8

Application of Therapeutic Phages in Medicine

Sanjay Chhibber* and Seema Kumari
Department of Microbiology, Basic Medical Sciences Building,
Panjab University, Chandigarh,
India

1. Introduction

For more than half a century, the doctors and clinicians have been relying primarily on antibiotics to treat infectious diseases caused by pathogenic bacteria. However, the emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal or agricultural usage has made antibiotics less and less effective (Fischetti, 2008; Perisien et al., 2008). These days scientists are now facing the threat of superbugs, i.e. pathogenic bacteria resistant to most or all available antibiotics (Livemore, 2004; Fischetti, 2006). During the last 30 years, no new classes of antibiotics have been found, even with the help of modern biotechnology such as genetic engineering. Pharmaceutical companies have mainly focused on the development of new products derived from the known classes of antibiotics (Carlton, 1999; Sulakvelidze et al., 2001) which is a cause of major concern. Thus, exploring alternative approaches to develop antibacterial products is also a worthwhile task, and re-examining the potential of promising older methods might be of value. One of the possible replacements for antibiotics is the use of bacteriophages or simply phages as antimicrobial agents (Shasha et al., 2004; Vinodkumar et al., 2008). Phage therapy involves the use of lytic phages for the treatment of bacterial infections, especially those caused by antibiotic resistant bacteria. In general, there are two major types of phages, lytic and lysogenic. Only the lytic phages (also known as virulent phages) are a good choice for developing therapeutic phage preparations (Sandeep, 2006; Borysowski and Gorski, 2008). The bactericidal ability of phages has been used to treat human infections for years as a complement or alternative to antibiotic therapy (Alisky et al., 1998; Matsuzaki et al., 2005; Kysela & Turner, 2007). Bacteriophages, nature's tiniest viruses and it is estimated that there are about 10^{31} phages on earth making viruses the most abundant life form on earth (Ashelford et al., 2000; Hendrix, 2002; Dabrowska et al., 2005). Bacteriophages not only help in the treatments of bacterial infections in animals and human beings but also used in birds, fishes, plants, food material and biofilm eradication (Flaherty et al., 2000; Goode et al., 2003; Leverentz et al., 2003; Park & Nakai, 2003; Curtin & Donlan, 2006).

2. Benefits of phage therapy over antibiotics

Phages appear to be better therapeutic agents as they have several advantages over traditional antibiotics (Pirisi, 2000; Sulakvelidze et al., 2001; Matsuzaki et al., 2005). Majority of them are summarized in the Table given below.

Bacteriophages	Antibiotics
Phages are highly effective in killing their targeted bacteria i.e., their action is bactericidal	Some antibiotics are bacteriostatic, i.e., they inhibit the growth of bacteria, rather than killing them (e.g., chloramphenicol).
Production is simple and cheap.	Production is complex and expensive.
Phages are an ‘intelligent’ drug. They multiply at the site of the infection until there are no more bacteria. Then they are excreted.	They are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection.
The pharmacokinetics of bacteriophage therapy is such that the initial dose increases exponentially if the susceptible bacterial host is available. In such cases, there is no need to administer the phages repeatedly.	Repeated doses of antibiotic is required to cure the bacterial disease.
The high selectivity/specificity of bacteriophages permits the targeting of specific pathogens, without affecting desirable bacterial flora which means that phages are unlikely to affect the “colonization pressure" of the patients	Antibiotics demonstrate bactericidal or bacteriostatic effects not only on the cause of bacterial disease, but on all microorganisms present in the body including the host normal microflora.. Thus their non-selective action affects the patient's microbial balance, which may lead to various side effects.
Because of phages specificity, their use is not likely to select for phage resistance in other (non-target) bacterial species	The broad spectrum activity of antibiotics may select for resistant mutants of many pathogenic bacterial species.
Humans are exposed to phages throughout life, and well tolerate them. No serious side effects have been described.	Multiple side effects, including intestinal disorders, allergies, and secondary infections (e.g., yeast infections) have been reported.
Phage-resistant bacteria remain susceptible to other phages having a similar host range.	Resistance to antibiotics is not limited to targeted bacteria.
Phages are found throughout nature. This means that it is easy to find new phages when bacteria become resistant to them. Selecting a new phage (e.g., against phage-resistant bacteria) is a rapid process and frequently can be accomplished in days.	Developing a new antibiotic (against antibiotic resistant bacteria) is a time consuming process and may take several years to accomplish.
Phages may be considered as good alternative for patients allergic to antibiotics.	If patient is allergic to antibiotic, treatment is very difficult

Table 1. Comparison of phages and antibiotics regarding their prophylactic and therapeutic use.

There are also some disadvantages with the phage therapy approach. These include:

- The problem which requires attention is the rapid clearance of phage by the spleen, liver and other filtering organs of reticuloendothelial system (Carlton, 1999). This can be taken care by doing serial passage in mice (Merril et al., 1996) so as to obtain a phage mutant capable of evading the reticuloendothelial system and therefore capable of long circulation in the blood. The minor variations in their coat proteins enable some variants to be less easily recognized by the RES organs, allowing them in the circulation for longer periods than the "average" wild-type phage.
- This therapy can not be used for intracellular bacteria as the host is not available for interaction.
- Theoretically development of neutralizing antibodies against phages could be an obstacle to the use phage therapy in recurrent infections. This needs to be confirmed experimentally. However, in the immunocompromised host where the immune system is depressed such as chronic infections, the phage therapy may work in this situation (Skurnik & Strauch, 2006).
- The shelf life of phages varies and needs to be tested and monitored.
- Phages are more difficult to administer than antibiotics. A physician needs special training in order to correctly prescribe and use phages.

3. Safety of the therapeutic phage preparation

During the long history of using phages as therapeutic agents through Eastern Europe and the former Soviet Union, there has been no report of serious complications associated with their use (Sulakvelidze & Morris, 2001). Phages are extremely common in environment and regularly consumed in foods (Bergh et al. 1989). In fact humans are exposed to phages from birth itself and therefore these constitute the normal microflora of the human body. They have been commonly found in human gastrointestinal tract, skin and mouth, where they are harboured in saliva and dental plaques (Bachrach et al., 2003). Phages are also abundant in environment including saltwater, freshwater, soil, plants and animals and they have been shown to be unintentional contents of some vaccines and sera commercially available in United States (Merril et al., 1972; Geier et al., 1975; Milch & Fornosi, 1975). Phages have high specificity for specific bacterial strains, a characteristic which requires careful targetting (Merril et al. 2003; Bradbury 2004). Therefore, phage therapy can be used to lyse specific pathogens without disturbing normal bacterial flora and phages pose no risk to anything other than their specific bacterial host (Lorch, 1999; Sulakvelidze et al., 2001; Duckworth & Gulig, 2002).

From a clinical standpoint, phage therapy appears to be very safe. Efficacy of natural phages against antibiotic-resistant *Streptococci, Escherichia, Pseudomonas, Proteus, Salmonella, Shigella, Serratia, Klebsiella* (Kumari et al., 2010), *Enterobacter, Campylobacter, Yersinia, Acinetobacter* and *Brucella* are being evaluated by researchers (Matsuzaki et al., 2005). However, in the last few years, modified phages are being explored increasingly, due to the limitations of phage therapy using lytic phages. The safety concerns regarding spontaneously propagating live microorganisms and the inconsistency of phage therapy results in the treatment of bacterial infections specifically induced scientists to explore more controllable phages (Skurnik et al., 2007). Phages can be modified to be an excellent therapeutic agent by directed mutation of the phage genome, recombination of phage genomes, artificial selection of phages *in vivo*, chimeric phages and other rational designs which confer new properties on the phages. These new modified phages have been shown to successfully overcome challenges to earlier phage therapy (Moradpour & Ghasemian, 2011).

As with antibiotic therapy and other methods of countering bacterial infections, endotoxins (lipopolysaccharide) are released by the gram negative bacteria as a component of outer membrane. This can cause symptoms of fever, or in extreme cases, toxic shock (Herxheimer reaction) (Theil, 2004). To address the endotoxin release issue, recombinant phage derived from *P. aeruginosa* filamentous phage Pf3 was constructed by genetic modifications and the results showed that this filamentous phages could be used as effective anti-infection agent (Hagen & Blasi, 2003; Hagens et al. 2004). This phage had the benefit of minimizing the release of membrane associated endotoxins during phage therapy (Parisien et al., 2008). In order not to compromise on the issue of the safe use of therapeutic phage preparation, rigorous characterizations of each phage to be used therapeutically should be done, in particular, especially looking for potentially harmful genes in their genome (Payne & Jensen, 2000; Carlton et al., 2005; Hanlon, 2007; Mattey & Spencer, 2008).

