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Bacteriophage therapy against antibiotic resistant bacteria

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Review

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Bacteriophages or phages are the most abundant microorganisms in our environment and are present in high numbers in water and foods of various origins. They are cultured in their respective host bacteria using conventional microbiological procedures and typically have very specific host ranges restricted to one or a few bacterial species. Phages have been used in a variety of applications to exploit their exquisite host specificity, including use as indicators of the presence of their bacterial hosts and as indicators of bacterial (manure) contamination. Typing phages have been widely used in identifying and classifying human bacterial pathogens. Phages offer potential for targeted biological control of bacterial pathogens in human, animal, and plant diseases. Phages were discovered in 1915 by British microbiologist Felix Twort, and independently in 1917, by French Canadian microbiologist Felix d'Hérelle Phages can be conventionally classified into two categories according to the strategies they use to escape their hosts: filamentous phages and lytic phages. Filamentous phages continuously extrude from their hosts without causing host lysis, whereas all other phages are lytic phages that encode gene products to compromise or destroy the bacterial cell wall. The aim of this review is to consider the current evidence on the effects of bacteriophages for detection and control pathogens. The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immunosuppressed patients. The concern that humankind is reentering the "pre-antibiotics" era has become very real and the development of alternative anti-infection modalities has become one of the highest priorities of medicine and biotechnology.

Key words: Antibiotic, bacteriophage, bacteriophage Therapy, drug resistance.

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 wide spread clinical, veterinary, and animal or agricultural usage has made antibiotics less and less effective (Grandgirard, 2008).These days' scientists are now facing the threat of superbugs, that is pathogenic bacteria are resistant to most or all available antibiotics (Livermore, 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, 1999a; Sulakvelidze *et al.*, 2001) which is a cause of

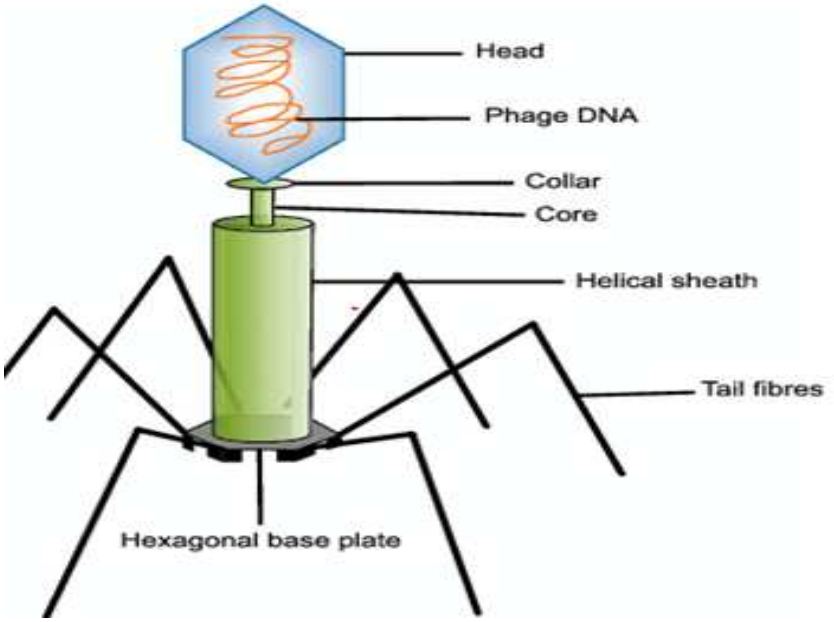


Figure 1. Morphology of phage (T4). **Source**: (Chernomordik, 1989).

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).

Phages were discovered in 1915 by British microbiologist Felix Twort, and, independently in 1917, by French Canadian microbiologist Felix d'Hérelle Phages can be conventionally classified into two categories according to the strategies they use to escape their hosts: filamentous phages and lytic phages. Phage therapy involves the targeted application of Bacteriophages that, upon encounter with specific pathogenic bacteria, can infect and kill them. As typically practiced phages then lyse those bacteria, releasing virion progeny that can continue the cycle, including migrating to other sites of infection anywhere in the body. The actual phage-mediated bacterial killing, however, occurs well prior to the lysis step. Example such as in the first minutes of infection for a phage such as phage T4as the phage converts the cell into a factory for making new phages. Phages are unique among antibacterial agents in their ability to increase their numbers when in the presence of bacterial targets. Of similar importance, phages only minimally impact non-target bacteria or body tissues (Kutter *et al*., 1994).

