Bacteriophages: A New (Yet Old) Weapon Against Infections

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Stephen K. Mathew and Reba Kanungo

8.1 Introduction

The alarming spread of antimicrobial resistance, identified by the WHO as a global threat, is drawing healthcare into the post-antibiotic era [1, 2]. Healthcare-associated infections (HAIs) are among the top five leading causes of morbidity and mortality in industrialized countries [3]. Infections by extensively drug-resistant bacteria are being increasingly reported: just one, methicillin-resistant *Staphylococcus aureus* (MRSA), kills more Americans every year than emphysema, HIV/AIDS, Parkinson's disease and homicide combined [4, 5].

Bacteria are extremely adept at developing mechanisms to survive hostile environments. This is underscored by the isolation of *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* strains resistant to even silver salts present in antibacterial preparations [6].

Development of new antibiotics has been hampered by rising costs of drug development coupled with relatively low returns of investment due to the rapid development of resistance to the new agent [7, 8]. In the face of ever-increasing resistance, this dearth of research and development has been called "the perfect storm" [9]. With only a few large multinational pharmaceutical companies involved in antibiotic discovery, the Infectious Diseases Society of America (IDSA) launched the "10 × '20 Initiative" with the aim of supporting the development of ten new systemic antibiotics by 2020, which was successful in identifying seven novel agents targeting Gram-negative bacilli [10, 11]. However, resistance against agents such as ceftolozane-tazobactam has already been observed [10, 12].

Surgical site infection (SSI) currently ranks as the most common cause of nosocomial infection, accounting for 31% of all hospital-acquired infections, and is associated with a mortality rate of 3% [13–16]. The additional cost of managing an SSI exceeds $20,000 per admission, and more than $90,000 per infection where an antimicrobial-resistant organism is responsible [14, 17]. The economic burden of antibiotic-resistant infections to the US healthcare system is estimated to be more than $20 billion each year [5].

Postoperative infection, though rare following plastic surgery, can significantly affect the cosmetic outcome, which also increases the risk of malpractice suits [13, 18]. It complicated approximately 1% of clean surgeries and 4% of clean contaminated surgeries [19]. As cosmetic surgery becomes increasingly popular, SSIs, particularly

S. K. Mathew (✉)
Department of Microbiology, Believers Church Medical College, Thiruvalla, Kerala, India

Department of Microbiology, Pondicherry Institute of Medical Sciences, Kalapet, Puducherry, India

R. Kanungo
Department of Microbiology, Pondicherry Institute of Medical Sciences, Kalapet, Puducherry, India

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those caused by MDR bacterial strains and are difficult to treat, become a pressing issue [18].

The "perfect storm" threatening to derail much of the progress in medicine could also be an opportunity for alternate antimicrobial modalities to emerge [20]. One of these modalities currently generating significant interest was discovered more than a century ago, in the pre-antibiotic era. Felix d'Herelle discovered bacteriophages and also realized their potential as antibacterial agents, following which it was used to contain human infections in several countries. The new world of antibiotics that was soon discovered, and the promise it brought—the end of infectious diseases—however, dwarfed much of the interest in phage therapy. Acceptance of bacteriophages as a therapeutic modality was further hampered by poorly designed studies generating conflicting results. Bacteriophage preparations available in the early twentieth century, apart from being of questionable quality, were also being marketed for pathologies not necessarily caused by bacterial infections [20]. While phage research died out in the western world, it remained an active area of research and use in parts of Eastern Europe and former Soviet Union.

Following the rediscovery of phages by the western world, the first randomized controlled trial (RCT) which was published in 2009 saw researchers treat chronic otitis and venous leg with bacteriophage-based preparations [21–23].