4. Clinical application of bacteriophages

4.1 Whole phage as antimicrobial agents

4.1.1 Phage therapy in Humans

However, although d'Hérelle carried out the first human therapeutic phage trial, the first article documenting phage therapy was on research conducted in Belgium by Bruynoghe and Maisin in 1921. They reported that phages when injected in six patients targeted staphylococcus near the base of cutaneous boils (furuncles and carbuncles), resulted in improvement within 48 hours and reduction in pain, swelling and fever. Merabishvili and workers (2009) used phage cocktail, consisting of exclusively lytic bacteriophages for the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections in burn wound patients in the Burn Centre of the Queen Astrid Military Hospital in Brussels, Belgium. The first controlled clinical trial of a therapeutic bacteriophage preparation (Biophage-PA) showed efficacy and safety in chronic otitis because of drug resistant P. aeruginosa in UCL Ear Institute and Royal National Throat, Nose and Ear Hospital, London, UK (Wright et al., 2009). Several clinical trials on phage therapy in humans were reported with the majority coming from researchers in Eastern Europe and the former Soviet Union (Abdul-Hassan et al., 1990; Sulakvelidze et al., 2001). One of the most extensive studies evaluating the application of therapeutic phages for prophylaxis of infectious diseases was conducted in Tbilisi, Georgia, during 1963 and 1964 and involved phages against bacterial dysentery (Babalova et al., 1968). The most detailed English language reports on phage therapy in humans were by Slopek and co workers who published a number of papers on the effectiveness of phages against infections caused by several bacterial pathogens, including multidrug-resistant mutants (Slopek et al., 1983, 1984, 1985; Kucharewicz-Krukowska et al., 1987; Weber-Dabrowska et al., 1987). Phages have been reported to be effective in treating various bacterial diseases such as cerebrospinal meningitis in a newborn (Stroj et al., 1999), skin infections caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus, E. coli* (Cislo et al., 1987), recurrent subphrenic and subhepatic abscesses (Kwarcinski et al., 1987), Staphylococcal lung infections

((Ioseliani et al., 1980; Kaczkowski et al., 1990), *Pseudomonas aeruginosa* infections in cystic fibrosis patients (Shabalova et al., 1995), eye infections (Proskurov, 1970), neonatal sepsis (Pavlenishvili & Tsertsvadze, 1993), urinary tract infections (Perepanova et al., 1995), and cancer (Weber-Dabrowska et al., 2001). Abdul-Hassan et al. (1990) reported on the treatment of 30 cases of burn-wound associated antibiotic-resistant *Pseudomonas aeruginosa* sepsis. Bandages soaked with 10^{10} phages/ml were applied three times daily. Half of the cases were found to be improved. Markoishvili et al., (2002) reported the use of PhagoBioDerm, the phage impregnated polymer, to treat infected venous stasis skin ulcers. To patients that had failed to respond to other treatment approaches, PhagoBioDerm was applied to ulcers both alone and, where appropriate, in combination with other treatment strategies. Complete healing of ulcers was observed in 70% of the patients. Mushtaq et al., (2005) reported that a bacteriophage encoded enzyme, endosialidase E (endo E) selectively degrades the linear homopolymeric a-2, 8-linked N acetylneuraminic acid capsule associated with the capacity of *E. coli* K1 strain to cause severe infection in the newborn infant. In one of the study, PhagoBioDerm (a wound-healing preparation consisting of a biodegradable polymer impregnated with ciprofloxacin and bacteriophages) was used in three Georgian lumberjacks from the village of Lia who were exposed to a strontium-90 source from two Soviet-era radiothermal generators they found near their village. In addition to systemic effects, two of them developed severe local radiation injuries which subsequently became infected with *Staphylococcus aureus*. Approximately 1 month after hospitalization, treatment with phage bioderm was initiated. Purulent drainage stopped within 2–7 days. Clinical improvement was associated with rapid (7 days) elimination of the *S. aureus* resistant to many antibiotics (including ciprofloxacin), but susceptible to the bacteriophages contained in the PhagoBioDerm preparation (Jikia et al., 2005). Leszczynski and co workers (2006) described the use of oral phage therapy for targeting Methicillin Resistant Staphylococcus aureus (MPSA) in a nurse who was a carrier. She had MRSA colonized in her gastrointestinal tract and also had a urinary tract infection. The result of phage therapy was complete elimination of culturable MRSA (Leszczynski et al., 2006).

4.1.2 Animal trials

In Britain, Smith and Huggins (1982, 1983) carried out a series of excellent, well-controlled studies on the use of phages in systemic *E. coli* infections in mice and then in diarrheic disease in young calves and pigs. Bogovazova et al., (1991) studied the effectiveness of specific phage therapy in non inbred white mice, caused by intraperitoneal injection of *K. pneumoniae* K25053 into the animals. Soothill, (1994) examined the ability of bacteriophage to prevent the rejection of skin grafts of experimentally infected guinea pigs. His findings demonstrated that the phage-treated grafts were protected in six of seven cases, while untreated grafts failed uniformly, suggesting that phage might be useful for the prevention of *P. aeruginosa* infections in patients with burn wounds. Phage therapy has been successfully used to remove *E. coli* 0157:H7 from livestock (Barrow et al., 1998; Kudva et al., 1999; Tanji et al., 2004). One of the most successful studies was carried out by Biswas and coworkers (2002). These workers suggested that a single i.p. injection of 3×10^8 PFU of the phage strain, administered 45 minutes after the bacterial challenge (vancomycin-resistant *Enterococcus faecium* (VRE) was sufficient to rescue 100% of the animals. Even when treatment was delayed to the point where all animals were moribund, approximately 50% of them were rescued by a single injection of the phage. The protective effect of bacteriophage was assessed against experimental *S. aureus* infection in mice. Subsequent intraperitoneal administration of purified ØMR11 (MOI of 0.1) suppressed *S. aureus*–induced lethality. This lifesaving effect coincided with the rapid appearance of ØMR11 in the circulation, which remained at substantial levels until the bacteria were eradicated (Matsuzaki et al. 2003). Benedict & Flamiano, (2004) evaluated the use of bacteriophages as therapy for Escherichia coli-induced bacteremia in mice. This experimental study showed clearly that a single dose of crude phage lysates administered by i.p. injection was enough to rescue bacteremic mice back to normal health after having been challenged with a lethal concentration of *E. coli*. Vinodkumar and co-workers (2005) studied the ability of bacterial viruses to rescue septicemic mice with multidrug resistant (MDR) Klebsiella pneumoniae isolated from neonatal septicemia. A single i.p. injection of $3x10^8$ PFU of the phage strain administered 45 minutes after the bacterial challenge rescued 100% of the animals. Wills and colleagues (2005) also demonstrated the efficacy of bacteriophage therapy against *S. aureus* in a rabbit abscess model. 2×10^9 PFU of staphylococcal phage prevented abscess formation in rabbits when it was injected simultaneously with S. aureus (8×10^7 CFU) into the same subcutaneous site. Phage multiplied in the tissues. The sewerage-derived bacteriophage reduced the abscess area and the count of *S. aureus* in the abscess was lowered in a bacteriophage dose dependent way (Will et al., 2005). Marza et al. (2006) reported the treatment of a dog with chronic bilateral otitis external that had consistently grown P. aeruginosa. This infection had failed to be resolved after repeated courses of topical and systemic antibiotics. After inoculation with 400 PFU of bacteriophage into the auditory canal there was a marked improvement in the clinical signs, 27 hours after treatment. Wang et al., (2006) examined the effectiveness of phages in the treatment of imipenem resistant *Pseudomonas aeruginosa* (IMPR-Pa) infection in an experimental mouse model. A single i.p. inoculation of the phage strain ØA392 (MOI > 0.01) at up to 60 min after the bacterial challenge was sufficient to rescue 100% of the animals. The workers demonstrated that the ability of the phage to rescue bacteremic animals was due to the functional capabilities of the phage and not to a non-specific immune effect. McVay and co-workers (2007) examined the efficacy of phage therapy in treating fatal *Pseudomonas aeruginosa* infections in mouse burn wound model. The results showed that a single dose of the *Pseudomonas aeruginosa* phage cocktail could significantly decrease the mortality of thermally injured, *Pseudomonas aeruginosa*-infected mice (from 6% survival without treatment to 22 to 87% survival with treatment) and that the route of administration was particularly important to the efficacy of the treatment, with the i.p. route providing the most significant (87%) protection. Watanabe et al. (2007) examined the efficacy of bacteriophage by using a gut-derived sepsis model caused by *Pseudomonas aeruginosa* in mice. Oral administration of a newly isolated lytic phage strain (KPP10) significantly protected mice against mortality with survival rates, 66.7% for the phage-treated group as compared to 0% survival in saline treated control group. Mice treated with phage also had significantly lower numbers of viable *Pseudomonas aeruginosa* cells and lower level of inflammatory cytokines (tumor necrosis factor alpha TNF-α, interleukin-1ß [IL-1ß], and IL-6) in their blood and different organs such as liver and spleen.

