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Bacteriophage: Clinical Applications

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Bacteriophages, also called phages are viruses that invade bacteria, disrupt their metabolic activities and kill the bacteria by lysing their cell wall. These characteristics have given phages antimicrobial properties and can also serve as good replacement for antibiotics that have an increased record of multiple-antibiotic resistance. Phage therapy involves the treatment of infectious bacteria with lytic bacteriophage. The antimicrobial properties of phages have obvious advantages. Phages are very selective to their host, and this minimizes attack on normal body flora unlike the commonly used antibiotics, which attack infectious bacteria and normal body micro flora, giving rise to opportunistic secondary infections. Phages act at the sight of action, making the required concentration of phages utilized, while antibiotics traverse the gastrointestinal tract making them prone to degradation before getting to the sight of action, thereby reducing the therapeutic effects. Adverse drug reactions, inflammatory effects and side effects that are associated with antibiotics are not found with phages. Resistance may actually occur with phages, but they have their natural way of evolving another phage to counter it. Phages are environmentally friendly. Some already exist in the body, freely. Lastly, it takes time and costs much to develop new antibiotics, but the production of phages is based on natural selection, isolation and identification of bacteria. Bacteriophage's gene can easily be manipulated by biotechnology approaches. This makes it possible to design bioengineered enzymes (genes) that can have any desired properties. To a large extent, this has addressed the potential clinical disadvantage with the specification of phages to their host bacteria. In this chapter, the utilization of bacteriophages in therapy vis-à-vis antibiotics will be discussed.

Keywords bacteriophage; phage therapy; antibacterial; bioengineered genes

1.0 Introduction

Chemotherapy, a discovery of the early 1900s is currently in a decline era. In fact, it has been projected that we are gradually heading to the "pre-antibiotics" where infections are no longer receptive to antibiotics treatment. This may not be unconnected to the high profile increase in antibiotics resistance with no attendant discovery of novel antibiotics. To fill this vacuum in the therapeutic world, bacteriophages have been tentatively identified as candidates. These bacterial viruses, arguably the most ubiquitous biological group on earth makes use of different bacterial species including the pathogenic ones as their hosts [1-4].

Their use in the treatment of bacterial infections, known as phage therapy was heralded by the works of two scientists: Frederick Twort and Félix d'Hérelle who independently discovered the virus in 1915 and 1917 respectively. In contrast to Twort who abandoned the work, d'Hérelle pursued researches in phage therapy and is remembered for the application of phage therapy in the treatment of dysentery and control of cholera [1]. While the advent of antibiotics led to the premature death of phage therapy researches in the Western world, the subject was relatively pursued in Eastern Europe. Now with the rise of antibiotics resistance and dearth of novel antibiotics, the attention of the world is gradually shifting to the use of bacteriophages as alternative antimicrobials [3, 5].

The antimicrobial properties of phages have obvious advantages over other antimicrobials. Phages are very selective to their host, and this minimizes attack on normal body flora unlike the commonly used antibiotics, which do not discriminate between infectious bacteria and normal body microflora, giving rise to opportunistic secondary infections [3]. In contrast to antibiotics, phages are able to undergo "auto-dosing" - adequate concentrations of phages are promptly produced only when they recognize their target species. With increase in phage population, bacterial cells are sequentially affected until they are cleared in the body [6]. Antibiotics traverse the gastrointestinal tract making them prone to metabolic degradation before getting to the site of action, thereby reducing the therapeutic effects. When their work in the body system has been completed, that is when the target pathogen is no longer present, phages are promptly cleared from the system with no side effects on the patient [1, 6]. Though bacteria may evolve to become resistance to phage therapy, these viruses have their own way of evolving to counter such resistance [7]. In fact, the evolution of bacteria/phage have been rightly described as an "arms race in a predator-prey system" [1]. Unlike their antibiotics counterparts, phage therapy has a high economic feasibility- it takes less time and cost, since it is based on natural selection, isolation and identification of bacteria containing the target phage [1, 8]. Lastly, the genomes of bacteriophgaes can easily be manipulated by biotechnology approaches to design phages that can also be used in the delivery of therapeutic cargos and specific diagnosis of bacterial infections.