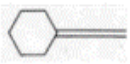
Phage therapy also involves the use of lytic phages for the treatment of bacterial infections, especially those caused by antibiotic resistant bacteria. In general, there are two major types of phages, lytic and lysogenic. Only the lytic phages (also known as virulent phages) are a good choice for developing therapeutic phage preparations (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; Kysela and Turner, 2007). Bacteriophages, nature's tiniest viruses and it is estimated that there are about 1031 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 and Nakai, 2003; Curtin and Donlan, 2006). Therefore, the objective of this paper was to present overview on bacteriophage therapy, explain the biotechnology of phage therapy against antibiotic resistant bacteria, and indicate the application and the account of phage therapy.

LITERATURE REVIEW

Morphology of phages

Most phages range in size from 24-200 nm in length. T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide (Figure1). All phages contain a

Table 1. Classification of Phage.

Shape	Order or family	Nucleic acid, particulars, size	Member	Number
	Caudovirales	dsDNA (L), no envelope		
	*Myoviridae*	Tail contractile	T4	1312
	*Siphoviridae*	Tail long, noncontractile	λ.	3262
	*Podoviridae*	Tail short	T7	771
	*Microviridae*	ssDNA (C), 27 nm, 12 knoblike capsomers	ϕX174	38
	*Corticoviridae*	dsDNA (C), complex capsid, lipids, 63 nm	PM2	3?
	*Tectiviridae*	dsDNA (L), inner lipid vesicle, pseudo-tail, 60 nm	PRD1	19
	*Leviviridae*	ssRNA (L), 23 nm, like poliovirus	MS2	38
	*Cystoviridae*	dsRNA (L), segmented, lipidic envelope, 70–80 nm	ϕ6	3
	*Inoviridae*	ssDNA (C), filaments or rods, 85–1950 x 7 nm	fd	66
	*Plasmaviridae*	dsDNA (C), lipidic envelope, no capsid, 80 nm	MVL2	5

Source: (Hanlon, 2007).

head structure, which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head encloses nucleic acid and acts as the protective covering. Some phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. T4 tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers (Sridhar, 2006).

Classification of Phages

There are currently 19 different classes of bacteriophage, that infects bacteria and archaea.Phages are currently classified on the basis of their nucleic acid type, single or double stranded DNA or RNA and on morphology. Tailed phages belong to the order Caudovirales. This order has three families, the Mycoviridae with contractle tails (Bradley's Group A), the Siphoviridae with long non-contractile tails (Bradley's Group B) and the Podoviridae with non-contractile short tails (Bradley's Group C). These phages contain linear, double stranded DNA (Hanlon, 2007).Further information on phage classification is summarized in (Table 1).

Life Cycle of Phage

Lytic phage or virulent phages: during lytic infection, virulent phages inject their nucleic acid into the host cell following attachment (Figure 2). Expression of the phage genome directs the cellular machinery of the host to