In recent years the phage therapy has received lot of attention due to an increase in the prevalence of antibiotic resistant strains in clinical settings. A numbers of recent experimental studies have proved the efficacy of phages in treating different infections. Chhibber & co workers (2008) had reported the therapeutic potential of phage SS in treating

Klebsiella pneumoniae induced respiratory infection in mice. A single intraperitoneal injection of (MOI of 200) phage (SS) administered immediately after i.n. challenge was sufficient to rescue 100% of animals from *K. pneumoniae*-mediated respiratory infections. The use of lytic bacteriophages to rescue septicemic mice with multidrug-resistant (MDR) *Pseudomonas aeruginosa* infection was evaluated (Vinodkumar et al., 2008). A single i.p. injection of 10^9 PFU of the phage strain, administered 45 min after the bacterial challenge (10^7), was sufficient to rescue 100% of the animals. Malik & Chhibber (2009) investigated the protective effect of *K. pneumoniae*–specific bacteriophage KØ1 isolated from the environment in a mouse model of burn wound infection caused by *K. pneumoniae*. A substantial decrease in the bacterial load of blood, peritoneal lavage, and lung tissue was noted following treatment with the bacteriophage preparation. Recently in other studies, workers have successfully employed well characterized phages to treat burn wound infection induced by *Klebsiella pneumoniae* in mice. In this study, a single dose of phages, intraperitoneally (i.p.) at an MOI of 1.0, resulted in significant decrease in mortality, and this dose was found to be sufficient to completely cure *K. pneumoniae* infection in the burn wound model. Maximum decrease in bacterial counts in different organs was observed at 72 hours post infection (Kumari et al., 2009). Kumari and co- workers (2010) evaluated the therapeutic potential of a well characterized phage Kpn5 in treating burn wound infection in mice as a single topical application of this phage was able to rescue mice from infection caused by *K. pneumoniae* B5055 in comparison to multiple applications of honey and Aloe vera gel (Kumari et al., 2010). Recently, Kumari and co-workers (2011) evaluated the efficacy of silver nitrate and gentamicin in the treatment of burn wound infection and compared it with phage therapy using an isolated and well-characterized *Klebsiella* -specific phage, Kpn5. Phage Kpn5 mixed in hydrogel was applied topically at an MOI of 200 on the burn wound site. The efficacy of these antimicrobial agents was assessed on the basis of percentage survival of infected mice following treatment. The results showed that a single dose of phage Kpn5 resulted in a significant reduction in mortality ($P<0.001$) as compared to daily application of silver nitrate and gentamicin (Kumari et al., 2011).

4.1.3 Phages in the eradication of biofilms

Biofilms are densely packed communities of microorganisms growing on a range of biotic and abiotic surfaces and surround themselves with secreted extracellular polymer (EPS). Many bacterial species form biofilms and it is an important bacterial survival strategy. Biofilm formation is thought to begin when bacteria sense environmental conditions that trigger the transition to life on a surface. The structural and physiological complexity of biofilms has led to the idea that they are coordinated and cooperative groups, analogous to multicellular organisms (Passerini et al., 1992). In humans biofilms are responsible for many pathologies, most of them associated with the use of medical devices. A major problem of biofilms is their inherent tolerance to host defences and antibiotic therapies. Therefore there is an urgent need to develop alternative ways to prevent and control biofilm-associated clinical infections (Azeredo & Sutherland, 2008). Bacteriophages have been suggested as effective antibiofilm agents (Donlan, 2009). Use of indwelling catheters was often compromised as a result of biofilm formation. Curtin and Donlan (2006) investigated if hydrogel-coated catheters pretreated with coagulase negative bacteriophage would reduce *Staphylococcus epidermidis* biofilm formation. In our laboratory**,** efficacy of bacteriophage was assessed alone or in combination with amoxicillin, for the eradication of biofilm produced by *Klebsiella pneumoniae* B5055 (Bedi et al., 2009). Similarly Verma *et. al*. (2009) also evaluated the efficacy of lytic bacteriophage KPO1K2 alone or in combination with another antibiotic, ciprofloxacin for eradicating the biofilm of *Klebsiella pneumoniae in vitro* (Verma et al., 2009). Despite the efficacy of antibiotics as well as bacteriophages in the treatment of bacterial infections, their role in treatment of biofilm associated infections is still under consideration especially in case of older biofilms. The ability of bacteriophage and their associated polysaccharide depolymerases was investigated to control enteric biofilm formation. The action of combined treatments of disinfectant and phage enzyme as a potentially effective biofilm control strategy was evaluated and the results showed that the combination of phage enzyme and disinfectant was found to be more effective than either of these when used alone (Tait et al., 2002). Since age of biofilm is a decisive factor in determining the outcome of antibiotic treatment, in one recent study, biofilm of *K. pneumoniae* was grown for extended periods and treated with ciprofloxacin and/or depolymerase producing lytic bacteriophage (KPO1K2). The reduction in bacterial numbers of older biofilm was greater after application of the two agents in combination as ciprofloxacin alone could not reduce bacterial biomass significantly in older biofilms (Verma et al., 2010).

4.2 Phage products or phage lysins

With the increasing worldwide prevalence of antibiotic resistant bacteria, bacteriophage endolysins represent a very promising novel alternative class of antibacterial in the fight against infectious disease. Pathogenic bacteria are increasingly becoming resistant to antibiotics. For nearly a century, scientists have attempted to treat bacterial infections with whole phages. Vincent Fischetti (1940) was the first, however, to focus on the deadly weapons, the potent and specific enzymes called lysins produced by these viruses. These lysins create lethal holes in bacterial cell walls. Fischetti has identified lysins that can kill a wide range of Gram-positive pathogenic bacteria, and have proven their effectiveness in both preventing and treating infections in mice, an important step towards their potential application in human disease (Fischetti, 2008). As an alternative to "classic" bacteriophage therapy, in which whole viable phage particles are used, one can also apply bacteriophage-encoded lysis-inducing proteins, either as recombinant proteins or as lead structures for the development of novel antibiotics. Phage endolysins, or lysins, are enzymes that damage the cell walls' integrity by hydrolyzing the four major bonds in its peptidoglycan component (Loessner et al., 1997; Lopez et al., 2004). A number of studies have shown the enormous potential of the use of phage endolysins, rather than the intact phage, as potential therapeutics. The great majority of human infections such as viral or bacterial start at a mucous membrane site (upper and lower respiratory, intestinal, urogenital, and ocular) which are the reservoir for many pathogenic bacteria found in the environment (i.e., pneumococci, staphylococci, streptococci), many of which are reported to be resistant to antibiotics (Young, 1994). Therefore, various animal models of mucosal colonization were used to test the efficacy of phage lysins to kill organisms on these surfaces. An oral colonization model was developed for prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a purified C1 phage lysin C1 (Nelson et al., 2001). Phage lytic enzymes have recently been proposed for the reduction of nasopharyngeal carriage of *S. pneumoniae* (Loeffler et al., 2001, 2003). In both these cases, when the animals were colonized with their respective bacteria and treated with a small amount of lysin specific for the colonizing organism, the animals were found to be free of colonizing bacteria two to five hours after lysin treatment. Group B streptococci are the leading cause of neonatal meningitis and sepsis all over the world. A vaginal model for group B streptococci was established to remove colonization of the vagina and oropharynx of mice with a phage lysin (named PlyGBS). A single dose of PlyGBS significantly reduced bacterial colonization in both the vagina and oropharynx (Cheng et al., 2005). These results support the idea that such enzymes may be used in specific high-risk populations to control the reservoir of pathogenic bacteria and therefore control the disease. These phage enzymes are so efficient in killing pathogenic bacteria that they may be considered as valuable tools in controlling biowarfare bacteria. To determine the feasibility of this approach, Schuch and co workers (2002) identified a lytic enzyme PlyG from the gamma phage that is specific for *Bacillus anthracis.* This approach may be used in post-exposure cases of anthrax, in which individuals can be treated intravenously with PlyG to control the bacilli entering the blood after germination because higher doses of phage lysin or multiple doses will result in nearly 100% protection. Recently, antimicrobial therapy of recombinant Cpl-1, a phage lysin specific for *Streptococcus pneumoniae* was reported to be effective in experimental pneumococcal meningitis using infant Wistar rats (Grandgirard et al., 2008).