8.2 Current Status of Skin Infections in Plastic Surgery

Between 2006 and 2009, a conservative estimate of 1.9% of surgical procedures in the United States was complicated by surgical site infections (SSIs) [17]. In 2006, the Surgical Care Improvement Project (SCIP) drafted nine measures to reduce surgical complications; of these, six focussed on prevention of postoperative infections [24]. However, a decade later, despite a high level of compliance with the core measures, infection rates remain largely unaffected and have only been further complicated by resistance to commonly used antimicrobial agents. SSIs now reportedly complicate over a tenth of inpatient and outpatient procedures [25–27].

The incidence of wound infections following breast plastic surgery, considered a "clean surgery", ranges from 3% to 30%, and is more than 50% among women undergoing reconstruction after treatment for breast cancer [13, 28]. Wound care is particularly problematic in burn patients, in whom 50% of all deaths are due to resultant infections [29, 30].

A rise in infective complications has been accompanied by a dramatic increase in the use of antibiotics. In plastic surgery alone, there has been up to a 200% increase since 1975 [31]. Prophylactic antibiotics are widely used even in procedures, such as rhinoplasties, that are rarely complicated by postoperative infections [32]. The overuse of antibiotics, due to lack of consensus, specific guidelines and a fear of litigation, has further contributed to antimicrobial resistance, and could paradoxically make empirical prophylactic antibiotics ineffective [32, 33].

8.3 History of Bacteriophage Therapy

While anecdotes of river waters possessing the ability to cure infectious diseases can be found in historical and religious texts, the idea of bacteriophages and their action as an antibacterial can be traced back to 1896, when British bacteriologist Ernest Hankin suggested the presence of an unidentified, heat-labile, filterable substance in the rivers Ganga and Yamuna in India with antibacterial activity against *Vibrio cholerae* which possibly helped to limit the spread of cholera [34].

Frederick Twort [34], a bacteriologist from England, reported a similar phenomenon almost 20 years later and advanced the possibility of this being due to a virus. Twort, however, did not pursue this finding and it was another 2 years before Felix d'Herelle, a microbiologist at the Institut Pasteur in Paris, France, officially discovered bacteriophages. He observed the bacteriophage phenomenon in 1910, in Mexico, while studying methods of controlling an epi-

zootic among locusts. D'Herelle, who a few years later was called to investigate an outbreak of severe dysentery among French troops, stationed on the outskirts of Paris, observed the appearance of small, clear areas on agar cultures when Shigella strains isolated from the patients were incubated with bacterium-free filtrates from the faecal samples. He termed these clear areas as "plaques", and proposed the name "bacteriophage" for a "virus parasitic on bacteria" [34]. Not long after, d'Herelle carried out what could be labelled a phase I trial when he along with his family members ingested phage preparations to demonstrate their safety before administering it to children with dysentery at the Hopital Des Enfants-Malades, Paris, all of whom exhibited signs of recovery [5]. However, the results of these studies were not immediately published and, therefore, the first reported use of phages to treat infectious diseases in humans came from Bruynoghe and Maisin in 1921, who used bacteriophages to treat staphylococcal skin disease [34, 35].

D'Herelle continued his studies on phages and some of his most sensational work was carried out in India, where he visited in 1927. There were reportedly no deaths among cholera patients in Calcutta and Lahore who received d'Herelle's phage preparations orally and intravenously, in contrast to a mortality rate of 40% among patients who received conventional injections of fluids and salts [36, 37].

Contrasting these successes, several scientists highlighted d'Herelle's failure to meet scientific standards for research. Combined with the introduction of penicillin to medical practice, this led to dwindling interest in d'Herelle's research [38].

