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Phage Therapy; A Review on the Biology and Therapeutic Application of Bacteriophage

Ashenafi Kiros

National Animal Health Diagnostic and Investigation Center (NAHDIC)Sebeta, Ethiopia.

Tadele Gashaw

WolaitaSodo University, School of Veterinary Medicine, Department of Microbiology, Public Health and Epidemiology, WolaitaSodo, Ethiopia

Ayichew Teshale

WolaitaSodo University, School of Veterinary Medicine, Department of Biomedical Science, WolaitaSodo, Ethiopia.

Abstract: *The emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal or agricultural usage has made antibiotics less and less effective. One of the possible replacements for antibiotics is the use of bacteriophages or simply phages as antimicrobial agents. Bacteriophages (phages) are viruses that infect only bacteria. They are non-toxic to other organisms, infecting, and in the case of lytic phages, multiplying rapidly within the bacterial host, ultimately killing it. Bacteriophages (or 'phages') were independently discovered by Twort and D' Herelle. Phages have a specialized structure with tunnel tails that allows them to bind to the surface of their bacterial targets. Once they are firmly attached the tail injects the viral DNA into the host cell. In a phage encoded protein, the holin, accumulates harmlessly in the cytoplasmic membrane until triggering at an allele-specific time to form micron-scale holes. The effectiveness of phage applications against pathogenic bacteria depends on several factors such as the bacteriophage/bacteria ratio, physico-chemical factors (pH, temperature), phage neutralization or resistance to phage. In the last few years, modified phages are increasingly being explored, mostly due to the limitations of phage therapy using lytic phages.*

Keywords: *Bacteriophage, phage therapy, antibiotic, resistance*

Back ground

Antibiotics are essential therapeutics, commonly used to control bacterial infections. They are one of the most significant contributions to modern science and have proved to be of vital importance in the dramatic rise in average life expectancy. However, the emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, or agricultural usage has made antibiotics less and less effective (Fischetti, 2008). Additionally, very frequent and inappropriate use of antibiotics, lack of educational awareness and regulatory authority regarding antibiotic usage, production, and marketing as well the lack of infection control in hospitals and inadequate water and sanitation in the community makes the situation worse **(**Zahraand Abdollah, 2011).These days' scientists are now facing the threat of superbugs, i.e.pathogenic bacteria resistant to most or all available antibiotics. 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 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 (Chhibber and Kumari, 2012).As natural killers of bacteria, phages are obvious candidates for exploitation as antibacterial agents. Phages have many intrinsic characteristics which make them attractive candidates for such applications. They cannot replicate in eukaryotic cells or incorporate their DNA into the genome of such cells. They are highly specific in their bactericidal

potential(Kearyet *al*., 2013).This article reviews the potential of phage therapy as a means of treating or preventing diseasesand draw attention to the comparison between the phage and antibiotic.

1. HISTORY OF PHAGE THERAPY

Bacteriophages (or 'phages') were independently discovered by Twort(1915) and D' Herelle(1917)although initial observations of these viruses date back to Hankin in 1896 Although D' Herelle was quickly convinced that phages were viruses, many scientists believed their bactericidal effect was the result of an enzyme Indeed during this early period, leading microbiologists such as Bordet and Gratia argued strongly against D' Herelle's virus hypothesis . Definitive proof that phages were able to propagate at the expense of their host came when performed their classic one-step growth curve experiment. If a single phage infects a bacterium, it replicates inside the host until a point when the viral progeny lyse the cell and diffuse into the environment (lysis from within or 'lytic infection'), also demonstrated that if many phages infect one bacterium simultaneously, they can kill their host without replication (Atterbury, 2009).

