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Review

Bacteriophage therapy revisited

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Phage therapy is a natural process of treating the bacterial infections of animals, plants and humans and it was used before the discovery and widespread of antibiotics. Even now this technology is widely used to increase the shelf life of fruits, vegetables, meat and harvested plants. In this mini review paper, we briefly describe the natural occurrence of bacteriophages; their discovery, history, emergence of phage therapy in the light of antibiotic resistance and their advantages over the traditional antibiotics. Preclinical studies of phage therapy in experimental animals/plants and its applications on the infectious diseases; wound infections emerged by the antibiotic resistant bacteria. Bacteriophage was first discovered by Ernest Hankin in 1896 from the water of Jumna river containing antibacterial substances which limited the spread of Cholera epidemics. In the initial stage of phage therapy, there were problems with the technology and now all these problems have been resolved by the advancement of science and technology. Now based on recent experience and results of bacteriophage applications against bacterial infections, this innovative technology for the treatment of various antibiotic resistant infections is successful.

Key words: Bacteriophage, phage therapy, food preservation, bacteriophage history, bacteriophage in agriculture, phage therapy human health**.**

INTRODUCTION

Amongst the kingdom of microorganisms, bacteriophages are highly abundant and chiefly concentrated in the niches of almost all natural environments on this planet (Breitbart et al., 2004). In nature they have been observed in open and coastal waters, marine sediments and particularly in terrestrial ecosystems such as soil. They are also commonly found in association with diverse higher organisms extending from insects to humans. This association is not only superficial, surface-mediated but extends within their bodies particularly the gastrointestinal tracts of humans, animals and insects (Ackermann, 2003). Conservative estimates of the prevalence of phages suggest an estimate of up to 10^{31} particles over the entire globe (Rohwer, 2003; Chibani-Chennoufi et al., 2004). This is equivalent to approximately 10^{9} mt in mass (Kropinski, 2006) and this value is ten times in excess of the bacterial mass (Strauch et al., 2007). Many phages are predators of bacteria (Rosamond and Allsop, 2000). Bacteriophage (phage) and the related viruses infect bacteria and thus obligate intracellular parasites that must multiply inside the prokaryotes by making use of some or all biosynthetic machinery of the host.

The anatomical features of a typical phage comprise of a head filled with genetic material, a syringe shaped tail and several fibers for the attachment to specific receptors on the surface of host bacteria. Phages bore into their relevance host bacteria and inject their genetic material in the form of either single stranded or double stranded nucleic acid genome which is enclosed in a protein or lipoprotein coat. For their replication in the host cells, phages contain yet unidentified lipolytic enzymes to facilitate the opening of the bacterial cell wall barrier and subsequently inject their DNA or RNA into the cytoplasm. The simplest phages code on average for 3-5 gene

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products, while the more complex phages can code for over 100 gene products. The variety of proteins and their amounts can vary significantly in different phage particles and the simplest phages have several copies of only one or two different proteins while more complex phages may have a markedly greater variation in the protein composition. These proteins function in both the infection process and act as a coat to protect their nucleic acid from nucleases in the environment. For entry into their host, phages have to negotiate, in the case of Gram-negative bacteria, two layers of lipid membranes typically separated by a peptidoglycan layer. In Gram-positive bacteria, two fused internal lipid bilayers and a thick peptidoglycan cell wall have to be traversed. The pene-tration may require specific entry enzymes, presently poorly characterized (Sulakvelidze et al., 2001; Kutter, 2005).

The proof that phages propagate at the expense of their host was first documented by (Ellis and Delbruck, 1939) who performed their classic one-step growth curve experiment. They showed that a single phage particle infected a bacterial cell, replicated inside the host until the viral progeny lysed the cell and was eventually released into the environment. This evidence was further substantiated in 1940 by Ruska who used the electron microscopy to observe phage particles (Pennazio, 2006). Depending on the species and conditions, each parent phage can produce approximately 100-300 progeny per lytic cycle from the targeted host cell.

These can further infect and kill a new generation of the target hosts and this exponential and cyclic replication continues until complete eradication of the targeted host ensues. In the absence of their targets, bacteriophages are simple non-living, inert coats of proteins containing their protected DNA. They can, nevertheless, be cannibalized by other microbes. Thus bacteriophages have the potential to kill bacteria that cause food spoilage, infectious diseases in humans as well as agriculturally important plants and animals (Carlton, 1999). Phages that inadvertently pene-trate into the bodies of higher organisms can be readily removed by normal physiological clearance process.

In this review, we briefly describe the history of bacteriophage therapy and their powerful applications in overcoming diverse microbial infections. We also discuss the future potential prospects of phage therapy in other applications.

HISTORY OF PHAGE DISCOVERY AND ITS USE AS THERAPY TOOL

The history of bacteriophage is quite controversial over claims of priority and discovery. A British bacteriologist, Ernest Hankin, (1896) portended that the water of the Ganges and Jumna rivers contained some unidentified antibacterial substance which was responsible for limiting the spread of cholera epidemics, endemic due to cremated bodies laid to rest in the holy waters. Two years later, the Russian bacteriologist Gamaleya observed similar occurrences while he was working with *Bacillus sublitis* as cited by (Samsygina and and Boni, 1984). About 20 years after the observations of Hankin; Frederick Tworts, a British medically-trained microbiologist and a superintendent of the Brown Institute for Animals, experimentally proved that the purported antibacterial agents passed through porcelain filters and had an obligatory need for bacteria for their growth (Twort, 1915). Unfortunately, he was unable to continue his work due to lack of funding from the Local Government Board and subsequent conscription in the World War I.