Commercial phage preparations began with d'Herelle as well, whose laboratory produced at least five phage preparations: Bacté-coli-phage, Bacté-rhino-phage, Bacté-intesti-phage, Bacté-pyo-phage, Bacté-staphy-phage—marketed by Societé Francaise de Teintures Inoffensives pour Cheveux (now, L'Oreal). Therapeutic phage preparations began to be available in the United States since the 1930s, with companies such as Eli Lilly and Abbott Laboratories taking an interest. Commercial production, however, was plagued with quality control issues: d'Herelle also reported that some preparations being marketed contained no detectable biologically active phage [37]. Though commercial production in the Western world declined with the advent of antibiotics, phage preparations were available in France till 1978 at d'Herelle's company, and at the Institut Pasteur till the 1990s [5, 37]. Phages continued to be used therapeutically in Eastern Europe and the former Soviet Union, centred around the Eliava Institute of Bacteriophages, Microbiology and Virology in Tbilisi, Georgia, and the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland [34]. The former was focussed on phage cocktail formulation and production (the Eliava Institute had a production capacity of up to two tons per week), and the work at the latter has been extensively documented [37].

8.4 What Is a Bacteriophage?

Bacteriophages are essentially viruses; as obligate parasites, they infect, replicate within and finally lyse the bacterium [20]. Over 6000 different bacteriophages have been discovered, which have been classified into 13 families depending on morphology, type of nucleic acid, and presence or absence of an envelope. About 96% of the discovered phages are "tailed", possessing an icosahedral head and a double-stranded DNA genome. Tailed phages, which comprise the order Caudovirales, are classified into 3 families based on the morphological features of the tail: Myoviridae (contractile tail), Siphoviridae (long, non-contractile tail) and Podoviridae (extremely short tail). The remaining 4% of the phages, classified into 10 families, may contain single-stranded or double-stranded RNA or DNA. These phages are cubic, filamentous or pleomorphic. Most therapeutic phages are tailed; some cubic and filamentous phages have also been used for therapy [21, 39].

Bacteriophages attach to receptors on the bacterial surface via tail fibres or base plate spikes, following which they inject their genome into the cell [40]. The nature of the receptor, its chemical

composition and spatial configuration, along with the structure of viral-receptor binding proteins play a major role in stabilizing the bacterial cell-bacteriophage interaction [41]. These receptors might be the same antigens determining the serotype of the bacteria, or transport channel proteins, or pili [7]. Importantly, receptor binding confers specificity on the bacteriophages. Termed the host range, this specificity is typically narrow—limited to a single bacterial species, or to a few strains within a species, or even a single strain.

Phages can also be divided roughly into two groups, according to their life cycle: lytic or lysogenic. In the lytic cycle, the bacterial cell machinery is hijacked to assemble and package progeny phages, which are released following death of the host cell and its rapid lysis with the help of holins and lysins [40]. Phages with a large burst size—the number of progeny phages released from each infected bacterial cell—are preferred for use in therapeutics [7]. Temperate phages undergo lysogeny, where the phage genome integrates with the bacterial genome and are transmitted vertically through successive generations of the bacteria. The genome of temperate phages may encode transmissible bacterial virulence factors, as seen with Corynebacterium diphtheriae where only isolates that harbour tox^+ phages produce diphtheria toxin [42]. At the same time, host genes for virulence and toxin production may be packaged into the bacteriophages during replication, which may in turn be transferred to other bacteria. As a result, temperate phages are thought to be less suitable than lytic phages for use in therapeutic preparations. However, it may be possible to inactivate genes responsible for lysogenicity and toxin production by genetic modifications, overcoming a disadvantage of lysogenic phages [39].

8.5 Why Should We Consider Bacteriophage Therapy?

Bacteriophages are a potent, natural antibacterial capable of inducing rapid bacterial cell lysis [21]. They are also ubiquitous, with up to 1×10^8 particles per gram of soil, and can be readily isolated from various environments. It is estimated that they destroy one-half of the bacterial population worldwide every 48 h [40]. Billions of years of this co-evolutionary predator-prey relationship have made bacteriophages a potentially rich source of antibacterial agents [29, 40].