The first use of phages as antibacterial agents proceeded relatively soon after their discovery, with the first phage therapy publication appearing in 1921. Approximately over the same period, d'Hérelle was observing a role for naturally occurring bacteriophages in the control of bacterial disease: "The disease is only definitely overcome at a time when the virulence of the bacteriophage is sufficiently high to dominate the resistance of the bacterium." "In all cases the fluctuations in the virulence, as well as the fluctuations in the resistance of the bacteria, parallel the stat be of the patient, and the onset of improvement coincides with the moment when the virulence of the bacteriophage dominates clearly the resistance of the bacterium." As described in some detail in Abedon et al, in other parts of the world—most notably the U.S.S.R., and particularly Georgia, but also Poland and France—the practice of phage therapy remained vibrant even as it faded in English-speaking nations. As the problem of antibiotic resistance became more apparent during the 1990s, however, numerous individuals as well as companies turned both to phage therapy and those institutions, most notably in the now independent former Soviet republic of Georgia, that still routinely practiced phage therapy. The result has been a growing interest in the potential to use phages as antibacterial agents within the context of medicine as well as veterinary medicine, agriculture, and other circumstances (Sulakvelidzeet *al*., 2001)

One of the best-known series of recent studies on the use of phages in veterinary medicine came from the laboratory of William Smith and his colleagues at the Institute for Animal Disease Research in Houghton, Cambridge shire, Great Britain. In one of their early papers, the authors reported the successful use of phages to treat experimental *E. coli* infections in mice (Sulakvelidzeet *al*., 2001).

During subsequent studies it was found that a single dose of specific *E. coli* phage reduced, by many orders of magnitude, the number of target bacteria in the alimentary tract of calves, lambs, and piglets infected with a diarrhea-causing *E. coli* strain. The treatment also stopped the associated fluid loss, and all animals treated with phages survived the bacterial infection (Smith and Huggins, 1983; 1987, Smith *et al*., 1987). The phage preparation was reported to be (i) efficacious in treating experimental infections of mice and (ii) nontoxic in mice and guinea pigs; i.e., gross and histological changes were not observed after intravenous (i.v.), intranasal, and intraperitoneal administration, even after a dose approximately 3,500-fold higher (estimated by body weight) than the human dose was given to mice during acute toxicity studies (Sulakvelidzeet *al*., 2001).

2. BIOLOGY OF PHAGES

2.1. Structure

Structurally, phages contain a core nucleic acid encapsulated with a protein or lipoprotein capsid which is connected with a tail that interacts with various bacterial surface receptors via the tip of the tail fibers. This interaction shows an affinity that is specific to certain group of bacteria or even to a particular strain. Phages are extremely diversified group and they are known to be the most abundant and self-replicating organisms on Earth,they are classified into three families: the *Myoviridae*(long contractile tail), the *Siphoviridae*(long non-contractile tail) and the *Podoviridae*(short non-contractile tail) (Hodgson, 2013).

2.2. Life cycle

Bacteriophages have the ability to interfere between two cycles lysogenic or lytic. In the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. Each phage follows a unique pathway to control bacteria. Some of them show a lytic infection cycle upon infecting their bacterial host. In this case, they grow in high numbers in bacterial cells, leading to cellular lyses. At the end of the cycle, a release of newly formed phage particles is observed. Using the lysogenic pathway, the phage genome integrates as part of the host genome. It stays in a dormant state as a prophase for extended periods of time. Adverse environmental conditions for the host bacterium may activate the prophase, turning on the lytic cycle. At the end, the newly formed phage particles are ready to lyse the host cell(Mandal*et al.*, 2014).

The life cycle of phages can be distinguished into four basic steps. First is an extracellular stage during which the virion capsid protects the phage genome such as from nucleases. This is preceded as well as preceded by an infection stage during which a majority of phage physiological aspects are observed (Black and Peng, 2006). Infection ends with release, usually through phage-induced bacterial lyses, thus initiating the extracellular phase. The extracellular phase ends and infection begins in the course of what variously is described as attachment, adsorption, uptake, penetration, ejection, injection, and/or translocation (Abedon, 2014).