5. Phage application in food industry

Food contamination is a serious issue because it results in foodborne diseases. Food contamination can be microbial or environmental, with the former being more common. Meat and poultry can become contaminated during slaughter through cross-contamination from intestinal fecal matter. Similarly, fresh fruits and vegetables can be contaminated if they are washed using water contaminated with animal manure or human sewage. During food processing, contamination is also possible from infected food handlers. Food contamination usually causes abdominal discomfort and pain, and diarrhea, but symptoms vary depending on the type of infection. At the present time, the leading causes of death due to foodborne bacterial pathogens are *Listeria* and *Salmonella*, followed closely by other foodborne pathogens such as *Escherichia coli* (*E. coli* O157:H7, in particular) and *Campylobacter jejunii*. Bacteriophages may provide a natural, non-toxic, safe, and effective means for significantly reducing or eliminating contamination of foods with specific pathogenic bacteria, thereby eliminating the risk, or significantly reducing the magnitude and severity, of foodborne illness caused by the consumption of foods contaminated with those bacteria (Meadet et al., 1999; Atturbury et al., 2003). The effectiveness of phage administration for the control of fish diseases and for food disinfection has also been documented. Nakai and co-workers (1999) and some other workers succeeded in saving the lives of cultured fish challenged by *Lactococcus garvieae* and *Pseudomonas plecoglossicida*, which are fish pathogens (Nakai et al., 1999; Nakai & Park, 2002; Park & Nakai, 2003). The need for control of pathogens during the manufacture of food is reflected by the incidence of foodborne bacterial infections. The use of phage or phage products in food production has recently become an option for the food industry as a novel method for biocontrol of unwanted pathogens, enhancing the safety of especially fresh and ready-to-eat food products (Hagens & Loessner, 2010). Phages were also shown to be effective for the elimination of food poisoning pathogens such as *Listeria monocytogenes* (Leverentz et al., 2003), *Campylobacter jejuni* (Atterbury et al., 2003) and *Salmonella* spp. (Leverentz et al., 2001; Goode et al., 2003) from the surface of foods. The bacterial spot pathogen of tomato plants, *Xanthomonas campestris* pv. *vesicatoria* was successfully controlled with bacteriophage (Flaherty et al., 2000).

6. Phages as antibacterial nanomedicines

Nowadays, apart from phage therapy, phages are also being used for phage display, DNA vaccine delivery, therapeutic gene delivery and bacterial typing Recently whole bacteriophage was constructed by fusing immunogenic peptides to modified coat proteins, which was found to be highly efficient DNA vaccine delivery vehicle (phage-display vaccination). Similarly the other approach has been incorporation of a eukaryotic promoter-driven vaccine gene within the phage genome (phage DNA vaccination) (Clark & March, 2006; Gao et al., 2010). Bacteriophages (phages) have been used for about two decades as tools for the discovery of specific target-binding proteins and peptides, and for almost a decade as tools for vaccine development. Drug-carrying phage represents a versatile therapeutic nanoparticle which because of tailoring of its coat can be equipped with a targeting moiety, and its massive drug-carrying capacity may become an important general targeting drug-delivery platform. In comparison to particulate drug-carrying devices, such as liposomes or virus-like particles, the arrangement of drug that is conjugated in high density on the external surface of the targeted particle is unique. A dense coating of the phage with aminoglycosides and other drugs might produce advantages that have been regarded as challenges in the application of phages as therapeutic agent. Most important issue in this field is the immunogenicity of bacteriophages on *in vivo* administration. This problem can be tackled as it has been shown that drug-carrying phages are hardly recognized by commercial antiphage antibodies and generate significantly lower antiphage antibody titers when used to vaccinate mice (in comparison to ‘naked' phages). Filamentous bacteriophages are the workhorse of antibody engineering and are gaining increasing importance in nanobiotechnology because of its nanometric dimentions (Yacoby et al., 2007). Vaks and Benhar (2011) described a new application in the area of antibacterial nanomedicines where antibody targeted, chloramphenicol drug loaded filamentous phage (M13) was used for inhibiting the growth of *Staphylococcus aureus* bacteria. Systemic administration of chemotherapeutic agents, in addition to its anti-tumor benefits, results in indiscriminate drug distribution and severe toxicity. Therefore to solve this problem, Bar and co workers (2008) used targeted anti-cancer therapy in the form of targeted drug-carrying phage nanoparticles. The bacteriophages are also being currently evaluated for their biosensor potential. In a recent study it has been proposed to develop a unique and innovative biosensor based on induced luminescence of captured Biowarfare bacterial agents and organic light emitting diode (OLED) technology. The system would use array of bacteriophage engineered to express fluorescent protein in infected Biowarfare agents (Gooding, 2006). The specificity of the phage provides capture of only targets of interest, while the infection of the bacteria and natural replication of the expressed protein will provide the detection signal. Using novel OLED arrays, a phage array chip can be constructed similar to DNA chips for multianalyte detection.

7. Conclusion

Phage therapy for eliminating multidrug resistant bacteria is gaining importance. The abundance of phages in the environment makes it a relatively simple task to isolate phages against any given pathogen which can be characterized using a series of known protocols. The timescale and costs for the development of a new phage(s) for therapy will be a fraction of those for introducing a new antibiotic. Currently, many pathogenic bacteria have acquired multiple drug resistance, which is a serious clinical problem. Phages, when properly selected, offer the most cost-effective alternative to antibiotics. These have proved to be efficient in bacterial elimination on single application and recently accepted for food treatment as well to counter food contamination during storage. Phages should be essentially free of contaminating bacterial toxin and also capable of evading the clearance by reticulendothelial system. Although some problems remain to be solved, many experts are of the opinion that phage therapy will find a niche in modern Western medicine in the future. Phage lytic enzymes have a broad application in the treatment of bacterial diseases. Whenever there is a need to kill bacteria, phage enzymes may be freely utilized. They may be used not only to control pathogenic bacteria on human mucous membranes, but may find application in the food industry to control disease causing bacteria. Phage lytic enzymes have yet to be exploited. Because of the serious problems of resistant bacteria in hospitals, day care centers, and nursing homes, particularly *staphylococci* and *pneumococci*, such enzymes may be of immediate benefit in these environments.