In this chapter we give a basic overview of the lifecycle and composition of bacteriophages which can be manipulated for clinical use. The applications of bacteriophages in medicine range from direct therapy using

monotherapeutic or phage cocktails; phage products such as endolysins and holins to delivery of therapeutic cargos and identification of important bacterial pathogens. These areas form the framework of this chapter. We conclude this article by examining the different modes of administering phage preparations, safety concerns of phage therapy and their attendant solutions.

2.0 Basic composition and life cycle

Like all viruses, phages are obligate intracellular parasites with either an RNA or DNA genome but not both nucleic acids at the same time. These nucleic acids may be double-stranded or single-stranded and are enclosed by an intact protein-rich capsid, which among other functions protect the nucleic acids from degradation [1, 4]. For some phages, their capsids are further covered by a lipid-rich envelope. Such phages are said to be "enveloped", while others lacking the envelope are said to be "naked".

Generally, their lifecycle begins with the attachment of the phage to specific receptors on the bacteria surface. This is followed by the penetration and injection of their genomes into the bacterial cytosol [9]. Inside the cell, the viral genome takes over the replication machinery of the host, creating multiple copies of its genome to the detriment of the host bacterium. While some viruses known as the lytic phages are able to rapidly end their infection cycle by lysing the bacteria cells, others known as the temperate or lysogenic phages are able to remain in a quiescent or latent phase by integrating their genomes with that of the host bacterium. Such integrated viral genomes are known as prophages and have been implicated with the resistance of the host bacterium to further phage infection. Some other phages are able to exist in the bacterial cytosol in a plasmid-like prophage form, a phenomenon colloquially known as pseudolysogeny [1, 4]. In contrast to lytic phages which are mostly used for therapeutic purposes, lysogenic phages are rarely used due to their ability to induce phage resistance, toxin production and transduction [10]. Specifically, the virulence of some pathogenic bacteria such as streptococci, clostridia and corynebacteria have been directly linked to lysogeny [2].

According to the International Committee on the Taxonomy of Viruses, there are more than ten different phage families which include but are not limited to myoviridae, siphoviridae, podoviridae [6], members of the order *Caudovirales*. They are the linear double-stranded DNA viruses, with a naked genome and a tailed morphology which may be contractile, non-contractile or short. They represent the most widely applied phages in the treatment of bacterial infections with about 96% of described lytic phages being their members [1, 10, 11].

3.0 Clinical applications

3.1 Monotherapy and phage cocktails

While phage monotherapy involves the use of a single phage preparation in treatment, phage cocktails involves the simultaneous administration of two or more different phage type [10]. There are two scenarios where monotherapeutic phage preparation are used. One is when prior identification of the target bacteria has been done, and two is in the treatment of diseases specifically linked to a particular pathogen. Also, with the discovery of broad-range phages, monotherapeutic phage preparations have been used in the treatment of several infections [1]. Several studies exist on the therapeutic potentials of phage preparations on different clinical models of infections (table 1.0). While phage specificity is very important to the success of the therapeutic procedure, there is another side of the story – phages have a limited host range for infectivity. The restricted spectrum must be identified and understood before the application of phages in medicine. Such studies are time-consuming and may not be economically feasible; herein phage cocktails come to the rescue. It employs two or more phages which may have different host range, allowing for the treatment of the bacterial infection prior to the identification of the specific etiologic agent [7, 12]. It is economically efficient and allows for the use of the phage preparation in some emergency conditions. In the direct clinical use of phages, care should be taken to purify the preparation and screen out lysogenic strains.

Table 1. 0 Direct therapeutic applications of phages.

