83.

Atterbury RJ, Van Bergen MA, Ortiz F, Lovell MA, Harris JA, De Boer A, Wagenaar JA, Allen VM, Barrow PA. 2007. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Appl Environ Microbiol, 73:4543–4549.

Babalova EG, Katsitadze KT, Sakvarelidze LA, Imnaishvili NSh, Sharashidze TG, Badashvili VA, Kiknadze GP, Meĭpariani AN, Gendzekhadze ND, Machavariani EV, Gogoberidze KL, Gozalov EI, Dekanosidze NG. 1968. Preventive value of dried dysentery bacteriophage. Zh Mikrobiol Epidemiol Immunobiol, 45:143–145. (In Russian)

Bentley R, Bennett JW. 2003. What is an antibiotic? Revisited. Adv Appl Microbiol, 52:303–331.

Bhan MK, Mahalanabis D, Fontaine O, Pierce NF. 1994. Clinical trials of improved oral rehydration salt formulations: a review. Bull World Health Organ, 72:945–955.

Born Y, Fieseler L, Marazzi J, Lurz R, Duffy B, Loessner MJ. 2011. Novel virulent and broad-host-range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and a relationship to *Enterobacteriaceae* phages. Appl Environ Microbiol, 77:5945–5954.

Bruynoghe R, Maisin J. 1921. Essais de the´rapeutique au moyen du bacteriophage. C R Soc Biol, 85:1120–1121.

Burrowes B, Harper DR, Anderson J, McConville M, Enright MC. 2011. Bacteriophage therapy: potential uses in the control of antibiotic-resistant pathogens. Expert Rev Anti Infect Ther, 9:775–785.

Capparelli R, Nocerino N, Iannaccone M, Ercolini D, Parlato M, Chiara M, Iannelli D. 2010. Bacteriophage therapy of *Salmonella enterica*: a fresh appraisal of bacteriophage therapy. J Infect Dis, 201:52–61.

Carlton RM. 1999. Phage therapy: past history and future prospects. Arch Immunol Ther Exp (Warsz), 47:267–274.

Chaudhry WN, Haq IU, Andleeb S, Qadri I. 2014. Characterization of a virulent bacteriophage LK1 specific for *Citrobacter freundii* isolated from sewage water. J Basic Microbiol, 54:531–541.

Chhibber S, Kaur S, Kumari S. 2008. Therapeutic potential of bacteriophage in treating *Klebsiella pneumoniae* B5055-mediated lobar pneumonia in mice. J Med Microbiol, 57:1508–1513.

Daniel A, Euler C, Collin M, Chahales P, Gorelick KJ, Fischetti VA. 2010. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother, 54:1603–1612.

Denou E, Bruttin A, Barretto C, Ngom-Bru C, Brüssow H, Zuber S. 2009. T4 phages against *Escherichia coli* diarrhea: potential and problems. Virology, 388:21–30.

Denyes JM, Krell PJ, Manderville RA, Ackermann HW, She YM, Kropinski AM. 2014. The genome and proteome of *Serratia* bacteriophage η which forms unstable lysogens. Virol J, 11:6.

d'Herelle F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. Cr Acad Sci (Paris), 165: 373–375. (In French)

Díaz E, López R, García JL. 1990. Chimeric phage-bacterial enzymes: a clue to the modular evolution of genes. Proc Natl Acad Sci U S A, 87:8125–8129.

Donnenberg MS. 2002. Evolution of pathogenic *Escherichia coli*. In *Escherichia coli*: virulence mechanisms of a versatile pathogen. Amsterdam: Academic Press. pp. 55–173.

Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. 2010. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. Lancet Infect Dis, 10:43–50.

Fischetti VA. 2008. Bacteriophage lysins as effective antibacterials. Curr Opin Microbiol, 11:393–400.

Gründling A, Bläsi U, Young R. 2000. Genetic and biochemical analysis of dimer and oligomer interactions of the lambda S holin. J Bacteriol, 182: 6082–6090.

Gründling A, Smith DL, Bläsi U, Young R. 2000. Dimerization between the holin and holin inhibitor of phage lambda. J Bacteriol, 182:6075–6081.

Gu J, Liu X, Li Y, Han W, Lei L, Yang Y, Zhao H, Gao Y, Song J, Lu R, Sun C, Feng X. 2012. A method for generation phage cocktail with great therapeutic potential. PLoS One, 7:e31698.

Gupta R, Prasad Y. 2011. Efficacy of polyvalent bacteriophage p-27/HP to control multidrug resistant *Staphylococcus aureus* associated with human infections. Curr Microbiol, 62:255–260.