In 1910, D' Herelle revisited the bacteriophage phenomena, when he was studying the microbiological means for controlling an epizootic epidemic of locusts in Mexico. He went on further to conduct an interesting investigation in 1915 on an outbreak of haemorrhagic dysentery where he made a filtrate of the fecal samples from patients and mixed them with *Shigella* strains isolated from other patients. A portion of the mixture was spread on agar medium which upon a period of incu-bation yielded small cleared areas which he described as plaques (D'Herelle, 1917) and he presented his findings to the Academy of Sciences (Summers, 1999).

The potency and efficacy of the phage preparation was further confirmed in 1919 when D'Herelle treated and cured a 12 year old boy with severe bacterial dysentery and later went on to successfully treat three additional patients with ages ranging from 3 to 12 years, all of whom successfully recovered within 24 h of treatment (Ho K, 2001; Sulakvelidze et al., 2001). He extended his work on phage therapy in various hospital trials. Thus, in 1925, he treated four bubonic phage patients in Egypt and dramatically reduced the mortality rate of cholera patients in India to about 30% in hospital trials (Sulakvelidze and Kutter, 2005). However, his findings were not immediately published and the earlier reports in 1921 on application of phages to treat infectious diseases of humans took the precedence (Bruynoghe and Maisin 1921).

Surprisingly, in 1931, the Council for the Pharmacy and Chemistry of the American Medical Association concluded that the use of bacteriophage in the treatment of infections was unproductive. This contention seriously retarded the willingness of the medical research community in the USA to further explore the value of phage therapy (Lorch, 1999).

However, this technique was extensively used and continued to be developed in the former Soviet Union countries for about 80 years (Kropinski, 2006). It was then widely recognized by the medical community to reduce the mortality of soldiers during World War 1. In his critical review, Chanishvili et al. (2001) states that after the discovery of phages as curing agents against wound

contagions, the therapy could also overcome miscellaneous infections in areas of ophthalmology, dermatology, pediatrics, gynecology, urology, pulmonology and surgery. One such example of significant progress in phage therapy was at the Institute of Bacteriophage Microbiology and Virology in Tblisi, Georgia in the former Soviet State Union where research from 1923 on phage sensitivity of *Staphylococcus, Streptococcus, Proteus, Pseudomonas aeruginosa* and *Clostridium*. According to the review of (Alisky et al., 1998) the known 27 publications from 1966 to 1996 relating to bacteriophages, they originated from the Russian and Polish research laboratories. Their continued interest on bacteriophage therapy and treatment against infections of *Staphylococcus*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Shigella*, *Salmonella* and *Streptococcus* had shown 80 to 85% success rate. Unfortunately, the publications of most of these findings in languages other than English and in the former Eastern 'block' journals failed to make the impact of these important discoveries on the Western Scientific communities. This ideology in the West was further compounded by the assumption that the use of bacteriophages as an effective agent for treatment of clinical disorders was of little value as systemically introduced phages could be rapidly cleared from the body.

More importantly, the key discovery of antibiotics took precedence as a major and significant milestone in the history of medical science. However, the emergence of multi-antibiotic resistant bacteria by over prescription of antibiotics for treatment of bacterial infections and patient failure to complete their courses of antibiotic treatment have led to many bacteria carrying a broad range of antibiotic resistance genes (Adamia et al., 1990). Additionally, the antibiotic resistance traits amongst bacteria have spread further due to ease of horizontal exchange of the genetic material among bacteria. With respect to this wide-spread global bacterial resistance, a recent special Task Force co-chaired by the CDC, FDA and NIH stated that the world as in the pre-antibiotic era may soon be faced with previously treatable diseases becoming untreatable (Thiel, 2004).

THE EMERGENCE OF PHAGE THERAPY IN THE LIGHT OF ANTIBIOTIC RESISTANCE

Much concern has been aroused since the emergence of the antibiotic resistant bacteria such as methicillin resistant *Staphylococcus aureus (*MRSA), and the newly emerging strains of *Clostridium difficile.* Despite the fact that since 1998 to 2003 several new classes of antibiotics have been introduced to counter these bacteria but only nine new antibiotics have been approved by FDA and surprisingly, of these only two had a novel mode of action, a critical consideration in the battle against antibiotic resistance.

Indeed, we are living in an ever more crowded and interconnected world in which resistant strains of microorganisms find opportunistic avenues to spread very rapidly. Modern science has also reciprocally plunged forward in designing new drugs and vaccines that are highly effective countermeasures to the bacterial diseases in humans and animals.

However, production of new drugs is a financially excruciating exercise and is also very time consuming. In addition, market failures have discouraged the development of new vaccines in the private sector. As a result, the American Institute of Medicine (Smolinski et al., 2003) has proclaimed that the world now faces a serious challenge in dealing with antibiotic resistant bacteria that were once thought to be eradicated by antibiotics and thus, the use of bacteriophages to combat bacterial infections has rekindled the interest of the general public and scientific communities (Alisky et al., 1998; Carlton, 1999; Osborne, 2000; Merril et al., 2003; Wagenaar et al., 2005).

The potential applications of phage therapy are already being actively sought for bacterial disease treatment (Pirisi, 2000) and a number of studies have also substantiated the many advantages in the potential use of phage for treatment of infectious diseases in plants (Fox, 2000), animals (Barrow et al., 1998), and humans (Weber-Dabrowska et al., 2000; O'Flaherty et al., 2005) and extending the longevity of the shelf life of foods and harvested plant products (Table 4) likely to be deteriorated by bacteria.