Bacterial pathogen	clinical infections	Therapeutic phage(s)	References
Staphylococcus aureus	Lung-derived septicaemia	S13'	[13]
	Mastitis	K	[14]
	Rhinosinusitis	CDP[1]	[15]
Chlamydia psittaci	Inclusion conjunctivitis[2]	øCPG1	[16]
Escherichia coli	Diarrhoea	T4	[17]
	Septicaemia	R	[18]
Klebsiella pneumonia	Burn wounds	Kpn5	[19]
	Pneumonia	SS	[20]
Pseudomonas aeruginosa	Lung infection	PAK-P1	[21]
	Gut-derived sepsis	KPP10	[22]
	Otitis	CDP[a]	[23]
	Burn wounds	CDP[a]	[24]
Mycobacterium ulcerans	Buruli ulcer	D29	[25]
Enterococcus faecalis	Sepsis	φEF24C	[5]

[1]Cocktail of different phages; [2]Clinical model of infection was not shown

3.2 Endolysin-Holin system

At the end of their lytic cycle, phages produce two different classes of proteins, holins and endolysins which work synergistically in the lysing of the bacterial cell wall. Though the endolysins lyse the cell wall, they lack a secretory signal factor and cannot access the cell wall without the action of holins. Holins are small hydrophobic proteins which are able to create "holes" in bacterial cell membrane, allowing the endolysins to access the cell wall [3, 26-28].

Depending on their bond specificity in the peptidoglycan layer, endolysins are classified into four different groups: endopeptidases, amidases, transglycosylases, and lysozymes [3, 29, 30]. Though these peptidoglycan hydrolases are endogenously synthesized, recombinant endolysins sometimes known as enzybiotics have found exogenous applications [26, 28]. Due to their species-specificity, they have been used in the de-colonization of the mucous membrane and treatment of experimental model of infections such as sepsis, pneumonia, meningitis and endocarditis [28, 31]. Gupta and Prasad [32] successfully purified an endolysin from phage P-27/HP and showed its ability (about 99.9%) to decolonize the spleens of treated mice from *S. aureus* 27/HP. Witzenrath *et al*. [33] demonstrated the therapeutic potential of endolysin Cp-l in the systemic treatment of fatal *S. pneumoniae*-induced pneumonia in mice.

When applied 24 hours after infection, lysin Cp-l was shown to drastically reduce the pulmonary bacterial load, prevent the development of bacteraemia and led to the rapid recovery of all the treated mice. This results were later correlated by Doehn *et al.* [34] who reported that the intranasal application of endolysin CP-l in a murine model successfully averted bacteraemia with an 80% mortality reduction. LysGH15, a broad-spectrum endolysin purified by Gu and team [35] (2010) prevented the development of a fatal *S. aureus*-induced bacteriamia in a murine model. Schmelcher and coworkers [36] successfully treated a multi-resistant *S. aureus* infection in a mouse model using nine different endolysins which include but are not limited to lysin K, twort, phi11 and phiSH2.

Endolysins are typically composed of two parts: The N-terminal cell wall-hydrolysing domain and the C-terminal cell wall-binding domain [27, 37]. Though there are broad-range endolysins with high thermostability [38, 39], advances in modern biology have led to the construction of two different classes of endolysins with improved host range, hydrolytic efficiency and stability. First is the chimeric endolysin formed from the fusion of the N-terminal domain of a lysin with the C-terminal domain of a different lysin. Such lysins combine the high hydrolytic activity of a lysin with the cell wall binding capacity of another lysin, thus broadening the host range of the lysin. Chimeric lysins synthesized from the endopeptidase domain of streptococcal endolysin λSA2 and the cell wall binding domain of either lysostaphin or LysK was shown to be effective in the treatment of S. aureus-induced mastitis and in the subsequent reduction of S. aureus load in the mammary glands of a mouse model [26]. Singh *et al.* [40] successfully used a chimeric lysin, Ply187AN-KSH3b to prevent endophthalmitis, an ocular infection in a mouse model. Daniel *et al.* [41]

obtained a novel chimeric lysin, ClyS, from the fusion of domains of two different *S. aureus* lysins: catalytic domain of Twort phage lysin and cell wall binding-domain of phiNM3 lysin. Within one hour of application, the modified lysin was found to cause a 2-log reduction in the viability of the tested methicillin-resistant *S. aureus*, with an attendant protective effect against death in a murine septicaemia model.