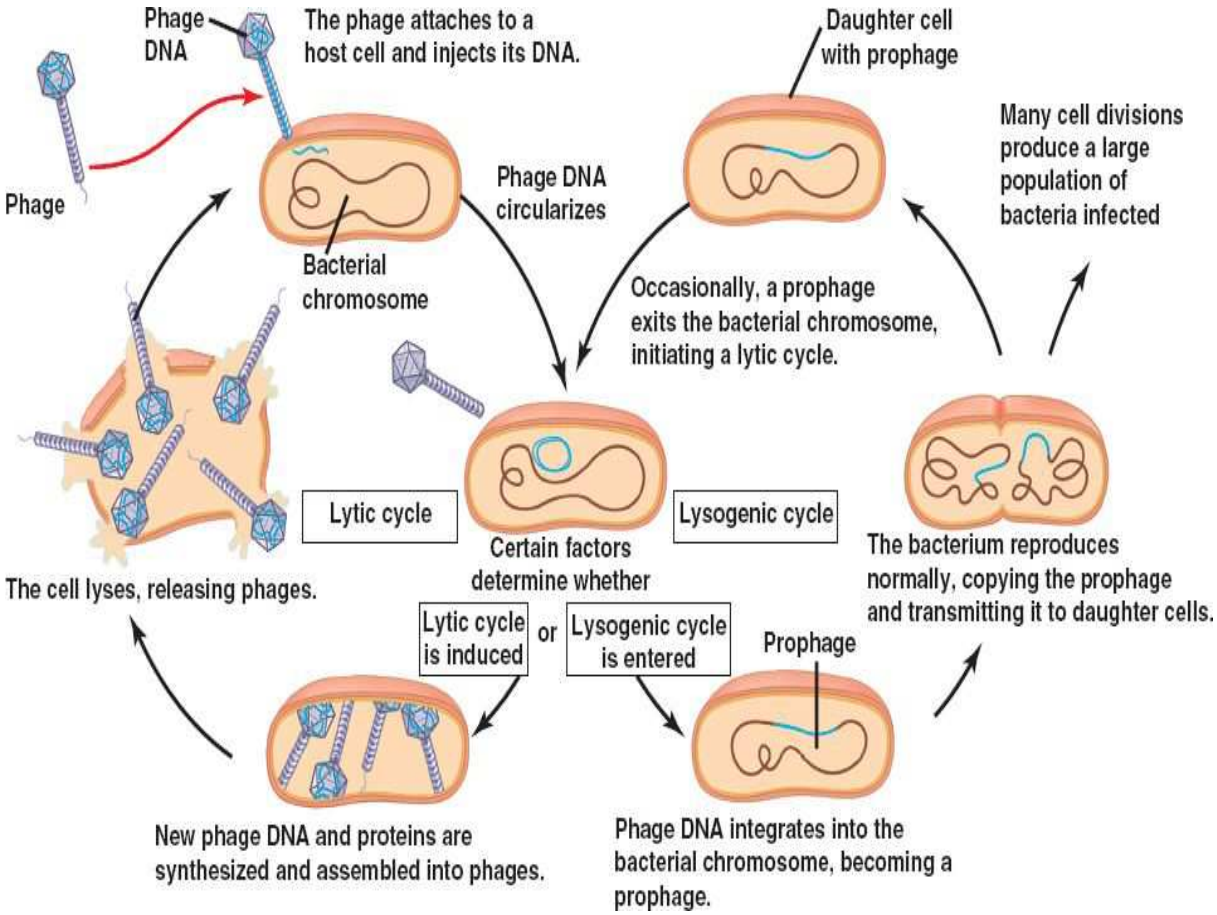


Figure 2. Life cycle of phages. Source: (Hanlon, 2007).

synthesis new phage capsule material. The resulting phage progeny are released by fatal cell lysis enabling the lytic cycle to continue as new cells are infected. Stress conditions such as ultraviolet light or chemical mutagens can induce a switch to the lytic cycle(Brussow, 2005; Sao-Jose *et al.,*2000; Xu,2005).

Lysogenic or Temperate Phages: lysogeny, or the lysogenic cycle, is one of two methods of viral reproduction (the lytic cycle is the other) (Figure 2). Lysogeny is characterized by integration of the bacteriophage nucleic acid into the host bacterium's genome or formation of a circular replicon in the bacterium's cytoplasm. In this condition the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called a prophage, can be transmitted to daughter cells at each subsequent cell division, and a later event (UV radiation or the presence of certain chemicals) can release it, causing proliferation of new phages via the lytic cycle) causing proliferation of new phages via the lytic cycle. Lysogenic cycles can also occur in eukaryotes, although the method of DNA incorporation is not fully understood (Campbell and Reece, 2005).

APPLICATION OF BACTERIOPHAGE THERAPY

Therapeutic Indication

Phage therapy is the application of bacteria specific viruses with the goal of reducing or eliminating pathogenic or nuisance bacteria. While phage therapy has become a broadly relevant technology, including veterinary, agricultural, and food microbiology applications, it is for the treatment or prevention of human infections that phage therapy first caught the world's imagination (Kutter *et al.,* 2010a,b and c). Bacteriophages in bioprocessing are used to reduce the bacterial load in foods usually in the minimally processed foods to avoid cooking associated flavor or texture (García *et al.,* 2010). Controlling pathogens of fruits and vegetables is of much concern as these foods cannot be further processed that would kill any pathogen present. Control of pathogens via phages is a non-thermal intervention by which growth of Salmonella and Campylobacter on chicken skin (Goode *et al.,* 2003). *Salmonella enteritidis* in cheese (Modi *et al.,* 2001). *Listeria monocytogenes* on meat (Dykes and

Moorhead, 2002) and fresh cut fruit (Leverentz *et al*., 2003) is reduced. Extending the shelf life of animal products, phage bioprocessing could be used (Greer and Dilts, 2002).