3. PHARMACOLOGY OF PHAGE THERAPY

3.1. Source of phage

Phage for a given bacterium can be isolated wherever that bacterium grows, such as in faeces, sewage, soil, hot springs oceans. Water from the Ganges (India) has been found to be a rich source of vibrio phages. The problem is not in isolating phage against particular bacteria but in selecting the ones most likely to be useful for clinical purposes. This includes lytic phages that have high efficacy and broad spectrum activity on clinically important strains. Phages should be such that they can be readily produced in large quantity and should be stable during storage.For therapeutic phages the ability to lyse host mutants resistant to other therapeutic phages in a given mixture is important (Mathur*et al*., 2003).

3.2. Pharmacodynamics and pharmacokenetics of phage therapy

Various pharmacological concepts require some modification to be fully applicable to phage therapy. First, movement into the blood is required only given systemic application and consequently often is not a goal with phage therapy, particularly of local infections. Second, movement for phages represents penetration to target bacteria and an important aspect of such penetration is into bacterial biofilms. Third, "metabolism" for phages logically includes not just inactivation but also activation particularly of phage bactericidal activity and also the often-associated in situ amplification of phage numbers. Phages as antibacterial agents, amplification takes place in the immediate vicinity of target bacteria. Lastly and as is true for antimicrobial agents in general, the concept of "body" in pharmacology includes not only host tissues but also microorganisms, including target bacteria for antibacterial treatment (Abedon, 2011).

Absorption and distribution can have the effect of increasing antibacterial concentrations within the vicinity of target bacteria, though also they have a diluting effect on dosages. Phage infection too can increase phage numbers within the vicinity of target bacteria. Together these pharmacokinetic mechanisms contribute to some peak phage density that may or may not be sufficient to substantially decrease densities of target bacteria. Particularly, peak densities must exceed some minimum effective concentration to effect net reductions in bacterial densities and these densities can be achieved through a combination of supplying sufficient phage numbers per individual dose, supplying multiple doses, and/or allowing for phages to replicate in. Lastly, various mechanisms exist whereby phage densities may decrease over time, which include what pharmacologically are described as metabolism and excretion, though as noted dosage dilution plays a role as well (Abedon,2014**).**

3.3. Interaction between phages and bacterium

The first step of the phage infection is adsorption of the phage particle to the bacterial cell wall by specific interactions between viral surface proteins and host cell receptors (Chatain, 2014). Phages have a specialized structure with tunnel tails that allows them to bind to the surface of their bacterial

targets. Once they are firmly attached the tail injects the viral DNA into the host cell. In a phage encoded protein, the holin, accumulates harmlessly in the cytoplasmic membrane until triggering to form micron-scale holes. This allows the soluble endolysin to escape from the cytoplasm to degrade the peptidoglycan. (Young, 2014)

The viral DNA injected through the tail into the host cell, directs the production of progeny phages, often over a hundred in half an hour. These 'young' phages burst from the host cell (killing it) and infect more bacteria.Meanwhile, lytic activity destroys the infected bacterium by overwhelming it with progeny phages that burst through the host cell(Chatain, 2014).

3.4. Spectrum of Phage

Each bacteriophage has a limited spectrum of infectivity against its bacterial targets, which must be understood in order to enable successful in vivo use. Thus, the development and adoption of clinical assays to rapidly identify causative bacterial pathogens and their susceptibility to phages are necessary.The bacterial host range of phage is generally narrower than that found in the antibiotics that have been selected for clinical applications. Most phages are specific for one species of bacteria and many are only able to lyse specific strains within a species. This limited host range can be advantageous, in principle, as phage therapy results in less harm to the normal body flora and ecology than commonly used antibiotics, which often disrupt the normal gastrointestinal flora and result in opportunistic secondary infections (Reza *et al.,* 2013).