Another group of synthetic lysins used in the medical world are the so-called artilysins. This innovation comes handy in counteracting the resistance of gram-negative bacteria to endolysin treatment. It is important to note that the lipopolysaccharide outer layer of gram negative bacteria hinders the exogenously-applied lysins from accessing the peptidoglycan cell wall. Artilysins are artificially constructed endolysins used to circumvent this problem. They are formed from the fusion of the N-terminal domain of a lysin with a lipopolysaccharide-penetrating polycationic peptide. Such peptides allow the enzyme to penetrate the outer membranes of gram-negative bacteria, enabling them to affect the peptidoglycan cell wall [42, 43].

3.3 Drug delivery

Through the field of nanotechnology and the technique of phage display, phages have been used as vehicles in the delivery of therapeutic cargos to target sites. Ideally, a drug delivery system should be able to enhance the efficiency of the drug by its controlled release of drugs at different rates, time and target cells [44]. Such systems should also be able to protect the drug from metabolic degradation and decrease the toxicity of the drug to mammalian cells which are not sites of interest. Phages fulfil such criteria for effective drug delivery [2, 42]. They have a non-toxic biodegradability nature and a small size distribution which allows for uniform drug dosing and effective endocytosis. Their ease of modification and mass-production is also an added advantage [45, 46]. Since nucleic acids are highly fragile in body fluids, phages have been projected as ideal carriers to protect infused nucleotide-based drugs and genes from metabolic degradation as they transverse through the body system [2]. Unlike eukaryotic viral groups such as lentiviruses, adenoviruses and retroviruses which have also been used in drug delivery, phages have not be implicated in the disruption of tumor suppressor genes, that is they are not tumor-inducing viruses [2].

As nanocarriers of therapeutic cargos, phages can be modified with various peptides which enable them to recognize target mammalian cells and undergo a complex receptor-mediated endocytosis [47, 48]. This is an important highlight in the modern delivery of drugs and therapeutic genes into target cells. In their classical studies, Poul and Marks [49] effectively engineered phage F5, a filamentous bacteriophage to deliver genes to mammalian cells using endocytosis and intracellular trafficking pathway. Here, the F5 phage was engineered with an antibody fragment (anti-ErbB2 scFv) which enabled it to recognize and bind to the growth factor receptor ErbB2 of breast tumour cells, leading to endocytosis and subsequent expression of the infused genes. Modified phages may have selective affinity for cancer cells than normal cells. For example, when modified with a peptide, SP94, virus-like particles (VLP) present on the surface of MS2 phage was found to exhibit a 10^4- fold higher selectivity for human hepatocellular carcinoma than for hepatocytes and other cells found in the liver [50]. Interestingly, Ashley and co-researchers [50] further found out that the modified MS2 phage VLPs loaded with drugs such as doxorubicin, cisplatin, and 5-flurouracil exhibited selective cytotoxicity to the carcinoma cell line even at drug concentrations less than 1 nM, while its encapsidation with a siRNA cocktail led to induction of apoptosis and growth arrest of the HCC cell line. This study demonstrates the unique ability of some phage VLPs such as that of MS2 to exhibit multivalent peptide display and carry diverse groups of therapeutic agents. More recently, Sun and team members [51] effectively delivered a therapeutic microRNA using a modified non-replicative phage PP7 virus-like particles. The PP7 VLPs was modified with a cell-penetrating peptide which allowed them to effectively target tumorous liver cells and was able to protect the microRNA from the hydrolytic activity of ribonucleases.

In addition to covalent and non-covalent methods used in the modification of phage capsids, they can also be ligated with other delivery systems for enhanced drug delivery [52]. Such complexes can be used in cancer therapy for enhanced internalization and uptake of drugs by cancer cells. When coated with poly (caprolactone-b-2-vinylpyridine) polymers, Suthiwangcharoen *et al.* [53] showed that folate-conjugated M13 phage capsid was an effective nanosized vehicle for the delivery of antitumor drugs such as doxorubicin.