Phages could be used as predators of pests (bacteria) found in association with plants, fungi or their products Phage mediated biocontrol of plant pathogens has successfully been attempted against *Xanthomonas pruni* associated bacterial spot of peaches to control infections of paces, cabbage and peppers. Phages have also been used to control *Ralstonia solanacearum* of tobacco. They have been successfully employed against *Xanthomonas campestris* which cause spots on tomatoes. Similarly bacterial blotch of mushrooms caused by *Pseudomonas tolaasii* can be treated with phages. Phages have also been considered as a means of controlling the biofouling of thermal power plants condenser tubes (Flaherty *et al.*, 2001).

Phages were reported to be effective in treating cerebro smeningitis in a newborn (Stroj *et al.*, 1999). Skin infection *Pseudomona, Staphylocococcus, Kl ebsiella, Proteus, E.coli* (Cislo *et al*., 1987), recurrent subphrenic and subhepatic abscesses (Kwarcinski*et al.,* 1994) and various chronic bacterial diseases (Kwarcinski *et al.,* 1990). In addition effective in the treatment of long term suppurative infections, phage therapy found, in a recent study. Normalize tumor necrosisfactor alpha (TNF-α) levels in serum and the production of TNF-αand interleukin-6by blood cell cultures (Weber-Dabrowska et al., 2000).

Phage typing is a popular tool to differentiate bacterial isolates, and is used in epidemiological studies with the aim of identifying and characterizing outbreak-associated strains. Although more sophisticated systems for differentiation are available, such as ribotyping, random amplified polymorphic DNA-PCR fingerprinting, or pulsed field gel electrophoresis of enzyme-digested DNA, the variable sensitivity to a set of bacteriophages (phage typing) remains a useful method because of its speed, relative simplicity, and cost-effectiveness. Studies on enterohemorrhagic *E. coli* (EHEC) and Campylobacter showed that phage typing can be highly useful, especially because any one typing method alone fails to produce all the relevant data pertaining to epidemiological relatedness (Sturino and Klaenhammer,2004; Hopkins et al.,2004).

Phages have been used as vehicles for the delivery of vaccines. Phage particles can be used directly carrying the vaccine antigens expressed on their surfaces. But in case of DNA vaccines the sequences that are essential for the vaccine antigen synthesis are incorporated into the phage genome and the phage would then act as vehicle for the delivery of DNA vaccine (Clark and March, 2004).

Bacteriophages are also used for the treatment of septicemia, furunculosis, and pulmonary and urinary tract infections and for the prophylaxis or treatment of postoperative and postraumatic infections. In many cases, phages are used against multidrug-resistant bacteriathat were refractory to conventional treatment with antibiotics (Slopek *et al*., 1987).

Mechanism of action

The few publications available on the subject (Bogovazova *et al*., 1991; Voroshilova *et al.*, 1992) suggest that phages get into the bloodstream of laboratory animals (after a single oral dose) within 2 to 4 hour and that they are found in the internal organs (liver, spleen, kidney, etc.) in approximately 10 hour. Also, data concerning the persistence of administered phages indicate that phages can remain in the human body for relatively prolonged periods of time that are up to several days (Katsitadze *et al*., 1968). However, additional research is needed in order to obtain rigorous pharmacological data concerning lytic phages, including full scale toxicological studies, before lytic phages can be used therapeutically. As for their bactericidal activity, therapeutic phages were assumed to kill their target bacteria by replicating inside and lysing the host cell via a lytic cycle. However, subsequent studies revealed that not all phages replicate similarly and that there are important differences in the replication cycles of lytic and lysogenic phages (Katsitadze *et al*., 1968).