Strain specificity, briefly mentioned earlier, allows for targeted therapy, limiting the deleterious effect on the normal microbial flora. This can help prevent adverse effects associated with antibiotic use, such as *Clostridium difficile* colitis, a leading cause of nosocomial diarrhoea particularly associated with the use of cephalosporins and clindamycin [33]. Bacteriophages also have little or no effect on eukaryotic cells, thus staving off more of the adverse effects associated with antibiotic use [21, 29]. Application in the nose and sinuses in an animal model did not alter the normal architecture of the mucosa [43]. Oral administration in patients with diarrhoea did not lead to adverse events [44, 45].

An added advantage, in contrast to antibiotics, is that the concentration of bacteriophages increases after reaching the site of infection due to self-replication [46]. As a result, the required dose of phages would generally be much less than that of antibiotics [47]. Economic considerations also favour bacteriophage therapy over conventional antibiotics, as the cost and complexity of developing a phage system is less than that of developing a new antibiotic [8]. While it is unlikely that bacteriophages will replace antibiotics, phage therapy could decrease antibiotic resistance by reducing the need for antibiotics [29]. Phages can also find use in situations where the necessary antibiotics are contraindicated, such as nephrotoxic antibiotics in patients with impaired renal function [37].

8.6 What Is the Evidence that Bacteriophages Work?

While bacteriophages as a therapeutic option failed to take off in the United States and Western Europe particularly following the discovery of antibiotics, clinical research with bacteriophages

continued in the former Soviet Union and Eastern Europe. These studies were published primarily in non-English languages, and as a result, not readily available to the global scientific community [34]. Interest in phages in the Western world was partly rekindled by the work of Smith et al. who demonstrated the effectiveness of a single intramuscular dose of phage in potentially lethal infections in animals by *Escherichia coli*. This was in contrast to the need for multiple doses of antibiotics such as tetracycline, ampicillin and chloramphenicol to control the infection. The emergence of phage-resistant strains of *E. coli* over the course of the experiments was however noted [48, 49].

Numerous experiments studying various infection models (bacteremia, central nervous system infection, sinus infection, lung infection, urinary tract infection, osteomyelitis, skin and wound infection, including burns) caused by bacterial pathogens such as *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* have since been conducted. Bacteriophage therapy decreased mortality in several studies. Where it was compared to antibiotics as controls, a more positive outcome was observed. No adverse effects were observed in mice following inoculation with high doses [21, 29, 30, 39, 43]. Phage treatment was shown to improve survival in mice infected with methicillin-resistant *Staphylococcus aureus* (MRSA), and lethal doses of *Vibrio vulnificus* [29, 50, 51]. Bacteriophages have also been shown to be effective against *Yersinia pestis*, responsible for plague, and against Burkholderia pseudomallei, a Category B bioterrorism agent that causes melioidosis [52, 53].

There is currently a lack of consensus on the most effective route of administration to target specific infections. Where some studies suggest that aerosolized formulations of phages are effective against respiratory infections, others have found systemic administrations to yield better access to bacteria in the lungs [54, 55]. This is also an important consideration in severe skin and soft tissue infections with a propensity to progress to septicaemia. Phages administered systemically offered better protection than when administered locally in a mouse burn wound model [30]. Orally delivered phages were effective against gastrointestinal infections caused by *E. coli* and *Campylobacter jejuni*, but concerns of phage inactivation due to gastric acidity need to be addressed [54].

Bacteriophages can also help to tackle biofilms which prove to be a significant challenge to conventional treatment. Biofilms are commonly associated with chronic, refractory infections, due to indwelling medical devices, and can be a thousand times more resistant to antibiotics than free-floating bacteria [29]. Treatment of silicone catheters with bacteriophage significantly reduced biofilm formation by *Staphylococcus epidermidis*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis* [54, 56]. Bacteriophages have several advantages over antibiotics in treating infections caused by biofilms. Replication at the site of infection allows for a high concentration of phages on the biofilm; they are able to infect dormant cells within the biofilm; and phages may possess or induce the bacterial cells to express enzymes capable of dissolving the biofilm matrix [20]. Phage treatment has been shown to significantly reduce biofilm biomass and cell density in experimental models [29].