8. References

Abdul-Hassan, H.S.; El-Tahan, K.; Massoud, B. & Gomaa, R. (1990). Bacteriophage therapy of Pseudomonas burn wound sepsis. *Annals Mediterranean Burn Club,* Vol.3, pp 262-4, ISSN 1592-9566

Alisky, J.; Iczkowski, K.; Rapoport, A. & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *Journal of Infection,* Vol.36, pp 5-15, ISSN 0163-4453

Ashelford, K.E.; Norris, S.J.; Fry, J.C.; Bailey, M.J. & Day, M.J. (2000). Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. *Applied Environmental Microbiology,* Vol.66, pp ISSN 4193 – 4199 1098-533

Atterbury, R.J.; Connerton, P.L.; Dodd, C.E.; Rees, C.E. & Connerton, I.F., (2003). Isolation and characterization of Campylobacter bacteriophages from retail poultry. *Applied Environmental Microbiology,* Vol.69, pp 4511–4518, ISSN 1462-2920

Azeredo, J. & Sutherland, I.W. (2008). The use of phages for the removal of infectious biofilms. *Current Pharmaceutical Biotechnology* Vol.9, No.4, pp 261-6, ISSN 1389-2010

Babalova, E.G.; Katsitadze, K.T.; Sakvarelidze, L.A.; Imnaishvili, N. S.; Sharashidze, T.G.; Badashvili, V. A.; Kiknadze, G. P.; Meipariani, A. N.; Gendzekhadze, N. D.; Machavariani, E. V.; Gogoberidze, K. L.; Gozalov, E. I. & Dekanosidze, N.G. (1968). Preventive value of dried dysentery bacteriophage. *Zhurnal Mikrobiologii Epidemiologii Immunobiologii*, Vol.2, pp 143–145, ISSN 0372-9311

Bachrach, G.; Leizerovici-Zigmond, M.; Zlotkin, A.; Naor, R. & Steinberg, D. (2003). Bacteriophage isolation from human saliva. *Letters in Applied Microbiology,* Vol.36, pp 50-53, ISSN 1365-2672

Bar, H.; Yacoby, I.& Benhar, I. (2008). Killing cancer cells by targeted drug-carrying phage nanomedicines. *BMC Biotechnology*, Vol.8, pp 37, ISSN 1472-6750

Barrow, P.; Lovell, M. & Berchieri, A. (1998). Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clinical and Diagnostic Laboratory Immunology*, Vol.5, pp 294 – 298, ISSN 1071-4138

Bedi, M.S.; Verma, V. & Chhibber, S. (2009.) Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. *World Journal of Microbiology and Biotechnology*, Vol.25, pp 1145–1151, ISSN 0959-3993

Benedict, L.R.N.; Flamiano, R.S. (2004). Use of bacteriophages as therapy for *Escherichia coli*-induced bacteremia in mouse models. *Philippines Journal of Microbiology and Infectious Diseases*, Vol.33, No.2, pp 47 – 51, ISSN 0115-0324

Bergh, O.; Borsgeim, G.; Bratbak, S. & Heldal, M. (1989). High abundance of viruses found in aquatic environments. *Nature*, Vol.340, pp 467-468, ISSN 0028-0836

Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A., Powell, B., Carlton, R., Merril, C. 2002. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium. *Infection and Immunity.* Vol. 70, pp 204-210, ISSN 1098- 5522

Bogovazova, G.G.; Voroshilova, N.N.;& Bondarenko, V.M. (1991). The efficacy of *Klebsiella pneumoniae* bacteriophage in the therapy of experimental Klebsiella infection. *Zhurnal Mikrobiologii Epidemiologii Immunobiologii*, Vol.4, pp 5 – 8, ISSN 0372-9311

Borysowski, J. & Gorski, A. (2008). Is phage therapy acceptable in the immunocompromised host?. *International Journal of Infectious diseases*, Vol.12, pp 466 – 471, ISSN 1201-9712

Bradbury, J. (2004). ''My enemy's enemy is my friend'': using phages to fight bacteria. *Lancet*, Vol.363, pp 624 – 625, ISSN 0140-6736

Brussow, H. & Hendrix, R.W. (2002). Phage genomics: small is beautiful. *Cell,* Vol.108 pp 13 -16, ISSN 0092-8674

Bruynoghe, R. &, Maisin J. (1921). Essais de thérapeutique au moyen du bactériophage du Staphylocoque. *Comptes Rendus des Séances et Mémoires de la Société de Biologie,* Vol.85, pp 1120-1, ISSN 0037-9026

Carlton, R. (1999). Phage therapy: past history and future prospects. *Archivum Immunologiae et Therapiae Experimentalis*, Vol.47, No.5, pp 267-274, ISSN 0004-069X

Carlton, R.M.; Noordman, W.H.; Biswas B. et al*.,* (2005). Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology*, Vol.43, pp 301 – 312, ISSN: 0273-2300

Cheng, Q.; Nelson, D.; Zhu, S. & Fischetti, V.A. (2005). Removal of group B streptococci colonizing the vagina and oropharynx of mice with a bacteriophage lytic enzyme. *Antimicrobial Agents and Chemotherapy*, Vol.49, pp 111–117, ISSN 1098-6596

Chhibber, S.; Kaur, S.; & Kumari, S. (2008). Therapeutic potential of bacteriophage in treating *Klebsiella pneumoniae* B5055-mediated lobar pneumonia in mice. *Journal of Medical Microbiology*, Vol.57, pp 1508 -1513, ISSN 0022-2615

Cislo, M.; Dabrowski, M.; Weber-Dabrowska, B. & Woyton, A. (1987). Bacteriophage treatment of suppurative skin infections. *Archivum Immunologiae et Therapiae Experimentalis*, Vol.2, pp 175–183, ISSN 0004-069X

Clark, J.R. & March, J.B. (2006). Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *Trends in Biotechnology*, Vol.24, pp 212–218, ISSN 0167-7799

Curtin J. J. & Donlan, R.M. (2006). Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis. Antimicrobial Agents and Chemotherapy*, Vol.50, No.4, pp 1268–1275, ISSN 1098-6596

Dabrowska, K.; Swita a-Jelen, K., Opolski, A.; Weber-Dabrowska, B. & Gorski, A. (2005). Bacteriophage penetration in vertebrates. *Journal of Applied Microbiology*, Vol.98, pp 7-13, ISSN 1365-2672

Donlan, R.M. (2009). Preventing biofilms of clinically relevant organisms using bacteriophage. *Trends in Microbiol*ogy, Vol.17, pp 66–72, ISSN 0966-842X

Duckworth, D. & Gulig, P. (2002). Bacteriophage: potential treatment for bacterial infections. *Biodrugs*, Vol 16, pp 57 – 62, ISSN 1179-190X

Fischetti, V.A. (2008). Bacteriophage lysins as effective antibacterials. *Current Opinion in Microbiology*, Vol.11, pp 393 – 400, ISSN 1369-5274

Fischetti, V.A.: Nelson, D. & Schuch, R. (2006). Reinventing phage therapy: are the parts greater than the sum? *Nature Biotechnology*, Vol.24, pp 1508 -1511, ISSN 1087-0156

Flaherty, J. E.; Jones, J.B.; Harbaugh, B .K.; Somodi, G .C. & Jackson, L.E. (2000). Control of bacterial spot on tomato in the greenhouse and field with h-mutant bacteriophages. *Bioscience,* Vol. 35, pp 882–888, ISSN 0006-3568

Gao, J.; Wang, Y.; Liu, Z. & Wang, Z. (2010). Phage display and its application in vaccine design. *Annals of Microbiology*, Vol. 60, pp 13-19, ISSN 1590-4261

Geier, M.R.; Attallah, A.F. & Merril, C.R. (1975). Characterization of *Escherichia coli* Bacterial viruses in commercial sera. *In Vitro,* Vol.11, pp 55 – 78, ISSN 1054-5476.

Goode, D.; Allen, V.M. & Barrow, P.A. (2003). Reduction of Experimental Salmonella and Campylobacter Contamination of Chicken Skin by Application of Lytic Bacteriophages. *Applied and Environmental Microbiology,* Vol.69, pp 5032-5036, ISSN 0099-2240.

Gooding, J.J. (2006). Biosensor technology for detecting biological warfare agents: Recent progress and future trends. *Analytica Chemica Acta*, Vol.559, pp 137-151, ISSN 0003-2670.

Grandgirard, D.; Loeffler, J.M.; Fischetti, V.A. & Leib, S.L. (2008). Phage lytic enzyme cpl-1 for antibacterial therapy in experimental pneumococcal meningitis. *Journal of Infectious Diseases,* Vol.197, pp 1519–1522, ISSN 1537-6613

Hagens, S. & Blasi, U. (2003). Genetically modified filamentous phage as bactericidal agents: a pilot study. *Letters in Applied Microbiol*ogy, Vol.37, pp 318 – 323, ISSN 1472-765X

Hagens, S. & Loessner, M.J. (2010). Bacteriophage for Biocontrol of Foodborne Pathogens: Calculations and Considerations. *Current Pharmaceutical Biotechnology*, Vol.11, pp 58-68, ISSN 1389-2010

Hagens, S.; Habel, A.; von Ahsen, U.; von Gabain, A. & Blasi, U. (2004). Therapy of experimental *Pseudomonas* infections with a nonreplicating genetically modified phage. *Antimicrobial Agents and Chemotheapy,* Vol.48, pp 3817 – 3822, ISSN 1098-6596.