PHAGE PREPARATION AND STORAGE

Pertain to medical significance there are five phage preparations against various bacterial infections. The preparations are called Bacterio-coli-phage, Bacterio-rhino-phage, Bacterio-intesti phage, Bacterio-pyo-phage and Bacterio staphyphage (Summers and Felix, 1999).
Phages are bacterium specific and it is, therefore, necessary in many cases to take a swab from the patient and culture it prior to treatment. Occasionally, isolation of therapeutic phages can require a few months to complete but supplies of phage cocktails for the most common bacterial strains in a geographical area are available (Wright *et al*., 2009).Phage can be freeze dried and turne d into pills without materially impacting efficiency.Temper ature stability up to 55°C and shelf life of 14 months was shown for some types of phages in pill forms. Application in liquid form is possible, stored pre ferably in refrigerated vials (Hanlon, 2007).

ROUTE OF ADMINISTRATION OF PHAGE THERAPY

Safety from a clinical stand point, phages appear to be innocuous. During the long history of using phages as therapeutic agents in Eastern Europe and the former Soviet Union (and, before the antibiotic era, in the United

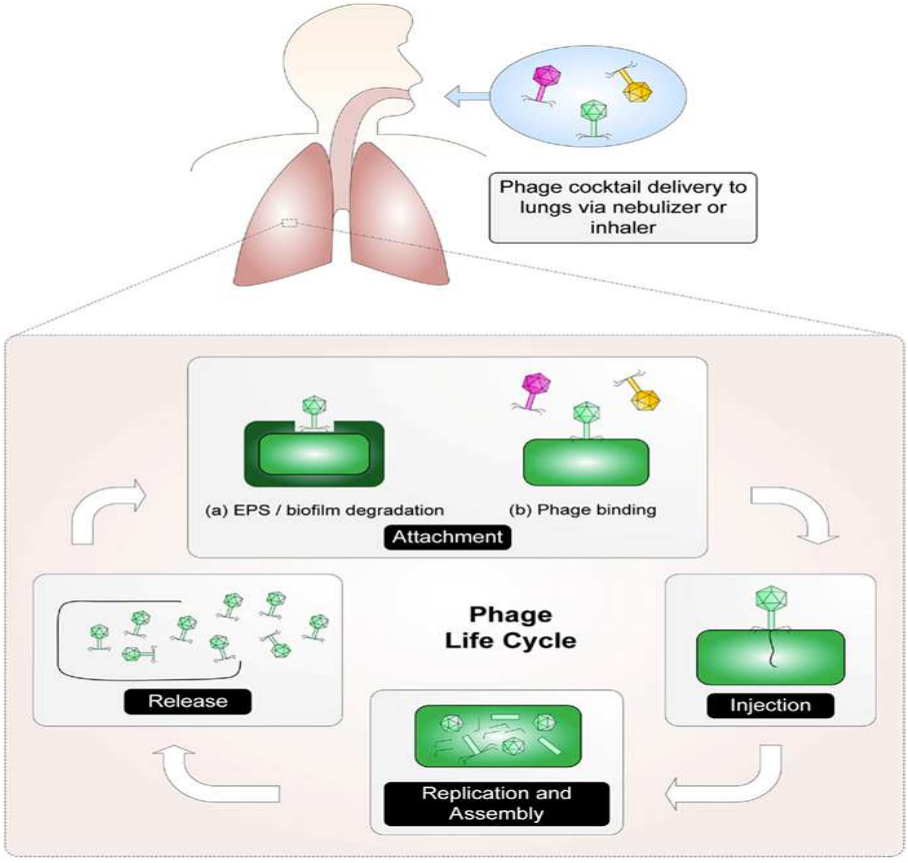


Figure 3. Therapeutic action of lytic phages during treatment of lung infections. **Source**: (Golshahi, 2008).

States), phages have been administered to humans (i) orally, in tablet or liquid formulations (105 to 1011 PFU/dose), (ii) rectally, (iii) locally (skin, eye, ear, nasal mucosa, etc.), in tampons, rinses, and creams, (iv) as aerosols or intrapleural injections, and (v) intravenously, albeit to a lesser extent than the first four methods, and there have been virtually no reports of serious complications associated with their use(Ochs *et al.,* 1992). The most commonly used phage administration methods will be discussed below:

Oral and topical administration

The oral route is generally the most convenient and carries the lowest cost. However, some phages can cause gastrointestinal tract irritation. For drugs that come in delayed release or time-release formulations, breaking the tablets or capsules can lead to more rapid delivery of the drug than intended (Borsheim and Heldal 1989).Topical phage delivering system almost donates the application of drugs directly to the site of action; the risk of systemic side effects is reduced. However, skin irritation may result, and for some forms such as creams or lotions, the dosage is difficult to control (Borsheim and Heldal, 1989).

