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Phage Therapy in the Post-Antibiotic Era: From Biology to Clinical Translation

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Abstract

Antibiotic resistance is perhaps the greatest threat to world health. Considering the escalating global crisis of antibiotic resistance, the search for alternative therapeutic strategies has become a paramount issue. The application of bacteriophages to target and eradicate bacterial infections, or phage therapy, is a promising strategy that has the potential to completely transform the treatment of infectious diseases. This paper provides a thorough analysis of the state of phage therapy as a competitive substitute for antibiotics, outlining its benefits, drawbacks, and therapeutic uses.

This study highlights the effectiveness and specificity of bacteriophages specifically targeting pathogenic bacteria while conserving the host microbiota through a thorough analysis of the literature. In addition, it delves into the dynamic interactions between bacteria and bacteriophages, including the mechanisms underlying phage resistance and possible approaches to reduce their prevalence. Furthermore, phage therapy's safety and therapeutic efficacy are evaluated by looking through current case studies and clinical trials, emphasising the promising results of the treatment of a variety of bacterial infections, including those that are antibiotic-resistant. The difficulties in implementing phage therapy are also covered in this review, such as the requirement for customised treatment plans, regulatory obstacles, and phage cocktail standardisation. To improve treatment outcomes and get around resistance mechanisms, it also looks at the synergistic potential of combining phage therapy with additional antimicrobial agents, including conventional antibiotics.

In the end, this review highlights how phage therapy has the potential to be a game-changer and a key component of future infectious disease control plans. This paper heralds a new era in the fight against antibiotic-resistant illnesses by offering insights into the mechanics, clinical efficacy, and obstacles of phage therapy. It also urges more research, standardisation, and integration of phage therapy into mainstream medical practice.

Keywords- Bacteriophage therapy, Anti-microbial resistance, Phage-antibiotic synergy, Multidrug-resistant infections

Introduction

The discovery of antibiotics back in 1928 was a revolutionary step in the field of medicine, and since then, antibiotics have been used to treat a wide range of infectious diseases. Over the past few decades, there has been a widespread increase and spread of antibiotic-resistant bacteria around the globe, which has become a major therapeutic threat [1]. The continuous acquisition of antibiotic-resistant traits resulted in the generation of multi-drug resistant (MDR), extensively drug resistant (XDR) and pan-drug resistant (PDR) pathogens [2].

A special group of bacteria that has been on the radar of various researchers, public health officials and clinicians, as they are responsible for causing multi-drug resistant (MDR) infections. This group includes bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. and is referred to by an acronym ESKAPE because of their capacity to escape the anti-microbial activity of antibiotics via multi-drug resistance [3]. The last 4 of the above-mentioned bacteria, especially the carbapenem- and cephalosporin-resistant strains, were recently included in a list issued by the World Health Organisation (WHO) as being the first concern for the research and development of new antibiotics. A few other species were also added to the high-priority list, including the pathogens of the digestive tract such as *Helicobacter pylori*, *Campylobacter* spp., and *Salmonella* spp., and the sexually transmissible agent *Neisseria gonorrhoeae* [4].

The patients that are highly susceptible to MDR infections are those with underlying medical conditions, immunocompromised individuals, patients undergoing surgery and old age people [5,6]. In this post-antibiotic era,

common infections can also be fatal. Viewing the current scenario of the world and the increasing antibiotic resistance in pathogens, it is important to promote new therapeutic alternatives and to revive the overlooked mechanisms, such as “phage therapy” [7].

1. Phage therapy: A brief history

Phages or bacteriophages are viruses that kill bacteria by replicating inside them using their cell machinery. They are present abundantly in the environment and are ubiquitous in nature [5]. Bacteriophages were discovered by British worker Frederick Twort in 1915. The term “bacteriophage” was first coined by Felix d’Herelle [6], meaning “bacterium eater,” [7].

The concept of using phages to treat bacterial illnesses, often known as "phage therapy," is not new; Twort and D'Hérelle discovered it in the early 1900s. D'Hérelle treated bacterial illnesses in people with phages. He discovered "invisible microbes" with antibacterial properties in the filtrates of stool from dysentery patients during his research. [7,8]. His work led to a widespread study about phage therapy carried out in humans [8]. He injected the phage suspension into himself, his colleagues, and finally his patients who were suffering from "cholera" and "bacillary dysentery" to verify the safety of the method. Following this, the phages were examined for numerous different infectious agents and wound diseases. [9,10].

Nevertheless, studies on phage therapy that were published questioned the biological properties of phage and included several errors, as well as no models for illnesses that affect animals [9]. Then World War II happened, and the antibiotics were discovered, which impeded the bacteriophage investigations. The benefits of antibiotics included their ease of synthesis, wide range of activity, and reliable manufacturing method. However, phage therapy was not completely defeated since two European nations (Germany and the Soviet Union) used phages to treat wound healing [11].

Since the beginning of phage therapy, numerous studies have been conducted to determine the causative agents of numerous human and animal diseases, including those caused by *Shigella, Acinetobacter, Citrobacter, Enterobacter, Enterococcus, Proteus, Pseudomonas, Staphylococcus*, and *Streptococcus* [11,12]. We live in those times where antibiotic resistance was declared a “global threat” by the WHO. The therapeutic use of phages would be the best possible alternative to respond to the increasing global need, which shall be discussed in further sections [11].