3.4 Phages in clinical diagnosis

Exploiting their high host specificity, phages have been used in the detection and diagnosis of bacterial infections [12, 54]. Such diagnostic method involves the use of a bacteriophage which recognizes and binds to a specific bacterial pathogen, leading to the identification of the pathogen in the clinical sample. The phage-bacteria binding can be visualized using traditional plaque assay, infusion of reporter genes and use of fluorescent-labelled phages [12, 55]. A detailed study of phage-based diagnostic methods has been reviewed in literature [54, 56]. In the reporter gene-based method, a recombinant mycobacteriophage is produced by incorporating a reporter gene such as luciferase gene into its genome. When introduced into a clinical sample, they specifically infect the target pathogen, leading to the expression of the reporter gene. In the case of phage modified with luciferase gene, light emission is used for endpoint detection.

For the traditional plaque assay, the amplification of the phage instead of the expression of a reporter gene indicates the presence of the tubercle pathogen. Here, the phage infects the target pathogen present in the clinical sample, leading

to the production of progeny viruses. The progeny phages are detected in the medium by the introduction of sensitive non-pathogenic tubercle bacteria, leading to the formation of lawns in agar plates. This gives an overview of the concentration of viable tubercle bacilli present in the original sample.

Phages have advantages over other clinical diagnostic tools due to their relatively inexpensive nature of production, high specificity for target bacteria and their unique ability to identify only viable pathogens [42, 54]. Interestingly, phage-based diagnostic technique has an added benefit over other methods since it is able to incorporate antibiotics susceptibility testing in its protocol. Here, cell suspension of the pathogen is incubated simultaneously in the presence or absence of the test antibiotic. This is followed by the introduction of the specific bacteriophage which binds to the pathogen. A comparison of the light emission or plaque formation in the presence or absence of the antibiotics gives an overview of the resistance or susceptibility of the organism [54]. This allows for the rapid antibiotics susceptibility profiling of the test pathogen.

Though phage-based diagnostic technique is mostly used in food biocontrol, it has also found application in the medical world especially in the detection of mycobacteria in sputum sample. Unlike other tuberculosis diagnostic methods which are hampered by the slow-growing nature of the pathogen, phage-based techniques using mycobacteriophages have been shown to be effective in the rapid detection of the tubercle pathogen in clinical samples.

Furthermore, such tests find optimum usage in resource-limited regions of the world which bears a huge burden of tuberculosis since they are economically feasible and do not require complex microbiological equipment [54]. Apart from its use in the diagnosis of tuberculosis [57-59], phage-based tests have also been used in the diagnostic detection of *Bacillus anthracis* [60], *Yersinia pestis* [61, 62], dysentery-causing *Shigella* spp. [63] and other pathogens.

3.5 Anti-biofilm activity

Biofilms, extracellular polymeric matrices, protect pathogenic organisms from the hostile external environment. In fact, they have been rightly described as “microbial shelters” which protect the composing microorganisms, usually in high densities, from the antagonistic activities of antibiotics [64]. They are responsible for the resistance of the bacteria to conventional antibiotics therapy and are implicated for the high rates of nosocomial infections when formed on medical devices such as catheters, contact lenses, cardiac valves, endotracheal tubes, dental plaques and other indwelling devices [1, 29, 65, 66]. Phages have been used in the treatment of biofilm-related infections. They have a good number of advantages over other conventional biofilm disrupting agents such as antibiotics. While the pharmacokinetics of most antibiotics tend to be independent of the concentration of the pathogen, the concentration of the phage increases with rise in the bacterial population – its pharmacokinetics varies with the bacterial cell concentration [67, 68]. Also, phages attack dormant or “persister” cells found in biofilms with their lytic activity being activated when the cells resume active metabolism [68]. Unlike other antimicrobials, phages have been arguably noted to maintain their anti-biofilm activity regardless of the age of the biofilm [65].