3.5. Routes of Administration

Phages can usually be freeze-dried and turned into pills without materially impacting efficiency. Temperature stability up to 55 °C and shelf lives of 14 months has been shown for some types of phages in pill form.Application in liquid form is possible, stored preferably in refrigerated vials. Phage preparations thus are more conservatively and readily applied more locally intraperitoneally, intrapleurally, inserted directly into a wound, etc. - as opposed to explicitly systemically.This either topical or less-directly systemic use of phages is less likely to be a problem because the phage move gradually from a local reservoir into the circulatory system and reproduce rapidly when they reach another collection of susceptible bacteria(Goodridge, 2010)

4. FACTORS AFFECTING EFFECTIVENESS OF TREATMENT IN PHAGES THERAPY

4.1. Phages/bacteria ratio

The use of bacteriophages against pathogenic bacteria has been studied using two different approaches, one passive, and the other active (Gill, 2010). In the case of the passive approach, the bacteriophages are added into the system at a level sufficient to ensure that all target bacteria are infected and lysed in a short period of time. On the contrary, active biocontrol relies on the addition of a small amount of phages. Bacterial elimination, in this case, supposes the replication of phages over several generations. The capacity of new replicated phages to access the target bacteria could be weakened by the biochemical and physico-chemical characteristics of the system (the viscosity for example). It appears that the passive treatment is more efficient than the active one (Chatain, 2014).

4.2. Environmental conditions and phage resistance

The survival and persistence of bacteriophages are affected by physico-chemical factors (pH, ions, temperature). The phage population is generally stable in relation to external factors. (Jonczyk*et al*., 2011). The proliferation of several phages is limited when pH is lower than 4.5, but the risk of pathogenic bacteria food contamination is also generally reduced below pH 4.5. In the case of phage oral injection, stomach acid can have a negative impact on the survival of phage which may lead to treatment failure (Watanable*et al*, 2007).

4.3. Accessibility to target bacteria

The diffusion of bacteriophages could be impaired or favored depending on the structure and the composition of the matrix and the environmental conditions (Marco *et al*., 2010). In solid media, the diffusion of bacteriophages could be limited, reducing phage adsorption on bacteria and, consequently, the phage infection capacity. The presence of other compounds could protect bacteria from phages. (Gill *et al*, 2006). In phage therapy, the escape of invasive pathogens into closed tissue

and organ compartments may block the effective use of bacteriophages, especially if the phage cannot actively follow the bacteria. (Sulakvelidze, 2005).

4.4. Circulation of phage and neutralization of phage by antibodies

Direct bactericidal effect of the phage was the principal determinant of the protective effect rather than any indirect effect such as a phage-stimulated immune response. Phage and bacterial numbers in the circulation were determined after the infection and showed that the bacterial load was much lower in the blood of phage-treated mice when compared to those that received only bacteria. It is also noticed that phage titers in mice infected with bacteria remained higher than the titers in mice that received only the phage. This suggested that the phage had replicated in the infected mice and consumed the bacteria (Matsuzaki*et al*., 2003).

4.5. Dose and moment of treatment

Another important factor that can modify the effectiveness of phage treatment is single dose versus multiple doses. Several studies have shown that multiple doses are better than a single dose**.** One study by Huff *et al.* found that treating chickens suffering from severe respiratory infections caused by *E. coli* was very helpful in clearing up symptoms. The application of bacteriophage was most useful very soon after the chickens had been exposed to the bacteria and that, if treated early, multiple doses were better than a single dose (Chatain, 2014

4.6. Phage administration

One advantage of phage use is the easy administration. Phages can be applied by oral, topical, intraperitoneal, intravenous, or intranasal administration (Gill and Hyman, 2010).Phage therapy has been used for the treatment of a variety of bacterial infections. They can be used in freeze-dried form and turned into pills without materially impacting efficiency. Oral administration works better when an antacid is included, as this increases the number of phages surviving passage through the stomach.However, it is difficult to conclude which mode of administration is the most effective. The effectiveness of treatment depends on various factors: the concentration of pathogenic bacteria on the infection site, phage preparation, and the dose applied medium composition and structure, and environmental conditions (Chatain, 2014).