THE ADVANTAGES OF PHAGE THERAPY OVER THE TRADITIONAL ANTIBIOTICS

Bacteriophages can be targeted to specific bacterial strains (Pirisi, 2000; Duckworth and Gulig, 2002; Weber-Dabrowska et al., 2003) and thus can targeted against pathogens or non beneficial bacteria in the gut without affecting the normal native bacterial flora of the body and thus spare patients from the chances of developing secondary infections (Chernomordik, 1998). Unlike the spread of antibiotic resistance from one strain to another, the high host-specificity of bacteriophages would alleviate this pattern of transmission. Nevertheless, it ought to be mentioned that the long-term usage of the same phage strain in the treatment may develop resistance.

Bacteriophages were considered to be safe during the long therapeutic history in Eastern Europe, former Soviet Union. Before antibiotic era so far, no major side effects have been reported with the exception of liberation of endotoxins from bacteria lysed by the bacteriophage therapy. Since bacteriophages are capable of exponential growth they can concomitantly accumulate and replicate at the site of infection and thus become available in abundance as a protectant. Phages have special advantages for topical applications because of their ability to penetrate deeper into the wound where the

infections are chronically incumbent. Bacteriophages are self-replicating as well as self-limiting with decrease in number of the specific bacteria concomitantly leading to their decrease and eventual elimination of both from the body without any harm (Abhilash et al., 2009). As the resistance of pathogenic organisms with antibiotics is increasing world widely and phage therapy may be an alternative to alleviate this risk. The therapeutic uses of bacteriophages in combination with antibiotics may be a more valuable approach (Skurnik and Strauch, 2006).

Unlike bacterial resistance to antibiotics, phages can mutate in step with evolving bacteria and if the bacteria become resistant to one phage, there is a natural abundance of phage species which can be targeted by other phages having a similar target range (Sulakvelidze et al., 2001).

Another major advantage of the bacteriophages as a therapeutic option for patients with antibiotic allergies, to date, is that no allergies have been reported against phage therapies.

Finally, phages are found throughout nature and it is relatively easy to find a new phage if a bacteria becomes resistant to it and involves a rapid and cheap process as compared to the approval and the costly manufacture of new antibiotics. Examples of many successful phage therapies in diverse experimental animal model systems are reviewed below in subsequent section.

PRECLINICAL STUDIES OF PHAGE THERAPY IN EXPERIMENTAL ANIMALS

On this planet, bacteria have dominated earth for over 3 billion years ago compared with the relatively recent arrival of humans. We have co- existed with them throughout our evolution and their transmitted pathogenicity associated with cholera, typhoid, tuberculosis and Methacillin Resistant *S. aureus* (MRSA), etc. For example, according to recent estimates, 22 million cases of typhoid occur each year causing 216,000 deaths predominantly in school-age children and young adults worldwide (Crump et al., 2004). In the United States and United Kingdom, the incidence of MRSA is around 42% (Gould and Baird, 2007) and mortality rates in excess of 1600 in 2005 to 2006 but has shown decline to about 1200. This can be overcome by potential application of bacteriophage therapy in view of the fact that the phage has a distinct bacteriolytic action in comparison with common bacteriostatic action of antibiotics. Therefore, the antibiotic resistance particularly in wound infected *S. aureus* can be reduced by phages as they have different mode of action for destroying the cells as compared to antibiotics.

A number of studies have been conducted in laboratory model animals to evaluate the efficacy of bacteriophages against diseases caused by various pathogenic bacteria Table 1 summarizes these findings.

Phage therapy in animal health

The history of phage therapy is older than chemotherapy. As mentioned earlier, in 1919 D'Herelle first used phage therapy for the treatment of a dysentery patient and followed its extensive use during First World War to save lives of numerous soldiers. In 1940, the discovery of the first antibiotic, penicillin, coupled with its industrial production enticed the health community to use this chemical drug and indeed it saved more lives as compared to any other known drug. However, the extensive overuse of antibiotics has led to an ever increasing population of pathogenic bacteria acquiring broad resistance against currently available antibacterial drugs. This is currently a serious problem in public health/ modern medicine, particularly in treating the immuno-compromised (AIDS) patients and the World has again entered the realm of the pre-antibiotic era. Due to these circumstances, phage therapy could indeed be very valuable to combat this looming crisis and, at present, the promise of this therapy is demonstrated continually by many successful studies which clearly demonstrate no adverse effects of the therapy. Some of these studies are listed in Table 2.

Phage therapy in farm animals

The farm animals are a source of transmission of pathogenic bacteria to humans according to Centre for Disease Control and Prevention (CDC). Gomez and Rhorer (2009) reported multi-state outbreaks of *Salmonella* from live poultry in United States and *Listeria monocytogenesis* infections from pasteurized milk from a local dairy of Massachusetts. In an another study Fey et al. (2000) isolated multi-resistant *Salmonella* including a Ceftriaxone resistant strain from a 12 year old child who acquired it from cattle.

In 1951, after the U.S. FDA approval use of common antibiotics and growth promoters in chicken feeds which improved the quality of the meat product with reduced fat and higher protein content. This instigated the pharmaceutical companies to mass produce of the antibiotics for farm animals (Boyd, 2001). By the 1970, nearly all birds commercially raised for meat in U.S. were being fed antibiotics to boost the meat yield (OoTAVT, 1979 Office of Technology Assessment). By the late 1990s, poultry producers were using 5 million Kg of antibiotics (see below) annually (Mellon et al., 2001). This extensive use of antibiotics in farm animals was not necessary for curing diseased animals and more than 90% were for promotion of weight gain in U.S farm pigs (U.S. Department of Agriculture, Animal and Plant Health inspection Service).