Inhalational administration

The application of inhalation technologies to phage therapy is one of the recent advances within the field. Taking into account the previous successes of bacteriophage therapy in local and systemic applications, bacteriophages could also be used to combat bacterial lung infections (Figure 3) (Huff, 2002; Golshahi, 2008).

ADVANTAGE AND DISADVANGE OF PHAGE THERAPY

Advantages

Comparison of phages and antibiotics; Lytic phages are similar to antibiotics in that they have remarkable antibacterial activity. However, therapeutic phages have

some at least theoretical advantages over antibiotics and phages have been reported to be more effective than antibiotics in treating certain infections in humans (Kochetkova *et al*., 1989; Meladze *et al.*, 1982; Sakandelidze, 1991) and experimentally infected animals (Smith and Huggins, 1982). Phages were used widely in the early 20th century to treat human and animal illness with varying degrees of success (Singleton and Sainsbury, 2002).

Phages are safe with no or less side effects (Hausler, 2007; Sulakvelidze *et al*., 2001). If bacteria become resistant to phages then phages do evolve naturally to infect the aforementioned resistant bacteria, hence minimizing the chances of bacterial escape, which scores another advantage of phage over antibiotics. After their administration phages can dissipate very quickly throughout the body reaching almost every organ; but the immune system swiftly clears syic phages which pose yet another problem to their acce ptance as therapeutic agent (Dabrowska *et al*., 2005; Clark and March, 2004).

Disadvantages

The therapeutic use of lytic phages is that the development of phage resistance may hamper their effectiveness. Bacterial resistance to phages will unquestionably develop, although according to some authors the rate of developing resistance to phages is approximately 10-fold lower than that to antibiotics. The rate of developing resistance against phages can be partially circumvented by using several phages in one preparation (much like using two or more antibiotics simultaneously). Most importantly, when resistance against a given phage occurs, it should be possible to select rapidly (in a few days or weeks) a new phage active against the phage-resistant bacteria (Carlton, 1999b).

The development of phage-neutralizing antibodies is another possible problem which may hamper phage effectiveness in lysing targeted bacteria in vivo. Indeed, the development of neutralizing antibodies after parenteral administration of phages has been well documented .It is also unclear how effective phages would be in treating diseases caused by intracellular pathogens where bacteria multiply primarily inside human cells and are inaccessible to phages. It is possible that phages will have only limited utility in treating infections caused by intracellular pathogens (Kucharewicz and Slopek, 1987).

In addition to these problems, various hypotheses have been advanced to explain cases in which phage therapy was not effective (Merril *et al*., 1996). That is reticulo endothelial system clearance of phages from the patient may be a potential problem because it might reduce the number of phages to a level which is not sufficient to combat the infecting bacteria (Merril *et al.*, 1996).

CONCLUSION AN D RECOMMENDATION

Bacteriophages have several characteristics that make them potentially attractive therapeutic agents. They are (i) highly specific and very effective in lysing targeted pathogenic bacteria, (ii) safe, as underscored by their extensive clinical use, and the commercial sale of phages, and (iii) rapidly modifiable to combat the emergence of newly arising bacterial threats. In addition, a large number of publications, some of which are reviewed in this mini review, suggest that phages may be effective therapeutic agents in selected clinical settings. Granted, many of these studies do not meet the current rigorous standards for clinical trials and there still remain many important questions that must be addressed before lytic phages can be widely endorsed for therapeutic use. However, there is a sufficient body of data and a desperate enough need to find alternative treatment modalities against rapidly emerging, antibiotic-resistant bacteria to warrant further studies in the field of phage therapy. Based on the above conclusion the following points are forwarded:

(a) Many researches proven that the emergency of antibiotic resistant bacteria is found to be considerable. Thus, application of phage therapy can be one of alternatives that mitigate the burden of this problem.

(b) The application of phage therapy is applied in a few countries so that it should be tried to apply widely as the emergency of antibiotic bacteria is increasing.

(c) In some extent the use of phage therapy is questionable pertain to its efficacy and host immune response against phages that need further study.

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