4.7. Specificity

Phages specifically infect the host bacteria species. This specificity can limit the effectiveness of phage use. To ensure that the bacteria can be lysed by the phage used, the bacterial strain isolated from the infection site will be tested for its sensitivity to the phage administrated. It is important also to verify if the phage is strongly lytic or not. However, polyvalent phages which can infect several bacteria strains of the same species do exist. The use of polyvalent phage allows the activity spectrum of phages to be increased. The polyvalent phage can be replaced by a cocktail of phages. Briefly, the phage by their specificity can infect only the target bacteria without effect on others bacteria flora, but the specificity may also have an ineffective treatment if the target bacteria are not lysed by the phages administered (Chatain, 2014).

4.8. Resistance to phage

As in the case of antibiotics, bacteria can develop resistance to phage, which may hamper the effectiveness of phage treatment. The first step of phage infection to bacteria is adhesion of phage on bacterial surface by surface proteins which act as receptors. If the bacterium loses the phage receptor, they become resistant to phage. Bacteria may also acquire horizontally a restriction-modification system that degrades the nucleic acid of the injected phage. In addition, phage resistance may be caused by a mutation in a gene, the product of which is essential for phage replication or assembly (Carlton, 1999).

5. THERAPEUTIC APPLICATION OF PHAGE

Bacteriophages (phages) are viruses that infect only bacteria. They are non-toxic to other organisms, infecting, and in the case of lytic phages, multiplying rapidly within the bacterial host, ultimately killing it. Lytic phages known as candidates for phage therapy, since they rapidly replicate into their host and lyse them. They are extremely specific for their targeted hosts and also are safe for animals and human because they have no activity against eukaryotic cells (Mandal*et al.*, 2014).

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, and which today is the primary motivator of the field. Although "phage therapy" has been historically associated with the use of bacteriophages in human medicine, phages also have been extensively used in veterinary medicine. The first-known therapeutic use of phages in veterinary medicine is associated with Felix d'Herelle (who was also the first to use phages to treat human infections, seeHuman Therapeutics), the co-discoverer of bacteriophages, who examined their efficacy in France, during 1919 – in preventing and treating*Salmonella*infections in chickens. Phages effectively reduced chicken mortality, which prompted other investigators to examine their possible usefulness in preventing and treating other naturally-occurring and experimental bacterial infections in animals. In that regard, phages have been reported to be a safe and effective preventive/treatment modality against numerous bacterial infections of animals(Jockers, 2014).

In natural environments they have a dominant role in controlling bacterial populations. Thus, in the era of the emergence and spread of multidrug resistant bacterial pathogens they are more and more often seen as promising antibacterial agents that could be an alternative to antibiotics. Bacteriophages' main advantages as therapeutics are their ability to target bacteria of certain strains or species, without any harmful effect on the rest of the bacterial microflora, as well as their self-limited propagation which is controlled by the availability of a sensitive host. Moreover, bacterial antibiotic resistance is not a barrier for phage infection (Sankar*et al*., 2014).

The ability of many phages to remain in a bacterium in the form of a prophage and increase its adaptive potential, as well as to participate in the horizontal gene transfer between bacterial cells, *a priori* precludes their use in therapy due to safety concerns. Factors that matter in the prediction of the remaining phages' therapeutic efficacy include host range and killing potential, adsorption kinetics and propagation efficiency, stability during storage and under "natural conditions", the ability to penetrate encapsulated cells or biofilms, easiness of purification (Hupfeldand Loessner, 2014).

5.1. Phage as antibiofilms

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.

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. Bacteriophages have been suggested as effective anti-biofilm agents (Chhibber and Kumari,2012).

5.2. Phage as biocontrol

The term biocontrol may be used to describe more food- or environment-oriented treatments. When phages are used as alternatives to antibacterial drugs in medicine or veterinary practice however, then this is what can be described specifically as phage therapy. While in principle all bacteria can be impacted by phages, in practice it is especially gastrointestinal afflictions, localized infections, and otherwise chronic infections that are treated within a phage therapy context(Tan *et al*., 2014).