According to (CDC) at least 17 classes of antimicrobials are approved as growth promotion for farm animals in United States (Anderson et al., 2003). These

Table 1. Phage therapy trials in mice as an experimental model.

Citation	Phage	Infection	Infectious agent	Phage dose (PFU)	Outcomes of treatment
Li et al. (2011)	3 Phages	Diarrhea	Pathogenic *E. coli*	3-2 Phages	Observed safe for rapid and effective preventing Pathogenic *E. coli* infections
Malik and Chhibber (2009)	Phage KØ 1	Burn wound infection by S/C	*K. pneumoniae*	10^{10}-10^{11}	100% survival of treated mice
Vinodkumar et al. (2008)	Phage CSV-31	Septicemia I/P	*P. aeruginosa*	10^{4}-10^{9}	With high dose post 24 h clear visibilities of healthy signs.
Capparelli et al. (2007)	Mutant phage M^{sa}	Abscesses I/V	*S. aureus*	10^{9}	Prevented abscesses formation and 97% rescued
McVay et al. (2007)	Phage Cocktail	Thermal injury infection S/C	*P. aeruginosa*	~10^{8}	Significance decrease in the mortality from 72- 78%.
Watanabe et al. (2007)	Phage KPP10	Gut sepsis O/I	*P. aeruginosa*	10^{10}	Reduced inflammatory response after 27 h inoculation with high survival rate
Wang et al. (2006)	Phage Ø9882	Bacteremia I/P	*E. coli*	10^{-4}	100% survival of animals post 40 min challenge cf with 0% survival in control
Wang et al., 2006	Phage ØA392	Bacteremia I/P	*P. aeruginosa*	10^{-2}	100% recovery of all mice cf with 100% mortality of all negative controls within 24h
Capparelli et al. (2006)	Phage Ø W	Infection I./P or orally	*E. coli*	10^{8}	Infection cleared within 48 h of inoculation.
Matsuzaki et al., 2003	Phage Ø MR11	Infection	*S. aureus*	10^{-2}	Suppressed *S. aureus*
Cerveny et al. (2002)	Phage CK-12	InfectionI/P	*V. vulnificus*	10^{8}	Appreciable therapeutic agent for localized and systemic infections.
Biswas et al. (2002)	Phages ENB6 and C33	Bacteremia I/P	*E. facillum*	10^{8}	Post 45 min. 100% rescue

S/C subcutaneous; O/I oral infection; I/P intra peritoneal; I/V intravenous; [1] Isolated 10 phages from the feaces of diarrhea chicken and propagated 3 and used for toxicity experiment and observed for rapid effective in preventing pathogenic E. coli infections. [2]Experimentally burnt mice infected by *K. pneumoniae* B5055 and treated by intra peritoneal injection of phage isolated from sewage. [3]Septicemia induced by *P. aeruginosa* YFN-58 and treated the infected mice by intra peritoneal-inducing bacteriophages isolated from raw sewage at a municipal treatment plant. [4]Experimentally-induced abscess inoculation of *S. aureus* A170 and treated by the phage. [5] Infected thermally-injured mice model by direct inoculation of *P. aeruginosa* PAO1(Rif) and treated by phage cocktail purchased from ATCC. [6]Experimental mice model with gut abscess induced by applying *P. aeruginosa* D4; treatment with phage derived from polluted river water; [7]Bacteremia infected and treated by phage; [8]Phage ØA392 isolated from hospital sewerage following infection with imipenem-resistant *P. aeruginosa;* [9]*E. coli* infection by intraperitoneal injection of phage ØW isolated from liquid bovine manure. [10]Treated MRSA infection by phage.[11]Treated the infection by injecting the phage intravenously isolated from estuarine sediments from Cedar Key, Fla. [12]Treated the infection by phage isolated from raw sewage.

include many families of antibiotics that are critical for treating human diseases, such as β-lactamase antibiotics including penicillins, lincosamides, macrolides, tetracycline, and erythromycin (Mellon et al., 2001). As bacteria become more resistant to the antibiotics fed to chickens and other

Table 2. Applications of phage therapy in animal health.

Citation	Phage	Infection	Infectious agent	Phage dose (PFU)	Outcomes of treatment
Leszczynski[1] et al. (2006)	Phages (Styloyiridae morphological group; 676/F,A3/R, A5/80)	Urinary tract infected	*S. aureus*	$7x10^8$	After treatment the rectal swab cultures was positive with only a single MRSA colony during the first week and after it become negative.
Marza[2] et al. (2007)	Phage (unspecified)	Burn skin graft infection	*P. aeruginosa*	$\sim10^3$	After 48 h post treatment of phage there was increase in the number of phages and after 3rd day there was no growth of culture.
Jika[3] et al. (2005)	Phage Bioderm ™	Radiation infection	*S. aureus*	Multiple films of phage	After 2 days of phage treatment significant decrease in pain as well as in purulent drainage from the wound. of both patients and after 7th day they fully recovered and went for skin grafting
Sundar[4] et al. (2008)	Host-specific bacteriophages	Used against human pathogenic bacteria	*S. typhimurium* *E. coli* *P. aeruginosa*		Lysed the *S. typhi*, *E. coli*, *P. aeruginosa* colonies. Regarding the host-specificity phages were specific for *S. typhi* and *E. coli* whereas, phage for *P. aeruginosa* was able to infect to both *P. aeruginosa* and *E. coli.*
Marza[5] et al. (2007)	Phage (unspecified)	Chronic bilateral otitis external	*P. aeruginosa*	$4x10^3$	The left ear dried after 27 h of treatment and the right cured after a long treatment