Hanlon, G.W. (2007). Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *International Journal of Antimicrobial Agents*, Vol.30, pp 118–28, ISSN 0924 -8579

Hendrix, R.W. (2002). Bacteriophages: evolution of the majority. *Theoretical Population Biology*, *Vol.*61, pp 471 – 480, ISSN 0040-5809

Ho, K. (2001). Bacteriophage therapy for bacterial infections. Rekindling a memory from the pre-antibiotics era. *Perspective in Biology and Medicine* , Vol.44, pp 1 -16, ISSN 1529-8795

Inal, J. (2003). Phage therapy: a reappraisal of bacteriophages as antibiotic. *Archivum Immunologiae et Therapiae Experimentalis*, Vol.51, pp 237-244, ISSN 0004-069X

Ioseliani, G.D.; Meladze, G.D.; Chkhetiia, N.S.; Mebuke, M.G. & Kiknadze, N.I. (1980). Use of bacteriophage and antibiotics for prevention of acute postoperative empyema in chronic suppurative lung diseases. Grudnaia khirurgiia, Vol.6, pp 63 – 67, ISSN 0017-4866

Jamalludeen, N.; Johnson, R.P.; Shewen, P.E. & Gyles. C. L. (2009). Evaluation of bacteriophages for prevention and treatment of diarrhea due to experimental enterotoxigenic *Escherichia coli* O149 infection of pigs. *Veterinary Microbiology*, 136, pp 135–141, ISSN 0378-1135

Jikia, D.; Chkhaidze, N.; Imedashvili, E.; Mgaloblishvili, I.; Tsitlanadze, G.; Katsarava, R.; Morris, J.G. & Sulakvelidze, A. (2005). The use of a novel biodegradable preparation capable of the sustained release of bacteriophages and ciprofloxacin, in the complex treatment of multidrug-resistant *Staphylococcus aureus*-infected local radiation injuries caused by exposure to Sr90. Clinical and Experimental Dermatology, Vol.30, pp 23-26, ISSN 1365-2230.

Kaczkowski, H.; Weber-Dabrowska, B.; Dabrowski, M.; Zdrojewicz, Z. & Cwioro, F. (1990). Use of bacteriophages in the treatment of chronic bacterial diseases. Wiadomosci Lekarskie, Vol.43, pp 136–141, ISSN 0860-8865

Kropinski, A.M. (2006). Phage therapy – everything old is new again. *Canadian Journal of Infectious Diseases and Medical Microbiology*, Vol.17, pp 297 – 306, ISSN 1712-9532

Kucharewicz-Krukowska, A. & Slopek S. (1987). Immunogenic effect of bacteriophage in patients subjected to phage therapy. *Archivum Immunologiae et Therapiae Experimentalis, Vol* 35, No. 5, pp 553–61 ISSN 0004-069X

Kudva, I.T.; Jelacic, S.; Tarr, P.I.; Youderian, P. & Hovde, C.J. (1999). Biocontrol of *E.coli* 0157 with 0157-specific bacteriophages. *Applied and Environmental Microbiology*, Vol.65, pp 3767 – 3773, ISSN 1098-5336

Kumari, S.; Harjai, K. & Chhibber, S. (2009). Efficacy of bacteriophage treatment in murine burn wound infection induced by *Klebsiella pneumoniae. Journal of Microbiology and Biotechnology*, Vol.19, No. 6, pp 622 – 628, ISSN 1738-8872

Kumari, S.; Harjai, K. & Chhibber, S. (2010). Topical treatment of *Klebsiella pneumoniae* B5055 induced burn wound infection in mice using natural products. *Journal of Infection in Developing Countries*, Vol.4, No.6, pp 367-377, ISSN 1972-2680

Kumari, S.; Harjai, K.& Chhibber, S. (2011). Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by *Klebsiella pneumoniae* B5055. *Journal of Medical Microbiology*, 60, pp 205-210. 0022-2615

Kwarcinski, W.; Lazarkiewicz, B.; Weber-Dabrowska, B.; Rudnicki, J.; Kaminski, K. & Sciebura, M. (1994). Bacteriophage therapy in the treatment of recurrent subphrenic and subhepatic abscess with jejunal fistula after stomach resection. Polski tygodnik lekarski, Vol.49, pp 535, ISSN 0032-3756

Kysela, D.T. & Turner, P.E. (2007). Optimal bacteriophage mutation rates for phage therapy. *Journal of Theoretical Biology*, Vol. 249, pp 411–421, ISSN 0022-5193

Leszczynski, P.; Weber-Dabrowska, B.; Kohutnicka, M.; Luczak, M. & Gorski, A. (2006). Successful eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) intestinal carrier status in a healthcare worker--case report. *Folia Microbiolica*, Vol.51, pp 236-8, ISSN 0015-5632

Leverentz, B.; Conway, W. S.; Camp, M. J.; Janisiewicz, W. J.; Abuladze, T., Yang, M.; Saftner, R. & Sulakvelidze, A. (2003). Biocontrol of Listeria monocytogenes on Fresh-Cut Produce by Treatment with Lytic Bacteriophages and a Bacteriocin. *Applied and Environmental Microbiology*, Vol.69, pp 4519-4526, ISSN 1098-5336

Leverentz, B.; Conway, W.S.; Alavidze, Z.; Janisiewicz, W.J.; Fuchs, Y.; Camp, M.J.; Chighladze, E. & Sulakvelidze, A. (2001). Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *Journal of Food Protection*, Vol.64, pp 1116 – 1121, ISSN 0362-028X

Livermore, D.H. (2004). The need for new antibiotics. *Clinical Microbiology and Infection*, Vol.10 (Suppl 4), pp 1 – 9, ISSN 1469-0691

Loeffler, J.M.; Djurkovic, S. & Fischetti, V.A. (2003). Phage Lytic Enzyme Cpl-1 as a Novel Antimicrobial for Pneumococcal Bacteremia. *Infection and Immunity* pp 6199–6204, ISSN 1098-5522

Loeffler, J.M.; Nelson, D. & Fischetti, V.A. (2001). Rapid killing of Streptococcus pneumoniae with a bacteriophage cell wall hydrolase. *Science*, Vol.294, pp 2170–2172, ISSN 1095-9203.

Loessner, M.J.; Maier, S.K.; Daubek-Puza, H.; Wendlinger, G. & Scherer, S. (1997). Three *Bacillus cereus* bacteriophage endolysins are unrelated but reveal high homology to cell wall hydrolases from different bacilli. *Journal of Bacteriology*, Vol.179, pp 2845–2851, ISSN 1098-5530

Lopez, R.; Garcia, E. & Garcia, P. (2004). Enzymes for anti-infective therapy: phage lysins. *Drug Discovery Today*, Vol.1, No.4, pp 469 – 474, ISSN 1740-6773

Lorch, A. (1999). "Bacteriophages: An alternative to antibiotics?" *Biotechnology and Development Monitor*, Vol.39, pp 14 -17, ISSN 0924-9877

Malik, R. & Chhibber, S. (2009). Protection with bacteriophage KØ1 against fatal Klebsiella pneumoniae–induced burn wound infection in mice. *Journal of Microbiology Immunology and Infection*, Vol.42, pp 134-140, ISSN 1684-1182

Markoishvili, K.; Tsitlanadze, G.; Katsarava, R.; Morris, G. & Sulakvelidze, A. (2002). A novel sustained-release matrix based on biodegradable poly (esteramide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. *Internation Journal of Dermatology*, Vol.41, pp 453 – 458, ISSN 0011-9059

Marza, J.; Soothill, J.; Boydell, P. & Collyns, T. (2006). Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. *Burns*, Vol.32, pp 644 – 646, ISSN 0305-4179

Mathur, M.D.; Bidhani, S. & Mehndiratta, P.L. (2003). Bacteriophage therapy: an alternative to conventional antibiotics. *Journal of Association of Physicians of India*, Vol.51, pp 593 – 596, ISSN 0004-5772