The actual practice of phage therapy is fairly straightforward. One or more phage types that are either thought to be effective against target bacteria or that have been shown to be effective following laboratory testing are administered in some manner to a patient. Ideally these phages can reach and then disrupt target bacteria. Disruption can be accomplished by killing bacteria, clearing biofilms, and perhaps also by increasing bacterial susceptibility to existing host immunity. Indeed, it has long been postulated that phages may play roles as components of a body's normal microbiota as a natural defense against bacteria (Abedon, 2014).

The therapeutic effect of the phages can be limited to a decrease in the pathogen's population down to a point at which the immune system can effectively control its reproduction. Several current strategies

to combat livestock-associated pathogens such as toxinogenic*E. coli, Campylobacter*, and *Salmonella* are direct extensions of "classical" phage therapy approaches in that they focus on targeting the bacteria in animals before slaughter. On the other hand, food contamination, for instance with *Listeria monocytogenes*, is more likely to occur during food processing, which consequently is the most reasonable time point for phage biocontrol of this pathogen (Golkar*et al*., 2014).

6. POSSIBLE CHALLENGES TO PHAGE THERAPY AND MODIFICATION

Modified phages

Despite numerous reports of successful in vitro phage treatment of infected cells, clinical application of this therapy has yielded conflicting and unpredictable results. Among the possible reasons are antibodies, gastric juices phage-resistant bacterial mutants and rapid phage clearance.

In the last few years, modified phages are increasingly being explored, mostly due to the limitations of phage therapy using lytic phages(Krylov, 2001).

The undesirable side-effects of phage therapy using lytic phages, 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. Directed mutation of the phage genome, recombination of phage genomes, artificial selection of phages in vivo, chimeric phages and other rational designs have conferred new properties on phages, including greater therapeutic potential. These new modified phages have been shown to successfully overcome challenges to earlier phage therapy, such as efficacyand safety issues**(**Zahra and Abdollah, 2014).

6.1. Strategies for enhancing phage lethality

Phage modification strategies often aim to construct lethal phages or to enhance the lethality of current phages to successfully kill antibiotic-resistant bacteria. Thanks to progress in the field ofsynthetic biology, these modified phages control bacteria more efficiently and may also be used with antibiotics as a combination biological–chemical treatment to reduce the chance of developing residence. In other words, engineered phages act like a strong adjuvant for antibiotic therapy. Engineered enzymatic phage able to express a biofilm-degrading enzyme during infection and consequently could attack the bacterial cells in the biofilm and the biofilmmatrix. In addition to targeting biofilm matrix, phages may be used to damage the bacterial cell wall. This damage allows antibiotics to pass through the outer membrane of Gram-negative bacteria, permitting access to their site of activity despite the drug hydrophobicity and large sizea filamentous phage-based strategy reduces the required effective dose of antibiotics. Specifically, in filamentous phage therapy, point mutations in the genes responsible for phage progeny extrusion increase damage to the bacterial outer membrane, enhancing antibiotic vulnerability.

Subsequent development of drug resistance and other unwanted side effects are thus likely to be reduced (Zahra and Abdollah, 2011).

6.2. Narrow host range of lytic phages and host specificity

Phages can be targeted far more specifically than most antibiotics to particular bacteria, resulting in much less damage to the body's normal microbial balance. Although genome rearrangements and mutations in specific genes, such as that encodingendolysin, can extend the host range of phages, phages are basically species-specific antibacterial agents. Indeed, the host specificity of phages is extremely refined, with each phage only invading one species or even a single bacterial strain. Therefore, a broad-host-range phage is of paramount importance for therapeutic application. To avoid failure in phage therapy resulting from narrow spectrum of phage host specificity, either an accurate diagnosis must be obtained prior to therapy or a pool of different phages must be applied. The most reliable method used to overcome this problem is the exploitation of phage cocktails. This technique entails administering a mixture of phages against the particular bacteria involved in a specific infection. Both naturally occurring isolates and modified constructs can be combined into cocktails with collectively enhanced capabilities, such as expanded host range.