[1]Urinary tract infection caused by MRSA transformed from gastrointestinal tract was successfully treated by three most efficient anti MRSA phages. [2]An uncured by burn skin graft infection by the treatment of appropriate antibiotics was successfully treated by phage. [3]Two severe cases of radiation wound infections of *S. aureus* resistant to several antibiotics (penicillin, streptomycin, chloramphenicol, ampicillin, oxacillin, gentamycin, erythromycy, doxycycline, ciprofloxacin, rocepin (trixon) and cefotaxime) was successfully treated by phage. [4]*In vitro* by the use of host specific phages the human pathogenic strains were treated. [5]Treated a dog infected with topical and systemic antibiotic resistant *Pseudomonas.*

animals raised for human meat consumption, this mode of resistance gets passed on to humans. The human food poisoning surveillance program of CDC suggested that this is the reason for an increase in antibiotic resistance in food borne diseases in the USA (Drexler, 2002).

This indiscriminate use of antibiotics may lead to the evolution of antibiotic resistance by selecting directly for drug resistant pathogens as well as for mobile genetic elements carrying resistance determinants in both human and non-human animal pathogens. These antibiotic resistant bacteria spread into the (i) environmental groundwater, air and farm soil and (ii) foods have an enormous impact on human health through consumption of meat and vegetables fertilized with raw manure, and water supplies contaminated by farm animals waste (Acar and Moulin, 2006). Therefore, the use of antibiotics on farm animals has the propensity to contaminate food during (i) slaughtering, milking, egg production, fermentation and (ii) processing, storage or packaging. Although in 1998, the European Union banned the use of a number of antibiotics of human importance in farm animals for sole purposes of growth promoters, their intimate use for treatment of infection developed resistancewith wide-spread antibiotics. Hence, many nations are in the process of legislating judicious powers on the use of antimicrobials as growth promoters in farm animals.

Bacterial resistant due to misuse of antibiotics has become a global issue and alternative methods are being sought that might decrease the use of antimicrobials in farm animals.

Table 3 summarizes some of the major studies

Table 3. Major phage therapy studies performed in farm animals.

Citation	Phage	Animal and mode of infection	Infectious agent	Phage dose (PFU)	Outcomes of treatment
Borie[1] et al. (2008)	3 different phage cocktails	Chicken Sprayed with phage	*S. enteritidis*	10^3	73% reduction of colonization cf with control 100%
Atterbury[2] et al. (2007)	Phages Ø 151, Ø 25, Ø 10	Chicken O/G	*Salmonella* Nal^r host strains *S. enteritidis*, S. *typhimurium* *S. hadar*	9.0 (Titer 1) or 11.0 (Titer 2) $\log_{10}$ of bacteriophages containing 30% (wt/vol) $CaCO_3$	Titer 2 reduced cecal colonization with in 24 hrs in *enteritidis* and *typhimurium* as compare to control. For 3rd strain *hadar* there was insignificant reduction
Raya[3] et al. (2006)	Phage CEV1	Sheep O/G	*E. coli* 0157:H7	10^{11}	By day 2 reduction of bacteria 10^2-10^3
Wills[4] et al. (2005)	Phage LS2	Rabbit by S/C	*S. aureus*	$2x10^9$	After 4th day of treatment only one out of 8 rabbits had an abscess , cf. 100% had abscess in untreated
Wagenaar[5] et al. (2005)	Phages 69(NTCC12669) 71(NTCC12669)	Chicken O/G	*C. jejuni*	71(NTCC 12669 $4x10^9$-$2x10^{10}$ to each group; A. treated 2 day before challenge; B. After 5 day of challenge; C. Treated only with phage; D. Challenged only with *C. jejuni*	A. Delayed colonization of *C. jejuni* and phage itself in the caeca, and *C. jejuni* count was lower as compare to control. B. Immediate reduction of *C. jejuni* 3 log CFU counts. C. Phage not established in the caeca in the absence of *C. jejuni*. No adverse effect of phage therapy on all groups.
Huff[6] et al. (2005)	Phages SPRO2 and DAF6	Chicken I/M	*Colibaccillosis E. coli*	10^8	Decreased bird mortality
Goode[7] et al. (2003)	Phage P125589, phage 29C and phage p22, H Tint	Chicken skin contamination	*S. enterica serovar Enteritidis and C. jejuni*	10^3	Significant reduction of bacterial population to 10^2 CFU in after 48 h
Huff[8] et al. (2003)	Phages DAF6 and SPRO2	Chicken (Cobb 500) I/M	*E. coli* (serotype-2) infection in the left thoracic airsac	$8x10^8$ and $3x10^9$	Immediate spray of phage reduced mortality from 50 to 20% cf to no efficacy after 24 or 48 h challenge
Barrow[9] et al. (1998)	Phage R	Chicken I/M	*E. coli* H247(018:K1:H7)	10^2-10^6	Protected morbidity and mortality; negative controls after 12 h positive symptoms

Table 3. Contd.

Barrow[10] et al. (1998)	Phage R	Calves I/M	*E. coli* H247(018:K1:H7)	$3x10^{10}$	Protected morbidity and mortality; in negative control symptoms appeared after 12 h challenged.