Hankin E. 1896. L'action bactéricide des eaux de la Jumna et du Gange sur le vibrion du choléra. Ann Inst Pasteur (Paris), 10:511–523. (In French)

Heithoff DM, Shimp WR, Lau PW, Badie G, Enioutina EY, Daynes RA, Byrne BA, House JK, Mahan MJ. 2008. Human *Salmonella* clinical isolates distinct from those of animal origin. Appl Environ Microbiol, 74:1757–1766.

Hung CH, Kuo CF, Wang CH, Wu CM, Tsao N. 2011. Experimental phage therapy in treating *Klebsiella pneumoniae*-mediated liver abscesses and bacteremia in mice. Antimicrob Agents Chemother, 55:1358–1365.

Iino T, Mitani M. 1967. Infection of Serratia marcescens by bacteriophage χ. J Virol, 1:445–447.

Larson EL, Cimiotti JP, Haas J, Nesin M, Allen A, Della-Latta P, Saiman L. 2005. Gram-negative bacilli associated with catheter-associated and non-catheter-associated bloodstream infections and hand carriage by healthcare workers in neonatal intensive care units. Pediatr Crit Care Med, 6:457–461.

Lazareva EB, Smirnov SV, Khvatov VB, Spiridonova TG, Bitkova EE, Darbeeva OS, Maĭskaia LM, Parfeniuk RL, Men'shikov D D. 2001. Efficacy of bacteriophages in complex treatment of patients with burn wounds. Antibiot Khimioter, 46:10–14.

Leverentz B, Conway WS, Alavidze Z, Janisiewicz WJ, Fuchs Y, Camp MJ, Chighladze E, Sulakvelidze A. 2001. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. J Food Prot, 64:1116–1121.

Levine OS, Levine MM. 1991. Houseflies (Musca domestica) as mechanical vectors of shigellosis. Rev Infect Dis, 13:688–696.

Loessner MJ. 2005. Bacteriophage endolysins--current state of research and applications. Curr Opin Microbol, 8:480–487.

Malik R, Chhibber S. 2009. Protection with bacteriophage KØ1 against fatal *Klebsiella pneumoniae*-induced burn wound infection in mice. J Microbiol Immunol Infect, 42:134–140.

Maragakis LL, Winkler A, Tucker MG, Cosgrove SE, Ross T, Lawson E, Carroll KC, Perl TM. 2008. Outbreak of multidrug-resistant *Serratia marcescens* infection in a neonatal intensive care unit. Infect Control Hosp Epidemiol, 29:418–423.

Matsushita K, Uchiyama J, Kato S, Ujihara T, Hoshiba H, Sugihara S, Muraoka A, Wakiguchi H, Matsuzaki S. 2009. Morphological and genetic analysis of three bacteriophages of *Serratia marcescens* isolated from environmental water. FEMS Microbiol Lett, 291:201–208.

McAuliffe O, Ross RP, Fitzgerals GF. 2007. The new phage biology: from genomics to applications. In Bacteriophage: Genetics and Molecular Biology (1st ed.). Mc Grath S and van Sinderen D. Norfolk, Engand: Caister Academic Press. pp. 1–42.

Merril CR, Scholl D, Adhya SL. 2003. The prospect for bacteriophage therapy in Western medicine. Nat Rev Drug Discov, 2:489–497.

Messerschmidt A, Prayer D, Olischar M, Pollak A, Birnbacher R. 2004. Brain abscesses after *Serratia marcescens* infection on a neonatal intensive care unit: differences on serial imaging. Neuroradiology, 46:148–152.

Niyogi SK. 2005. Shigellosis. J Microbiol, 43:133–143.

Pang T, Savva CG, Fleming KG, Struck DK, Young R. 2009. Structure of the lethal phage pinhole. Proc Natl Acad Sci U S A, 106:18966–18971.

Pastagia M, Schuch R, Fischetti VA, Huang DB. 2013. Lysins: the arrival of pathogen-directed anti-infectives. J Med Microbiol, 62:1506–1516.

Paterson DL. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Med, 119:S20–28.

Payne RJ, Phil D, Jansen VA. 2000. Bacteriaphage therapy: the pecculiar kinetics of self-replicating pharmaceuticals. Clin Pharmacol Ther, 68: 225–230.

Phalipon A, Sansonetti PJ. 2007. *Shigella*'s ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? Immunol Cell Biol, 85:119–129.

Qian ZW, Yue QA, Tian FL. 2007. Study overview of phagotherapy. Med Recapitulate, 13:1256−1258. (In Chinese)

Ramanculov E, Young R. 2001. Genetic analysis of the T4 holin: timing and topology. Gene, 265:25–36.

Reed CA, Langlais C, Kuznetsov V, Young R. 2012. Inhibitory mechanism of the Qβ lysis protein A_2. Mol Microbiol, 86:836–844.