In addition to their encoded endolysins, phages produce depolymerases which are able to degrade the polysaccharide-rich matrices of biofilms, allowing the permeation of the phage (or other antimicrobial in the case of combination therapy) into the deeper layers of the biofilm [65, 66]. These depolymerases seem to be a common feature of tailed bacteriophages and have been demonstrated in the tail spikes of *Pseudomonas putida* phage AF where they effectively disrupted their biofilms [69]. In their classical work, Shen *et al.* [70] reported that PlyC, a streptococcal-specific endolysin was more efficient than the tested antibiotics (penicillin and erythromycin) in the direct lysing and death of *Streptococcus pyogens* cells found within a biofilm matrix. This interesting result was suggested to be due to the ability of the endolysin to exhibit bactericidal activity by hydrolysing more than one bond in the cell wall. More recently, Schuch *et al*. [71] found out that bacteriophage lysin CF-301 totally removed *S. aureus* biofilms from the test catheters within 1 h, with eradication of the released bacteria within 6 h. Phage therapy has also been applied in the disruption of *S. aureus* biofilms and the attendant treatment of chronic wounds caused by such biofilms [72].

Though phages and their products have been reported to have extensive biofilm-disrupting activity, they can be used synergistically with other antimicrobials to target biofilm-forming bacteria [1]. A good example is the study done by Rahman and his group [73] who reported that co-application of phage SAP-26 and rifampicin drastically disrupted *S. aureus* biofilms leading to a cell survival of only 0.001%. Recently, combination therapy of phage vB_PaeM_P6 with ciprofloxacin was shown to eradicate biofilms formed by *Pseudomonas aeruginosa* [74]. Lysostaphin has been co-administered with lysin CF-301 in the eradication of *S. aureus* biofilms formed on different medical devices [71].

4.0 Clinical mode of administration

Although rarely discussed in literature, the success of phage therapy depends among other things on timing of treatment, dosage and delivery route. Depending on the target site of bacterial infection, different routes have been used in the administration of therapeutic phages into the patient [10, 11]. Ideally, routes for phage therapy are chosen in order to ensure the effective delivery of the phage to the target site of infection [75]. Clinical administration of phage therapy may be broadly divided into two groups which are parenteral and local administration [44]. Parenteral route involves the systemic administration of the phage preparation from where it rapidly circulates into various organs of the body

[11]. It is the most widely used route and is most likely to elicit immune responses. It may be intramuscular (when administered through the muscles), intravenous (through the veins), intra-arterial (through the arteries) or subcutaneous (underneath the skin). Local administration involves the direct application of the therapeutic phages to the target organ of action. It may be done orally (for gastrointestinal infections), inhalation (for respiratory infections) and topically (for skin-related infections). Topical phage therapy has been used in the treatment of otitis, a chronic ear infection caused by *P. aeruginosa* [23]; chronic wound infections in a model simulating diabetes mellitus [76]; colonized burn wounds [77] and *P. aeruginosa*- induced keratitis [78].

Respiratory infections are usually chronic in appearance due to the inability of the orally or parenterally administered drugs to access the deeply buried pathogenic species in the airways [79]. Of recently, Bodier-Montagutelli *et al*. [10] reviewed inhaled phage therapy as a very effective tool in the treatment of respiratory infections caused by bacteria. It involves the direct introduction of the therapeutic phage into the lungs, through the airways. This ensures the high proximity of the phage to the target respiratory pathogen, with attendant heavy losses on the bacteria population.

Technically, inhalable phage formulations (due to their dry state nature) have a higher shelf-life than liquid phage preparations used in other routes of delivery. Thus, they can be stored for a longer period with little or no loss of stability. However, this mode of administration suffers a number of technical setbacks dominant among which is the inactivation of the phage due to mechanical stresses which they face during the stringent formulation process. One of the major techniques used in the formulation of inhalable phage powders is spray-drying, a technique which to the best of our knowledge was first reported by Matinkhoo and his group [7]. Here, the phage is dispersed in excipients of inhalable particle sizes with subsequent dehydration into dry powders. In a very recent study, Leung *et al.* [75] successfully produced an environmentally-stable inhalable powder by spray-drying phage PEV2, a phage active against antibiotics-resistant P. aeruginosa, into powder matrices containing trehalose, mannitol or L-leucine. Trehalose was found to be a better stabilizer than other tested agents, with a trehalose-containing matrix (more than 40%) effectively preserving the pulmonary phage formulation for one year at a storage condition of 4 ºC. This agrees with a previous work done by Vandenheuvel *et al.* [80] who showed trehalose to be an effective excipient in the protection of phages from the shear stress generated during the spray-drying process of producing inhalable phage powders.