G oral gavage, I/M intramuscular; [1]Treated the colonization of *Salmonella* by 3 cocktail phages isolated from sewage. [2]Treated the colonization of *Salmonella* Nal^r host strains *S. enteritidis* P125109, S. *Hadar* 18, S. *Typhimurum* 7/74 by phages. [3]Treated by orally gavages of phage isolated from sheep resistant to colonization of *E. coli* 0157:H7. [4]After phage treatment only one rabbit got the abscess and its area was (64mm^2) while in untreated all suffered with an area of (32 to 144mm^2). [5]Treated the infection of *C jejuni* by phage. [6]*Colibaccillosis* caused by *E. coli* was treated by phages SPRO2 and DAF6 isolated from poultry and found significant results.[7]Experimentally contaminated skin was treated by phages isolated from sewage campylobacter phage 12673. [8]Treated the experimentally-infected chickens by phage isolated from waste-water treatment plant at 2, 24 or 48 hrs of post challenge. [9]Treated the experimentally induced septicemia and meningitis caused by injecting *E. coli* H247 (018:K1:H7) by introducing the phage R isolated from human sewage. [10]Treated septicemia and meningitis experimentally phage R isolated from human sewage.

investigated to eradicate pathogenic bacteria from the food-producing animals by using the bacteriophages.

Antibiotic growth promoters are used to aid growing animals to capture the food resources more efficiently, get maximum benefit from them and allow them to develop into strong and healthy individuals. Although the mechanism of antibiotic action is unclear it is thought that the promoters suppress sensitive population of bacteria in intestines. For example (Walsh et al., 2007) suggested that as much as 6% of the net energy in the pig diet could be lost due to microbial fermentation and therefore, treatment with antibiotics reduces this loss and in turn increases body weight. Table 3 summarizes some of the major studies undertaken in the use of bacteriophages to eradicate pathogenic bacteria from food-producing animals

NATURAL OCCURRENCE OF PHAGES IN FOODS

Bacteriophages occur naturally in fermented products as well as unprocessed vegetables in correlation with a target host bacteria. These have been isolated in many cases and used as bio-control for safety of the food products.

Lu et al. (2003) isolated 26 different phages from four commercial sauerkraut (cabbage fermented by *Lactobacilli*) fermentation plants. Atterbury et al. (2005) isolated *Campylobacter* phages at a level of $4x10^6$ PFU/g^{-1} from chicken, Gautier et al. (1995) isolated phage infecting *Propionibacterium freudenreichii* from a Swiss cheese at a level of up to $7x10^5$ PFU/g and Suarez et al. (2002) isolated 61 natural phages up to level of 10^9PFU/ml from Argentinean dairy farms products (yogurt and cheese) that were typed to thermophylic lactic acid bacteria with a remarkably short latency and high burst size.

Allwood et al. (2004) isolated *Escherichia coli* phages from a number of retail products such as pork, mushrooms, raw vegetables (lettuce) ground beef, fresh chicken, chicken pie and delicatessen food with a frequency of as high as 10^4 PFU/g. In New Zealand, Tsuei et al. (2007) isolated *E. coli* and *Campylobacter* phages from vegetables and chicken in more than 90% of the samples at a titer of 250 PFU/g^{-1}. In 1987 Whitman and Marshall in 1971 used the bacteriophages host system to study a variety of refrigerated products. They isolated the bacteriophages at a high frequency from chicken in (50% samples), beef (64.8%), pork sausage (57.15%), oysters (100%), and raw skimmed milk (25%), there were no phages isolates in 5 samples of luncheon and 2 samples of egg white. The range of isolated bacteriophages from different products was between $<10^2$ PFU/g to $6.3x10^7$/g and bacterial contamination was $2.2x10^5$ CFU/g in all samples except one. These studies signifies the wide spread occurrence of phages in a variety food consumed by human and may play a significant role in protection against infection.

USE OF BACTERIOPHAGE TECHNOLOGY IN FOOD

Bacteria are ever-present in our environment and all the living things are in contact with them. Our food becomes contaminated with the human pathogenic bacteria and by consuming this food we acquire these food-borne diseases. According to estimates of World Health Organization International Food Safety Authority Network (INFOSAN 2008), every year the global deaths of children are 1.5 million and a considerable proportion of which is thought to be food-borne. Even in the developed countries like USA, the people affected from food born diseases are approximately 7.6 million; 325,000 hospitalized and 5,000 deaths recorded. In England and Wales this accounts for 2,366,000 cases, 21,138 hospitalization and 718 deaths annually.

There are a number of physico-chemical procedures currently available for the decontamination of food from the food-borne diseases

Table 4. Summary of phage therapy studies performed on foods.

Citation	Phage	Food	Infection agent (*in vitro*)	Phage dose (PFU)	Outcomes of treatment
Sharma[1] et al. (2009)	Phage ECP-100	Fresh cut iceberg lettuce	*E. coli* O157:H7	10^4	After 2 days almost complete eradication cf with untreated control.
Sharma[2] et al. (2009)	Phage ECP-100	Cantaloupe (melons)	*E. coli* O157:H7	10^7	Almost complete removal after 2 day but titre varied to 10^3 CFU by day 7 cf with infected.
Ellis and Delbruck[3] (1939)	Wild type phages (WHR)	Carcasses of chickens	*S. typhimurium* *S. enteritidies*	10^9	Great reduction of both *S. enteritidies* and *S. typhimurium*
Ellis and Delbruck[4] (1939)	Double stranded DNA phage of *V.harveyi* i	*35000 Nauplii of Penaeus monodon (prawns)*	*V. harveyi*	$2x10^5$	86% survival of *P. monodon in* phage treated; while 40% survival rate with antibiotics treated
Higgins[5] et al. (2005)	Phage PHL-1 to 72	Carcasses of broiler chicken	*S. enteritidis* PT13A	$10^6 – 10^{10}$	Reduced frequency of *S. enteritidis* recovery cf to control
Leverentz [6] et al. (2001)	*Salmonell*a-specific phage	melons and apples	*S. enteritidis*	$5x10^7$	Reduction of *S. enteritidis* to 10^3 on melons but not in apples
Fiorentin[7] et al. (2005)	Phage CNPSA1, CNPSA3 and CNPSA4	Chicken cuts	*S. enteritidis* PT4 (P1(25589)	10^3	3.5 orders of magnitude in cecal content.
Guenther[8] et al. (2009)	Phages A511 and P100	Ready-to-eat food	*L. monocytogenesis*	$3x10^6$ to $3x10^8$ (stored at 6°C for 6 days)	Phage concentration was recorded $3x10^8$ PFU/g and very efficient for specific biocontrol of *L. monocytogenesis* in ready-to-eat foods
O'Flynn[9] et al. (2004)	Cocktail phages e11/2, e4/1c and pp01	Beef	*E. coli* O157:H7	$2x10^8$	Observed 7 of the 9 samples were free of *E. coli* O157:H7 and in the another 2 samples have less then 10CFU/ml