Lysin, a phage genome-encoded protein expressed by tailed phages, which enables liberation of progeny phages from infected bacteria, is also a candidate therapeutic agent against Gram-positive bacteria because of its ability to destroy peptidoglycan, a vital component of the cell wall [39].

Bacteriophages have complex pharmacokinetics that are yet to be fully elucidated. Most researchers observed that phages afforded best protection when given within a few hours of bacterial inoculation, but computerized models have predicted that inoculations given too early could either be less effective or fail completely [51, 57, 58]. Paradoxically, some antibiotics can even diminish the effectiveness of phages [58]. Available data from animal experiments suggests that phages enter the bloodstream following a single oral dose within 2–4 h and are found in the internal organs within 10 h. Phages were

preferentially compartmentalized to the liver and spleen, irrespective of the route of administration [29]. In the human body, administered phages can remain for up to several days [5]. A better, if not complete, understanding of the behaviour of bacteriophages in vivo is necessary to achieve consistent and predictable results with bacteriophage therapy.

8.7 Concerns with Bacteriophage Therapy

Since phages capable of infecting across bacterial species or genera are few in number, rapid and precise identification of the pathogenic bacteria is necessary in order to select an appropriate bacteriophage from an established phage library [39]. Such an individualized approach has been by and large successfully followed in Poland [37]. Use of phage cocktails targeting commonly encountered bacterial species and strains can potentially tackle this shortcoming. However, these cocktails would have to be re-formulated regularly taking into account prevalent species and strains [21]. Even when mixed to form cocktails, the host range can remain relatively narrow [37]. This also limits the role of bacteriophages in empirical therapy [7]. Experiments have extended the host range of phages through genetic modifications that allow them to overcome barriers to adsorption and infection [59]. There is also much to be understood of the interaction between phages and the target bacterial hosts at the site of infection, as opposed to under laboratory conditions [60].

Phages administered intravenously can activate the immune system. Subsequently, phage titres may fall due to innate immunity and phagocytosis in the blood and liver. While non-neutralizing antibodies have been observed following certain phage injections, clinical and animal trials have not demonstrated serious immunological reactions. Long-term intrasinus application of phages did not alter the local profile of immune cells in an animal model [43]. The immunological response against every phage considered for parenteral therapy, however, would need to be studied [7]. The large size of the phage particles, when compared to antibiotic molecules, also limits the concentrations that can be achieved in therapeutic preparations—solutions may become viscous at high concentrations, more than 10^{13} phages per mL [7]. Models created to calculate dosage requirements would need to take into account the complex pharmacokinetics of bacteriophages [47].

Some bacteriophages, though mostly temperate phages, enhance virulence by transferring genetic elements vital to the bacteria. The ability to produce exotoxin in *Vibrio cholerae* is carried and transferred by phages, as is Shiga toxin production in *E. coli*, as well as virulence determinants in *P. aeruginosa*, *Shigellae* and *S. aureus* [20, 61]. This potential problem with therapeutic bacteriophages may be overcome by selection of phages incapable of such transfer, or by genetically modifying them. The genome sequence of therapeutic phages needs to be characterized, which would also help to confirm the absence of undesirable genetic elements. The safety and efficacy of phages should also be demonstrated [20, 39, 40, 46].

A possible side effect of phage therapy, also seen with bactericidal antibiotics, is the release of cell wall components which are mediators of septicaemia, such as endotoxins from Gram-negative bacteria [62]. Patients receiving phages have occasionally experienced right hypochondrial pain and fever a few days into treatment, possibly due to the release of endotoxins [63]. Genetically modified, non-replicating phages designed to digest bacterial genomic DNA kill bacteria with minimal release of endotoxin. The survival rate of mice infected with *P. aeruginosa* was significantly higher with non-replicating phages that do not cause endotoxin release, than with lytic phages; this was also correlated with lower levels of inflammation [62].