Recently, phages have been adapted to new bacterial host cells while maintaining strong lytic activity.Engineered phages against H. pylori express a recombinant protein comprising both a component derived from a phage surface protein and a component consisting of the variable region of

an antibody, providing a bacterial antigen-binding site. The second part renders thephage capable of binding to and thereby inhibiting the growth ofbacterial cells involved in infection (Zahra and Abdollah, 2011).

6.3. Rapid phage clearance by host immune system

To address the latter issue in particular, some studies have sought to develop phages that can avoid inactivation by the host defense system and persist inthe body. Serial passaging is one method that prolongs phage viabilityin vivo. During phage circulation, spontaneously occurring phage mutants are selected and grown from serial blood samples. A sample of these remaining phages is produced at a high titer and injected into a second animal. This protocol is then repeated several times. After such progressive clonal purification and selection, the resultant phage strain can survive at least 15% longer in the body than the wild-type.

Phages have also been genetically modified to delay inactivation in vivo. A genetically engineered phage expressing a recombinant complement-antagonizing peptide on its surface is one example.The modified phage construct is made by in vivo recombination in the host bacterium. During this process, the normal surface protein genein the genome of phage is replaced with a fusion gene composed of complement-antagonizing peptide and phage surface protein genes. Anti-host-defense engineered phages could incorporate and express other categories of genes as well, including those encoding interleukins, other cytokines, and inhibitors of various cellular activating or inhibitory factors (e.g., inhibitors of macrophage activating factor). Beyond new gene expression, mutations may also be induced to reduce host clearance of phages in conjunction with serial passaging. In addition to changing the phage genome to delay inactivation, phenotypic modification by attaching a polymer to phage surface proteins, which effectively masks those antigens, can enable longer circulation (Zahra and Abdollah, 2011).

6.4. Release of endotoxin and progeny into media

A concern with any lytic bacterial treatment is that the rapid and massive destruction of bacteria in vivo may release endotoxins and superantigens that stimulate an inflammatory response that can cause significant morbidity. In order to reduce the risk of this happening, phages have been selected or engineered to be lysis-deficient and/or non-replicative. These approaches can significantly decrease the levels of endotoxin and inflammatory mediators generated during phage therapy and thus improve survival. For example, Hagens and Blasi engineered filamentous phages to express restriction endonucleases and holins in E. coli. These phages were toxic to bacteria but did not cause cell lysis and thus released minimal levels of endotoxin(Lu and Koeris, 2011).

7. COMPARISON OF PHAGE THERAPY AND ANTIBIOTICS

Phages have specific properties which give them advantages as therapeutic agents. They are self-replicating as well as self-limiting. They continue to multiply and penetrate deeper as long as local infection as present. This is in sharp contrast to antibiotics which decrease in concentration below the site of infection. Phages are lytic against specific bacteria so they can be targeted more specifically than antibiotics which are active against a group of bacteria. Phages do not harm normal intestinal microflora while antibiotics have side effects which can be serious (Nakaiand Park, 2002).

Compared to antibiotics, phages go deeper into the infected area. Antibiotics, on the other hand, have concentration dependent that quickly decrease as they go below the surface of the infection. The replication of phages is concentrated on the infected area where they are needed the most, while antibiotics are metabolized and removed from the body (Borysowski*et al.*, 2014).Phage therapy has distinct advantages over antibiotics because the use of “wild type” phage with a multitude of different types of phages is too variable for bacterial resistance to form. Additionally, the phages keep intact healthy microflora and maintain a good microorganism count by preventing against small intestinal bacterial overgrowth (Jockers, 2014).