Matsuzaki, S.; Rashel, M.; Uchiyma, J.; Ujihara, T.; Kuroda, M.; Ikeuchi, M.; Fujieda, M.; Wakiguchi, J. & Imai, S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *Journal of Infection and Chemotherapy*, Vol.11, pp 211 – 219, ISSN 1437-7780

Matsuzaki, S.; Yasuda, M.; Nishikawa, H.; Kuroda, M.; Ujihara, T.; Shuin, T.; Shen, Y.; Jin, Z Fujimoto, S.; Nasimuzzan, M.D.; Wakiguchi, H.; Sugihara, S.; Sugiura, T.; Koda, S.; Muraoka, A. & Imai. S. (2003). Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage ΦMR11. *Journal of Infectious Diseases*, Vol.187, pp 613 – 624, ISSN 0022-1899

Mattey, M. & Spencer, J. (2008). Bacteriophage therapy - cooked goose or Phoenix rising? *Current Opinion in Biotechnology*, Vol.19, pp 1 – 5, ISSN 0958-1669

McVay, C.; Velasquez, S.M.; & Fralick, J.A. (2007). Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.6, pp 1934 -1938, ISSN 1098-6596

Mead, P.S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M. & Tauxe, R.V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases,* Vol.5, No.5, pp 607-25, ISSN 1080-6059

Merabishvili, M.; Pirnay, J.P.;; Verbeken, G.; Chanishvili, N.; Tediashvili, M.; Lashkhi, N.; Glonti, T.; Krylov, V. et al. (2009) Quality-controlled small-scale production of a well- defined bacteriophage cocktail for use in human clinical trials. PLoS ONE Vol.4, pp e4944, ISSN 1932-6203

Merril, C.R.; Friedman, T.B., Attallah, A.F.M.; Geier, M.R.; Krell, K. & Yarkin, R. (1972). Isolation of bacteriophages from commercial sera. *In Vitro*, Vol.8, pp 91 – 93, ISSN 1071-2690.

Merril, C.R.; Biswas, B.; Carlon, R.; Jensen, N.C.; Creed, G.J.; Zullo, S.; & Adhya, S. (1996). Long-circulating bacteriophage as antibacterial agents. *Proceedings of the National Academy of Sciences of the United States of America* Vol.93, pp 3188 – 3192, ISSN 0027-8424

Merril, C.R.; Scholl, D. & Adhya, S.L. (2003). The prospect for bacteriophage therapy in Western medicine. *Nature Reviews Drug Discovery*, Vol.2, pp 489–497, ISSN 1474-1776

Milch, H. & Fornosi, F. (1975). Bacteriophage contamination in live poliovirus vaccine. *Journal of Biological Standardization*, Vol. 3, pp 307 – 310, ISSN 0092-1157

Moradpour, Z. & Ghasemian, A. (2011). Modified phages: Novel antimicrobial agents to combat infectious diseases. *Biotechnology Advances*, Vol.29, pp 732–738, ISSN 0734-9750

Mushtaq, N.; Redpath, M.B.; Luzia, J.P. & Taylor, P.W. (2005). Treatment of experimental *Escherichia coli* infection with recombinant bacteriophage derived capsule depolymerase. *Journal of Antimicrobial Chemotherapy*, Vol.56, pp 160 -165, ISSN 0305-7453

Nakai, T. & Park, S.C. (2002). Bacteriophage therapy of infectious disease in aquaculture. *Research in Microbiology*, Vol.153, pp 13 – 8, ISSN 0923-2508

Nakai, T.; Sugimoto, R.; Park, K. H.; Matsuoka, S.; Mori, K.; Nishioka, T. & Maruyama, K. (1999). Protective effects of bacteriophage on experimental *Lactococcus graviae* infection in yellow tail. *Diseases of Aquatic Organisms*, Vol.37, pp 33-41, ISSN 0177-5103

Nelson, D.; Loomis, L. & Fischetti, V.A. (2001). Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. *Proceedings of the National Academy of Sciences of the United States of America*, *Vol.*98, pp 4107–4112, ISSN 0027-8424

Oliveira, A.; Sereno, R. & Azeredo, J. (2010). *In vivo* efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses. *Veterinary Microbiology*, Vol.146, No.(3-4), pp 303-308, ISSN 0378-1135.

Parisien, A..; Allain, B.; Zhang, J.; Mandeville, R. & Lan, C.Q. (2008). Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. *Journal of Applied Microbiology*, Vol.104, pp 1 -13, ISSN 1365-2672

Park, S.C., Nakai, T. (2003). Bacteriophage control of Pseudomonas plecoglossicida infection in ayu Plecoglossus altivelis. *Diseases of Aquatic Organisms,* Vol.53, pp 33–39, ISSN 0177-5103

Passerini, L.; Lam, K.; Costerton J.W. & King, E.G. (1992). Biofilms on indwelling vascular catheters. *Critical Care Medicine, Vol.* 20, pp 665-673, ISSN 1530-0293

Pavlenishvili, & Tsertsvadze, T. (1993). Bacteriophagotherapy and enterosorbtion in treatment of sepsis of newborns caused by gram-negative bacteria. *Pren Neon Infection*, Vol.11, pp 104, ISSN 0975-5241

Payne, R.J.H. & Jansen, V.A.A. (2000). Phage therapy: the peculiar kinetics of self replicating pharmaceuticals. *Clinical Pharmacology and Therapeutics*, Vol.68, pp 225-230, ISSN 0009-9236

Perepanova, T.S.; Darbeeva, O.S.; Kotliarova, G.A.; . Kondrat'eva, E.M.; Maiskaia, L.M.; Malysheva, V.F.; Baiguzina, F.A. & Grishkova, N.V. (1995). The efficacy of bacteriophage preparations in treating inflammatory urologic diseases. Urologica e Nefrologica, Vol.5, pp 14-17, ISSN 0393-2249

Pirisi, A. (2000). Phage therapy - advantages over antibiotics? *Lancet*, Vol.356, pp 1418, ISSN 0140-6736

Platt, R.; Reynolds, D.L. & Phillips, G.J. (2003). Development of a novel method of lytic phage delivery by use of a bacteriophage P22 site-specific recombination system". *FEMS Microbiology Letter*s, Vol.223, pp 259-265, ISSN 1574-6968

Proskurov, V.A. (1970). Use of staphylococcal bacteriophage for therapeutic and preventive purposes. *Zhurnal Mikrobiologii Epidemiologii Immunobiologii,* Vol.2, pp 104-107, ISSN 0372-9311

Rohwer, F. (2003). Global phage diversity. *Cell,* Vol.113, pp 141, ISSN 0092-8674

Sandeep, K. (2006). Bacteriophage precision drug against bacterial infections. *Current Science*, Vol.90, pp 361 – 363, ISSN 0011-3891

Schuch, R.; Nelson, D. & Fischetti, V.A. (2002). A bacteriolytic agent that detects and kills *Bacillus anthracis*. *Nature*, Vol 418, pp 884–889, ISSN 0028-0836

Shabalova, I.A.; Karpanov, N.I.; Krylov, V.N.; Sharibjanova, T.O. & Akhverdijan, V.Z. (1995). *Pseudomonas aeruginosa* bacteriophage in treatment of *P. aeruginosa* infection in cystic fibrosis patients, p. 443. *In* Proceedings of IX International Cystic Fibrosis Congress. International Cystic Fibrosis Association, Zurich, Switzerland.