As with antibiotics, the development of resistance by the bacterial targets could blunt the efficacy of phages. Resistance to phages is often due to changes, as a result of mutations or acquisition of genes, in the receptors on the bacterial surface [30]. However, phages rapidly co-evolve with bacteria and bacteriophages capable of overcoming protective bacterial systems have

been isolated [7, 39]. Phage cocktails effective against various bacterial strains and possible mutants arising during therapy could pre-empt the rise of resistance [54]. Bacteriophages can multiply within bacteria only if their density exceeds a threshold [47]. On the other hand, a higher-than-necessary concentration would lyse the target bacteria before secondary phage multiplication can be initiated, necessitating multiple doses to eradicate infection [47]. Dosage would also depend on duration of infection at initiation of therapy. Phage preparations administered up to 10 days after infection have been successful [54].

Therapeutic preparations will need to be stable and viable during transportation and storage [40]. Purified phages remained stable for up to 2 years when maintained at 4 °C [52, 53, 64]. Further research is required on phage delivery formulations, and on long-term stability of the phages within formulations [54].

The pharmaceutical industry has largely stayed away from phage therapy probably because it does not see large investments being profitable [60].The risk of mutations developing during the course of therapy also challenges large-scale production as it would require rigorous monitoring [46]. Institutes such as the Eliava Institute, Georgia, and Queen Astrid Military Hospital, Brussels, Belgium, have shown that small-scale production of bacteriophage cocktails, following strict quality-control protocols, is possible [64].

Current regulations requiring full clinical trials for each therapeutic bacteriophage make it difficult for bacteriophages to find their way to routine clinical use. While stringent legislation is necessary for any therapeutic product licensed for human use, factors unique to phages need to be taken into account. Regulatory authorities would need to discuss and consider whether phage therapy merits a distinct set of rules [7, 46].

8.8 Non-human Uses of Bacteriophages

Strain specificity has an already established use in the laboratory in typing systems used for identification of bacterial strains and newer diagnostic tests such as KeyPath (MicroPhage, Inc., Longmont, Colorado) to rapidly identify MRSA from blood cultures [65]. The Eliava Institute has been using phages to track enteric pathogens in the environment, and for rapid detection of anthrax and brucellosis [66].

Anti-Listeria phage cocktails were among the first phage products to obtain a Generally Recognized as Safe (GRAS) status from the United State Food and Drug Administration (FDA) [59]. ListShield™ and LISTEX™ P100 are marketed as food additives intended to disinfect processed poultry products and meat. Omnilytics, Inc. (US) specializes in supplying customized phage preparations (Omnilytics' Agriphage™) tailored against the prevalent crop pathogens for agricultural use [67]. *Staphylococcus aureus* phage lysate Staphage Lysate® has been shown to be effective in treating and controlling recurrent pyoderma in dogs [68].

8.9 Human Applications of Bacteriophages

Work with phages carried out in the 1930s report successful treatment of skin infections, surgical infections, typhoid fever, Salmonella and Shigella spp.-related colitis, septicaemia, and urinary tract infections [21, 69]. One of the largest studies, involving 30,769 children, on the effectiveness of phages against bacterial dysentery was conducted in Tbilisi, Georgia, in 1963–1964. Children living on one side of the streets were given anti-Shigella phages orally and those on the other side served as the controls. The incidence of dysentery was 2.6-fold higher in the control group [70].

Numerous reports of successful topical applications of phages, particularly from Eastern European countries, are available [54]. Oral administrations may be useful in fighting enteric infections due to *C. difficile* [40]. Nestlé Research Centre and other subsidiaries of Nestlé S.A. have conducted RCTs on patients, including children, suffering from diarrhoea [44, 45].