[1]Assessed the efficacy of phage on experimentally contaminated food by (spot inoculation) storing for 2 days 4°C. [2]Treated the experimentally contaminated food by phage spray and evaluated its efficacy between 2, 5, 7 day after storing it at 4-20°C. [3]Treated the enterobacter by phage isolated waste water treatment plant and noticed great reduction in both the strains of *Salmonell*a. [4]Treated the infected arthropods by double stranded DNA phage isolated from shrimp farm water and found better than antibiotics treatment. [5]Treated the experimentally contaminated carcasses of broiler chicken by phage PHL-4 out of PHL-1 to PHL-72 isolated from municipal water treatment plant.[6]Treated the *Salmonella* from fresh cut slices of melons and apples stored at various temperature by phage. [7]Experimentally contaminated chicken cuts by *S. enteritidis* PT4 P1 (25589) by phage isolated from feaces of free ranged chicken. [8]Treated the different types of food stored at 6°C for 6 days by phage and found satisfactory results. [9]Experimentally contaminated beef meat treated with phages and observed 7 samples without contamination and only in 2 samples there <10 CFU/ml.

causing pathogenic bacteria and or extension of the shelf life of food from spoilage bacteria. By use of physical treatment, there is obvious potential change in the sensory quality of food and by chemical means, there is a possibility of residues remaining in the food and, then consumption by humans would, in turn, develop resistance to them.

To avoid these risks, the intensity of the treatment has to be limited which in turn limits their effectiveness. To overcome these problems the use of bacteriophages to control the pathogenic and spoilage bacteria in or foods has attracted a growing interest in the food industry (Hudson et al., 2005).

Bacteriophages are host specific and can be selected on the basis of the type of food and on the environment to which the food was exposed. For example *Listeria* specific phages could be used to biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses (Guenther and Loessner, 2011).

However, recently in USA with the authorization of the use of bacteriophage six cocktail by the FDA to control *L. monocytogenes* on ready to eat meats (United States Food and Drug Administration, 2006) and has opened doors to new applications of these natural bacterial killers. The major studies performed on food decontamination are reviewed in Table 4.

Table 5. Phage therapy in agriculture.

Citation	Phage	Plant	Infectious agent	Phage dose (PFU)	Outcomes of treatment
Obradovic and Jones [1] (2004)	6 different specific phages to *X. campestris pv.race* T3	Tomato c.v.BHN 555, 6 week old	*X. campestris pv Vesicatoria* 10^8 CFU by spray	10^{10}	Treatments significantly reduced severity of disease; no correlation with fruit yield
Obradovic and Jones [2] (2004)	6 different specific phages to *X. campestris pv.race T3*	Tomato cv. BHN444 5 week old	*X. campestris pv. Vesicatoria* 10^8 CFU By spray	10^{10}	Treatments significantly reduced severity of disease; yield fruit increased
Balogh and Jones[3] (2003)	h-mutant phage	Tomato	*X. compestris Pv. Visicatova*	10^{10}	Greenhouse
Flaherty[4] et al. (2000)	Phage h-mutant specific to Xcp isolated from soil, water & sludge	"Maverick Red" geranium (21 strains tested)	*X. yanthomonas compestries* 10^8 CFU by spray	10^{11}	6 days post-treatment lytic response destroyed all strains.
Flaherty[5] et al. (2000)	!ecific for tomato race 1 (T1) and (T3)	'Sunbeam' tomato (Lycopersicon esculentum Mill	*X. anthomonas campestris pv. Vesicatoric* by spot	10^8	Repeated doses reduced the incidence and severity of bacterial spot disease; increased fruit yield.

[1]Treated the 6 months old tomato c.v.BHN 555 by 6 different specific phages. [2]5 week old tomato BHN 444 was treated by 6 different specific phages. [3]Treated Greenhouse infected tomato of by h-mutant phage. [4]Treated the Maverick red infection by h-mutant phage isolated from soil, water and sludge. [5]Sunbeam tomato infection treated by specific h-mutant phage for tomato race.

RECENT APPLICATIONS OF BACTERIOPHAGE IN AGRICULTURE

Bacterial plant diseases affect important agricultural crops and result in considerable damage and serious economic loss worldwide. They are becoming more difficult to control because present day bactericide use isnot as effective as in the past. The antibiotic resistance of bacteria to copper has been reported for many bacterial pathogens that affect important crops such as cherry (Sudin et al., 1989; Sudin and Bender, 1993), ornamental plants (Scheck and Pscheidt, 1998), pear (Loper et al., 1991), pepper (Marco and Stall, 1983) and deciduous woody plants (Scheck and Pscheidt, 1998).