Optimal wound concentration and efficacy of antibiotics is also often difficult or impossible to achieve by means of local antibiotic therapy due to their dilution by inflammatory exudates, neutralization by enzymes and other inflammatory mediators, and inability to penetrate adequately into the tissues. The reproductive ability of bacteriophage, in contrast, avoids this problem since they continue to replicate and penetrate into tissue in the presence of susceptible bacteria. This makes

phages ideal for wound treatment, in contrast to antibiotics, whose concentration decays rapidly with distance from the source or, when used systemically, the blood vessel(Abedon and Thomas, 2010).

Phages, by contrast and in particular, generally have low toxicities plus can have relatively low per-unit costs, meaning that large phage quantities can be employed in circumstances where increasing antibiotic concentrations (such as to overcome uptake, clearance, and penetrability to target bacteria concerns) is not an option for reasons of toxicity or cost. The cost issue is especially relevant in developing nations where per capita health expense is relatively low. Also contributing to low phage toxicity, in comparison to antibiotics, is that phage concentrations are self-regulatory: They are quickly flushed from the body and/or inactivated by the immune system when their host is no longer present. This latter aspect is linked with challenges in administering phage therapy systemically, however, where a large bolus of antigen enters the circulatory system at once. Even if applied locally, or *per os*, phage particles often enter into systemic circulation, which can be viewed as advantageous in terms of phage penetration to localized or more systemic infections (Goodridge, 2010)

The antibacterial effects of phages in purulent wounds are more manageable than those of antibiotics. This is because the penetration of antibiotics into infected tissue, as well as their concentration there, is directly related to their systemic concentration, with increase of this concentration having very obvious limits. These limits are defined by combinations of antibiotic toxicity along with their rates of uptake and clearance.Phages, by contrast and in particular, generally have low toxicities (Sulakvelidze*et al*., 2001)plus can have relatively low per-unit costs, meaning that large phage quantities can be employed in circumstances where increasing antibiotic concentrations (such as to overcome uptake, clearance, and penetrability to target bacteria concerns) is not an option for reasons of toxicity or cost.

The cost issue is especially relevant in developing nations where per capita health expense is relatively low, such as in Georgia. In Georgia, in fact, one practice is to employ phages in concert with the more expensive antibiotics, with antibiotics applied systemically in standard relatively low densities while the phages are applied in high densities locally. Also contributing to low phage toxicity, in comparison to antibiotics, is that phage concentrations are self-regulatory: They are quickly flushed from the body and/or inactivated by the immune system when their host is no longer present. This latter aspect is linked with challenges in administering phage therapy systemically, however, where a large bolus of antigen enters the circulatory system at once. Even if applied locally, or *per os*, phage particles often enter into systemic circulation, which can be viewed as advantageous in terms of phage penetration to localized or more systemic infections. Phage preparations thus are more conservatively and readily applied more locally – intraperitoneally, intrapleurally, inserted directly into a wound, etc. – as opposed to explicitly systemically.

This either topical or less-directly systemic use of phages is less likely to be a problem because the phage move gradually from a local reservoir into the circulatory system and reproduce rapidly when they reach another collection of susceptible bacteria(Gill and Hyman,2010).

8. CONCLUSIONS AND RECOMMENDATIONS

We have reached a critical point in treating infectious diseases: new drugs are not being developed at anywhere near the pace necessary to keep ahead of the natural ability of bacteria to evolve and defend themselves against antibiotics. The result is that some of our most powerful drugs are becoming useless. Phage therapy for eliminating multidrug resistant bacteria is gaining importance. Theabundance of phages in the environment makes it a relatively simple task to isolate phages. Phages, when properly selected, offer the most cost-effective alternative to antibiotics. Bacteriophages' main advantages as therapeutics are their ability to target bacteria of certain strains or species, without any harmful effect on the rest of the bacterial microflora, as well as their self-limited propagation which is controlled by the availability of a sensitive host. Moreover, bacterial antibiotic resistance is not a barrier for phage infection.

Thus based on this the following recommendations are forwarded

- Enhancing wide range of research to accelerate in use of phage therapy.
- Developing effective phages especially for antibiotic resistant bacteria.
- Promote studies on phage treatment modification.

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