The first fully regulated, placebo-controlled, double-blinded, randomized Phase I/II trial on phage therapy was conducted in the UK in 2009

on patients suffering from chronic *P. aeruginosa-otitis*. A single local application of a cocktail of phages (Biophage-PA) resulted in decreased colony counts on culture, improved symptoms and clinical indicators, without adverse reactions [23].

Much of the published research on human application of phages is from case studies subject to experimenter's bias [37]. This can be traced back to d'Herelle's first known use of phages at the Hospital Des Enfants-Malades where phages were administered to all the sick patients without a placebo group [5]. In order to conclusively demonstrate the consistent efficacy of phages, more double-blind, randomized controlled trials, complying with regulatory and ethical guidelines, are required for greater acceptance of phage therapy [71].

8.9.1 Bacteriophage Therapy for Skin Infections

Topical application of bacteriophages is the most studied route of administration. Phages have been successfully used against ulcers, pyogenic infections, burns, and wounds [22, 37, 63, 72].

PhageBioDerm®, a commercially successful biodegradable wound dressing consisting of a stabilized hydrogel system impregnated with ciprofloxacin, benzocaine, chymotrypsin, bicarbonate, and 6 lytic phages against *S. aureus*, *Streptococcus spp*., *P. aeruginosa*, *E. coli*, and Proteus spp., was approved for human use in Georgia in 2000 [40, 54]. Studies have reported successful treatment of ulcers that failed to respond to conventional therapy [72]. Phase I trials on chronic venous leg ulcers and burn wounds have not reported any adverse events associated with bacteriophage use [22, 37].

Phages against *Propionibacterium acnes* involved in the pathogenesis of acne have displayed a broad ability to kill clinical isolates of *P. acnes* [73]. These phages, incorporated into an aqueous cream, retained their antibacterial activity up to 90 days when stored appropriately [74]. A number of trials evaluating phage therapy for burn wound infections, diabetic foot, and acne have been registered in the USA over the past few years [75–77].

8.10 Future Possibilities for Bacteriophage Therapy

Phage therapy can be an important component of personalized medicine, tailored against bacteria isolated from the site of infection [46]. This approach has been shown to be successful at some centres, but would require facilities for phage susceptibility testing [37]. Bacteriophages have been used to transfer gene cassettes that confer susceptibility to antibiotics, thereby reversing drug resistance in bacteria [21].

Phages bearing chloramphenicol on the surface have been shown to specifically target *S. aureus* in vitro [40]. Preparations of bacteriophage lysins could be effectively used in infections caused by Gram-positive bacteria: lysins against *Bacillus anthracis*, *Enterococcus spp.*, and *Streptococcus pneumoniae* have been identified [40]. Liquid-based phage skin disinfectants could be formulated to target difficult-to-treat nosocomial pathogens such as MRSA, *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, without affecting the normal flora [78].

8.11 Conclusions

Bacteriophages have been shown to be potent antibacterial agents targeting most of the known human bacterial pathogens. Animal and human studies have so far not reported serious adverse effects. Local applications of phages have been effective in treating ulcers, wound and burn infections. Commercially successful wound dressings such as PhageBioDerm® have been in use for almost two decades. Similar topical formulations against local infections could be among the first to gain widespread use.

While using bacteriophages therapeutically appears promising, care must be taken to ensure that resistance does not develop. One of the ways that this may be done is to ensure that adequate concentrations of the phages are maintained at the site of infection during therapy. Therapeutic use must be preceded by rigorous clinical trials. Regulations, definitions and standards need to be established by internationally recognized organizations.

Though it is unlikely that phages will replace conventional antibiotics anytime in the near future, robust studies providing reliable and reproducible results will enable bacteriophage therapy to complement antibiotics. Pharmaceutical companies play a critical role in bringing phage therapy to patients suffering from infectious diseases.

While we move towards developing and adopting new weapons to fight infections, it is imperative that we avoid the mistakes that led to the development and spread of antimicrobial resistance.

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