The isolation of bacterial pathogens resistant to other agrochemicals, for example, streptomycin resistant strains from apple (Burr et al., 1988; Chious and Jones, 1991), pepper (Ritchie and Dittapongpitch, 1991), tomato (Stall and Thayer, 1962) and woody plants (Scheck et al., 1996) further emphasizes a steady serious evolving agricultural problem. As stated earlier, it is clear that antibiotic resistance in animals and plant bacterial pathogens can be transferred to humans via the food chain and are potential reservoirs of antibiotic resistant bacteria for humans.

Shortly after the discovery of bacteriophages, they were used for control of plant disease (Okabe and Goto, 1963) and represented a novel way to control the growth of plant-based bacterial pathogens and are now a great replacement for the chemical control measures Table 5.

PROBLEMS OF EARLY PHAGE THERAPY

In the early days of phage therapy, the greatest limitation was the highly inadequate scientific approaches, methodologies and equipment used by the scientists. Carlton states that there was "failure to conduct placebo-controlled studies to remove endotoxins from the phage preparations and to reconfirm phage viability after addition of sterilizing agents to the preparations".

In recent years, with the development of genome-based technologies, it is convenient to target bacteriophage products against specific bacteria (Rosamond and Allsop, 2000). Verthe et al. (2004) assessed the lytic activity of *Enterobacter aerogenes* BEI/gfp strain expressing a green fluorescent protein on treatment with

bacteriophage UZI in an intestinal microbial ecosystem.

BARRIERS/PROBLEMS

In the early stage of this innovative technology (bacteriophage) there was a lack of modern scientific methodologies in the field of molecular biology. The medical practitioners used the phages without conducting placebo-controlled studies, failed to remove endotoxins from the preparation and also failed to reconfirm phage viability after addition of sterilizing agents to the pre-paration. In fact, phage therapy was used in emergency in a very crude manner often with uncharacterized phages at unknown concentrations to the patients without specific bacteriological diagnosis (Ackerman and DuBow, 1987). For example, claims of benefit for cholera were based on the administration of phage therapy to all inhabitants of villages who had diarrhoea and in another trial, on simply administrating undisclosed amounts of phage added to the village water wells and the number of cases were assessed subjectively (Barrow and Soothill, 1997). Phage therapy has become a broadly relevant technology, including veterinary, agriculture and food microbiology applications; it is for the treatment or prevention of human infections (Kutter et al., 2010).

EVIDENCES OF SAFETY OF PHAGE THERAPY

As described at the start of this review, our environment is full of enormous numbers of phages and even millions exist in our digestive systems. Moreover, we regularly consume millions of phages in our food and water. In addition phages are abundantly present in common environments and we regularly consume phages from naturally unpolluted water which contain as many as $2x10^8$/ml bacteriophages (Bergh et al., 1989). The use of bacteriophage therapy as prophylaxes is safe and during its long history from discovery to present day, no mishaps have been reported.

Specific phage safety-related issue have been undertaken by (Carlton et al., 2005) who employed an oral toxicity approach in the high doses $5x10^{11}$ PFU /ml of *Listeria*-phage listex P100 orally for 5 consecutive days to disinfect the albino rats of *L. monocytogenesis* infections. They monitored them for up to 8 days and found no morbidity and mortality, no abnormal physical or behavior changes in the treated rats and their body weight remained the same as compared to control.

E. coli phage T4 was administered orally at a low dose (10^3 pfu/ml) and a high dose (10^5 pfu/ml) to 15 healthy volunteers in their drinking water. A week after two days course there was no considerable phage T4 replication on the commensal *E. coli* population and serum transaminase level remained in normal range, and neither T4 phage nor T4-specific antibodies were observed (Bruttin and Brussow, 2005). In another study adenosine deaminase deficient patients in the United States given bacteriophage Ø174 orally and their humeral immune response against the phage was normal (Ochs et al., 1992).

CONCLUSIONS

Phages have been proved as a potential means to eradicate pathogens like *Campylobacter* in raw food (Mangen et al., 2007) and *Listeria* in fresh food to decrease food spoiling bacteria (Mc Grath and Van Sinderen, 2007). In agriculture phages have been used against *Campylobacter*, *Escherichia* and *Salmonella* and in farm animals, *Lactococcus* and *Vibrio* in fish and *Erwinia* and *Xanthomonas* in plants. Phages have been used against diarrhea causing organisms *E. coli*, *Shigella* and wound causing pathogens of skin like *Staphylococcus* and *Streptococci*.

On the basis of the review of literature on phage therapy in animals (experimental and food producing), humans, food and plants we can conclude that bacteriophage is an effective treatment against a wide range of bacteria in humans, animals, plants and foods of commercial interest.

As much of the evidence of phage therapy against Gram-negative bacteria such as *P. aeruginosa*, *E. coli*, *Vibrio vulnificus*, *Klebsiella, C. jujeni* and *Salmonella* spp; and Gram-positive bacteria such as *S. aureus*, have been conducted and shown to be effective significant prophylaxis. Thus phage therapy can reduce unavoidable complications of chemotherapy such as the appearance multidrug resistance of bacteria. Regulation of phage therapy is necessary to allow more wide spread and ease of use of phage therapy.

In the 1980s, interest in phage therapy slowly resurged in the West and in 1997 a North American stockbroker founded a company in Georgia Research Institute Inc (GRI) and started a commercial manufacturing company under the name of Phage Therapeutics International Inc. at a plant in Seattle. Phage therapy and its uses as a disinfectant are expected to spread in water cleansing and sanitation utilities in a wide areas as well as industrial applications.

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