Samsygina GA, Boni EG. 1984. Bacteriophages and phage therapy in pediatric practice. Pediatriia, 4:67–70. (In Russian)

Savva CG, Dewey JS, Deaton J, White RL, Struck DK, Holzenburg A, Young R. 2008. The holin of bacteriophage lambda forms rings with large diameter. Mol Microbiol, 69:784–793.

Sarker SA, McCallin S, Barretto C, Berger B, Pittet AC, Sultana S, Krause L, Huq S, Bibiloni R, Bruttin A, Reuteler G, Brüssow H. 2012. Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. Virology, 434:222–232.

Savarino SJ, Hall ER, Bassily S, Wierzba TF, Youssef FG, Peruski LF Jr, Abu-Elyazeed R, Rao M, Francis WM, El Mohamady H, Safwat M, Naficy AB, Svennerholm AM, Jertborn M, Lee YJ, Clemens JD. 2002. Introductory evaluation of an oral, killed whole cell enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine in Egyptian infants. Pediatr Infect Dis J, 21:322–330.

Sharma M, Patel JR, Conway WS, Ferguson S, Sulakvelidze A. 2009. Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettucet. J Food Prot, 72:1481–1485.

Shi Y, Yan Y, Ji W, Du B, Meng X, Wang H, Sun J. 2012. Characterization and determination of holin protein of *Streptococcus suis* bacteriophage SMP in heterologous host. Virol J, 9:70.

Si XD. 1955. Bacillary dysentery therapy using dysentery phage. Nat Med J China, 41:824−834. (In Chinese)

Smith HW, Huggins MB. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves, piglets and lambs. J Gen Microbiol, 129:2659–2675.

Snyder JD, Merson MH. 1982. The magnitude of the global problem of acute diarrheal disease: a review of active surveillance data. Bull World Health Organ, 60:605–613.

Subekti D, Oyofo BA, Tjaniadi P, Corwin AL, Larasati W, Putri M, Simanjuntak CH, Punjabi NH, Taslim J, Setiawan B, Djelantik AA, Sriwati L, Sumardiati A, Putra E, Campbell JR, Lesmana M. 2001. *Shigella* spp. surveillance in Indonesia: the emergence or reemergence of *S. dysenteriae*. Emerg Infect Dis, 7:137–140.

Sulakvelidze A, Alavidze Z, Morris Jr JG. 2001. Bacteriophage therapy. Antimicrob Agents Chemother, 45: 649–659.

Summers WC. 1999. Bacteriophage discovered, in Felix d'Herelle and the origins of molecular biology. New Haven, CT: Yale University Press. pp. 47–59.

Tanaka S, Clemons WM Jr. 2012. Minimal requirements for inhibition of MraY by lysis protein E from bacteriophage φX174. Mol Microbiol, 85:975–985.

Tsay RW, Siu LK, Fung CP, Chang FY. 2002. Characteristics of bacteremia between community-acquired and nosocomial *Klebsiella pneumoniae* infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. Arch Intern Med, 162:1021–1027.

Twort FW. 1915. An investigation on the nature of ultra-microscopic viruses. Lancet, 189:1241−1243.

Verma V, Harjai K, Chhibber S. 2010. Structural changes induced by a lytic bacteriophage make ciprofloxacin effective against older biofilm of *Klebsiella pneumoniae*. Biofouling, 26:729–737.

Verma V, Harjai K, Chhibber S. 2009. Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment. J Antimicrob Chemother, 64:1212–1218.

Wall SK, Zhang J, Rostagno MH, Ebner PD. 2010. Phage therapy to reduce preprocessing *Salmonella* infections in market-weight swine. Appl Environ Microbiol, 76:48–53.

Xu M, Struck DK, Deaton J, Wang IN, Young R. 2004. A signal-arrest-release sequence mediates export and control of the phage P1 endolysin. Proc Natl Acad Sci U S A, 101:6415–6420.

Yang Q, Wang H, Chen M, Ni Y, Yu Y, Hu B, Sun Z, Huang W, Hu Y, Ye H, Badal RE, Xu Y. 2010. Surveillance of antimicrobial susceptibility of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in China: the 2002-2009 study for monitoring antimicrobial Resistance trends (SMART). Int J Antimicrob Agents, 36:507–512.

Yasuike M, Sugaya E, Nakamura Y, Shigenobu Y, Kawato Y, Kai W, Fujiwara A, Sano M, Kobayashi T, Nakai T. 2013. Complete genome sequences of *Edwardsiella tarda*-lytic bacteriophages KF-1 and IW-1. Genome Announc, 1: e00089-12.

Young R. 1992. Bacteriophage lysis: mechanism and regulation. Microbiol Rev, 56:430–481.

Young R, Bläsi U. 1995. Holins: form and function in bacteriophage lysis. FEMS Microbiol Rev, 17:191–205.