Shasha, S.M.; Sharon, N. & Inbar, M. (2004). Bacteriophages as antibacterial agents. *Harefuah*, Vol.143, pp 121–125, ISSN 0017-7768

Skurnik, M. & Strauch, E. (2006). Phage therapy: facts and fiction. *International Journal of Medical Microbiology*, Vol.296, pp 5–14, ISSN 1438-4221

Skurnik, M.; Pajunen, M. & Kiljunen, S. (2007). Biotechnological challenges of phage therapy. *Biotechnology Letters*, Vol.29, pp 995 – 1003, ISSN 0141-5492

Slopek, S.; Durlakowa, I.; Weber-Dabrowska, B.; Kucharewicz-Krukowska, A.; Dabrowski, M. & Bisikiewicz, R. (1983). Results of bacteriophage treatment of suppurative bacterial infections. I. General evaluation of the results. *Archivum Immunologiae et Therapiae Experimentalis,* Vol.31, pp 267–291, ISSN 0004-069X

Slopek, S.; Kucharewicz-Krukowska, A.; Weber-Dabrowska, B. & Dabrowski M. (1985). Results of bacteriophage treatment of suppurative bacterial infections. V. Evaluation of the results obtained in children. *Archivum Immunologiae et Therapiae Experimentalis,* Vol.33, pp 241–259, ISSN 0004-069X

Slopek, S.; Durlakowa, I.; Weber-Dabrowska, B.; Dabrowski, M. & Kucharewicz-Krukowska, A. (1984). Results of bacteriophage treatment of suppurative bacterial infections. III. Detailed evaluation of the results obtained in a further 150 cases. *Archivum Immunologiae et Therapiae Experimentalis,* Vol.32, pp 317–335, ISSN 0004-069X

Smith H.W. & Huggins M.B. (1983). Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves, piglets and lambs. *Jouranl of General Microbiology*, Vol.129, pp 2659 – 2675, ISSN 0022-1287

Smith, H.W. & Huggins, M.B. (1982). Successful treatment of experimental *Escherichia coli* infections in mice using phages: its general superiority over antibiotics *Jouranl of General Microbiology*, Vol.128, pp 307 – 318, ISSN 0022-1287

Soothill J. (1994). Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa. Burns*, Vol.20, pp 209 – 211, ISSN 0305-4179

Stroj, L.; Weber-Dabrowska, B.; Partyka, K.; Mulczyk, M. & Wojcik, M. (1999). Successful treatment with bacteriophage in purulent cerebrospinal meningitis in a newborn. Neurologia i neurochirurgia polska, Vol.3, pp 693 – 698, ISSN 0028-3843

Sulakvelidze, A. & Morris, J.G. (2001). Bacteriophages as therapeutic agents. *Annals of Medicine*, Vol.33, pp 507 – 509, ISSN 1365-2060

Sulakvelidze, A.; Alavidze, Z. & Morris, J. (2001). Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy*, Vol.45, pp 649 – 659, ISSN 1098-6596

Tait, K.; Skillman, L.C. & Sutherland, I.W. (2002). The Efficacy of Bacteriophage as a method of biofilm eradication. *Biofouling,* Vol.18, No. 4, pp 305–311, ISSN 0892-7014

Tanji, Y.; Shimada, T.; Fukudomi, H.; Miyanaga, K.; Nakai, Y. & Unno, H. (2005). Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. *Journal of Bioscience and Bioengineering, Vol.*100, pp 280–287, ISSN 1389-1723

Tanji, Y.; Shimada, T.; Yoichi, M.; Miyanaga, K.; Hori, K. & Unno, H. (2004). Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Applied Microbiology and Biotechnology,* Vol.64, pp 270 – 274, ISSN 0175-7598

Teuber, M. (2001). Veterinary use and antibiotic resistance. *Current Opinion in Microbiology*, Vol.4, pp 493–499, ISSN 1369-5274

Thacker, P.D. (2003). Set a microbe to kill a microbe: drug resistance renews interest in phage therapy. *Journal of American Medical Association*, Vol.290, pp 3183 – 3185, ISSN 1538- 3598

Thiel, K. (2004). Old dogma, new tricks — 21st century phage therapy. *Nature Biotechnology*, Vol.22, pp 31-36, ISSN 1087-0156

Vaks, L. & Benhar, I. (2011). Antibacterial application of engineered bacteriophage nanomedicines: antibody-targeted, chloramphenicol prodrug loaded bacteriophages for inhibiting the growth of *Staphylococcus aureus* bacteria. *Methods in Molecular Biology*, Vol .72, pp 187-206, ISSN 1064-3745

Verma, V.; Harjai, K. & Chhibber, S. (2009). Restricting ciprofloxacin induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment. *Journal of Antimicrobial Chemotherapy*, *Vol.*64, pp 1212–1218, 0305-7453,

Verma, V.; Harjai, K. & Chhibber, S. (2010). Structural changes induced by a lytic bacteriophage make ciprofloxacin effective against older biofilm of *Klebsiella pneumoniae*. *Biofouling*, Vol.26, No. 6, pp 729-737, ISSN 0892-7014

Vinodkumar, C.S.; Kalsurmath, S. & Neelagund Y.F. (2008). Utility of lytic bacteriophage in the treatment of multidrug-resistant *Pseudomonas aeruginosa* septicemia in mice. *Indian Journal of Pathology and Microbiology*, Vol.5, No. 3, pp 360 – 366, ISSN 0974-5130, ISSN 0377-4929

Vinodkumar, C.S.; Neelagund, Y.F. & Kalsurmath, S. (2005). Bacteriophage in the treatment of experimental septicemic mice from a clinical isolate of multidrug resistant *Klebsiella pneumoniae*. *Journal of Communicable Diseases*, Vol.37, No. 1, pp 18 – 29, ISSN 0019-5138

Wang, J.; Hu, B.; Xu, M.; Yan, Q.; Liu, S.; Zhu, X.; Sun, Z.; Reed, E.; Ding, L.; Gong, J.; Li, G.Q. & Hu, J. (2006). Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa. International Journal of Molecular Medicine*, Vol. 17, pp 309 - 317, ISSN 1107-3756.

Watanabe, R.; Matsumoto, T.; Sano, G.; Ishii, Y.; Tateda, K.; Sumiyama, Y.; Uchiyama, J.; Sakurai, S.; Matsuzaki, S.; Imai, S. & Yamaguchi. K. (2007). Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. *Antimicrobial Agents and Chemotherapy*, Vol 51, No. 2, pp 446 – 452, ISSN 1098-6596

Weber-Dabrowska, B., Dabrowski, M. & Slopek, S. (1987). Studies on bacteriophage penetration in patients subjected to phage therapy. *Archivum Immunologiae et Therapiae Experimentalis,* Vol.35, No.5, pp 563–68, ISSN 0004-069X

Weber-Dabrowska, B.; Mulczyk, M. & Górski, A. (2001). Bacteriophage therapy for infections in cancer patients. *Clinical and Applied Immunology Reviews*, Vol.1, pp 131–134, ISSN 1529-1049

Wills, Q.; Kerrigan, C. & Soothill, J. (2005). Experimental bacteriophage protection against *Staphylococcus aureus* abscesses in a rabbit model. *Antimicrobial Agents and Chemotherapy,* Vol.49, pp 1220 – 1221, ISSN 1098-6596

Wright, A.; Hawkins, C.H.; Anggard, E.E.& Harper, D.R. (2009). A controlled Quality-controlled small-scale production of a well- defined bacteriophage cocktail for use in human clinical trials. clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibi- otic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clinical Otolaryngology, Vol.34, pp 349–357, ISSN 1749-4486

Yacoby, I.; Bar, H. & Benhar, I. (2007). Targeted drug-carrying bacteriophages as antibacterial nanomedicines. *Antimicrobial Agents and Chemotherapy*, Vol.51, No. 6, pp 2156–63, ISSN 1098-6596

Young, R. (1992). Bacteriophage lysis: mechanism and regulation. *Microbiology and Molecular Biology Reviews*, Vol. 56, pp 430–481, ISSN 1092-2172.

Bacteriophages
Edited by Dr. Ipek Kurtboke

ISBN 978-953-51-0272-4
Hard cover, 256 pages
Publisher InTech

Published in print edition March, 2012

Bacteriophages have received attention as biological control agents since their discovery and recently their value as tools has been further emphasized in many different fields of microbiology. Particularly, in drug design and development programs, phage and prophage genomics provide the field with new insights. Bacteriophages reveals information on the organisms ranging from their biology to their applications in agriculture and medicine. Contributors address a variety of topics capturing information on advancing technologies in the field. The book starts with the biology and classification of bacteriophages with subsequent chapters addressing phage infections in industrial processes and their use as therapeutic or biocontrol agents. Microbiologists, biotechnologists, agricultural, biomedical and sanitary engineers will find Bacteriophages invaluable as a solid resource and reference book.

Sanjay Chhibber and Seema Kumari (2012). Application of Therapeutic Phages in Medicine, Bacteriophages, Dr. Ipek Kurtboke (Ed.), ISBN: 978-953-51-0272-4, InTech, Available from: http://www.intechopen.com/books/bacteriophages/therapeutic-bacteriophages

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821