Though oral phage therapy is used in the treatment of gastrointestinal infections, a drawback to their clinical application is the susceptibility of the phage protein coat to low pH of the stomach. To remedy this situation, different approaches have been demonstrated. This include the neutralization of the stomach pH prior to oral administration of the phage preparation, bio-prospecting and bio-engineering of acid-tolerant phages as well as the encapsulation of the therapeutic phage in polymeric particle. The later approach has received much attention in the scientific world. This may be due to their feasibility with minimal side effects on the patient. Using an alginate-based microsphere in the encapsulation of *S. aureus* phage K, Ma *et al.* [81] reported an enhanced survival of the phage in a simulated gastrointestinal environment. While the free phage was completely destroyed in such environment, the encapsulated phage showed only a 2.4 log unit reduction in viability when incubated for 1h. Remarkably, a similar result was reported by Tang and co-workers [82] who demonstrated the high stability of phage K to acidic conditions when encapsulated in alginate-whey protein microspheres.

5.0 Safety concerns and challenges

The lysis of bacteria by phages may lead to the release of their toxic lipopolysaccharide outerlayer and pyrogens into the body. This glycolipid is an endotoxin which may elicit different adverse effects such as septic shock and toxic shock syndrome in the patients. To remedy the situation, proper quality control measures and downstream processing are carried out to removal pyrogens and other bacteria cell lysates in phage preparations [10]. Such purification techniques include ultrafiltration, Casecium-chloride density centrifugation and chromatography. Also, following the discovery of the "unusual" filamentous phages which release new particles without lysis of the bacterial cell, non-replicative genetically modified therapeutic phages have been used [28, 83-86]. Such phages efficiently exhibit their lethal activity without bacterial cell lysis. This represents a huge advancement in the field of phage therapy.

In contrast to other antimicrobial agents, phages are "live" preparations [87] which have diverse pharmacokinetic properties. This forms a strong barrier to the regulatory approval and clinical usage of phages in humans [10]. Due to this, proper delineation of phage pharmacokinetics should be carried out by scaling "preclinical" phage therapy to clinical trials. When stored over time, phage preparations like other biological agents lose their storage stability and subsequent efficacy. This concern is addressed by the composition of the phage preparation with stabilizing agents such as sugars, surfactants and other polymers which protect the phage from dehydration and denaturation [10, 81].

Different ways in which bacteria acquire resistance to phage infections has been reviewed by Lu and Koeris [12]. These include modification of their receptors preventing initial phage adsorption to their surfaces; blocking the release of phage genomes into their cytosol; lysogeny and the use of restriction modification system which targets the phage genome. In contrast to other antimicrobial agents, phages can evolve to counteract the bacterial resistance. Also, due to the established notion that different phages may have different mechanism of killing their host bacteria, phage cocktails can also be administered, minimizing the chances of bacterial resistance to the therapeutic procedure. Bacterial resistance can also be tackled by administering the phage preparations in combination with other antimicrobial agents

[12]. Another challenge in the field of phage therapy is the ability of some phages to transfer bacterial genes from one host to another. This phage-mediated process, known as transduction involves the random packaging of bacterial genes into the phage genome at the late stages of lysogeny. It is obligately related to the virulence and toxicity of several pathogenic bacteria such as *Corynebacterium*. To solve this problem, the genomics and proteomics of prospective therapeutic phages are thoroughly studied to make sure that they are not lysogenic strains.

6.0 Conclusion

The rising importance and applications of bacteriophages in the medical world cannot be over-emphasized. From their use in the treatment of infections, modern biotechnology advances have led to their use in the delivery of therapeutic cargos such as drugs and genes, and in the specific diagnosis of infections. Depending on the target site of action, bacteriophages can be applied parenterally or locally. These different routes of administration have different effects on the therapeutic efficacy of the administered phages and surely needs more exploration. The private and public sector should come together and explore the seemingly unlimited clinical potentials of bacteriophages for the benefit of humanity.

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