2. Phage biology and how they kill bacteria

Bacteriophages, or phages, are viruses that infect bacteria. They are non-living biological entities consisting of either DNA or RNA genomes encased in a protective protein shell known as a capsid [12]. Since phages cannot replicate independently, they are entirely dependent on bacterial hosts to complete their life cycle.

Based on their replication strategy, phages are broadly classified into lytic phages and lysogenic (temperate) phages (Figure 1) [13]. Lytic phages infect bacterial cells, hijack the host machinery to replicate their genomes and structural proteins, assemble progeny virions, and ultimately cause bacterial lysis. The bursting of the host cell releases large numbers of newly assembled phages, which can go on to infect surrounding bacteria. Lysogenic phages, in contrast, adopt a different strategy. Their DNA integrates into the host chromosome as a prophage, where it can persist for extended periods in a dormant state [15]. Prophages are widely distributed across bacterial genomes—many bacteria harbours at least one, and some species carry multiple prophages. These integrated viral elements are not silent passengers: they can actively shape bacterial physiology, influence metabolism, regulate stress responses, enhance pathogenicity, and even confer resistance to subsequent phage infections. Such outcomes are possible because prophages can express accessory genes, some of which encode virulence factors or fitness determinants that benefit the bacterial host.

The lysogenic cycle is maintained by turning off genes that drive lysis, thereby promoting the persistence of the prophage [15]. While this relationship can be stable, lysogenic conversion may also have undesirable consequences. During this process, bacteria may acquire new characteristics such as increased virulence [25] or the incorporation of antibiotic resistance genes [26]. For this reason, the therapeutic application of temperate phages is generally discouraged. However, exceptions exist: for example, in Clostridium difficile, temperate phages have been engineered or repurposed to provide therapeutic benefit [27]. Thus, while strictly lytic phages remain the cornerstone of phage therapy, specific cases highlight the potential of controlled temperate phage use.

The advancement of phage therapy has been propelled by mechanistic studies of lytic phages and their bactericidal proteins. A hallmark of these phages is their ability to breach the bacterial cell envelope using specialised proteins. Central to this process are holins and endolysins [17]. Holins are small, hydrophobic proteins that accumulate in the bacterial inner membrane during the phage replication cycle. At a precisely regulated time, holins undergo oligomerisation to form large pores, which destabilise the cytoplasmic membrane and allow endolysins to access their substrate [15,16]. Endolysins are peptidoglycan-degrading enzymes that enzymatically dismantle the bacterial cell wall [20]. Their catalytic

domains enable them to hydrolyse specific bonds within peptidoglycan, employing activities such as endopeptidase, amidase, glycosidase, or lytic transglycosylase cleavage [21,22]. By disrupting the structural integrity of the bacterial wall, endolysins facilitate osmotic lysis, resulting in the release of phage progeny at the culmination of the replication cycle.

The structural architecture of endolysins varies with bacterial type. Gram-positive bacteria, with their exposed thick peptidoglycan layer, are targeted by modular endolysins that typically contain both an enzymatically active domain (EAD) and a cell wall-binding domain (CBD). The CBD anchors the enzyme to the cell wall, ensuring specific and efficient hydrolysis of peptidoglycan by the EAD [19,23]. In contrast, Gram-negative bacteria possess an additional outer membrane that restricts access to peptidoglycan. Their endolysins are generally globular enzymes composed of a single EAD, adapted to penetrate or exploit temporary breaches in the outer membrane [21]. These differences highlight the evolutionary adaptation of endolysins to the structural features of their bacterial hosts.

While the holin–endolysin system is the most well-characterised lytic strategy, some phages employ additional proteins. Spanins, for example, span the inner and outer membranes of Gram-negative bacteria and mediate fusion of these layers to complete cell lysis. Amurins represent another class, functioning as single proteins that inhibit peptidoglycan synthesis rather than directly degrading it [17]. These diverse mechanisms underscore the versatility of phage-mediated lysis. Among holins, the lambda phage protein S105 is one of the best-studied. It forms lesions in the lipid bilayer with diameters averaging over 300 nm, dramatically compromising membrane integrity and facilitating rapid bacterial death [15].

Through the coordinated action of holins, endolysins, and accessory lysis proteins, phages achieve highly efficient killing of bacterial hosts. Most lytic phages relevant to human infections belong to the order caudovirales or the family Microviridae, and their replication cycles culminate in the orchestrated destruction of bacterial cells [24]. These processes form the biological foundation upon which modern phage therapy is being developed and optimised.

3. Comparison to Antibiotics

Phages are potent, non-living entities that are the foundation of phage-based treatments because of their lytic cycle. Conversely, antibiotics are substances that can specifically interfere with the physiological functions of bacteria, like the creation of proteins or cell walls. The antibiotics and phages' mechanisms of action have striking differences; some elaborative comparative points are discussed as follows:

3.1. Specificity

Phages are highly specific for their hosts. However, it was discovered recently that phages can “jump” hosts (facilitated by microbiota in the gut), which means that phage-host specificity may change and evolve [29], which is not the same as antibiotics. In cases like wound infections, phage therapy could be less effective because wounds are usually infected with more than one bacterium. This limitation can be overcome by creating phage cocktails, which are effective against more than one type of bacteria [30].

3.2. Safety

Phage therapy directly aims at pathogenic bacteria, whereas antibiotics affect not just the pathogen but also the other microbiota, which play a significant role in the body. Due to this, antibiotics might cause disturbances in the microbiome, such as mucosal candidiasis, antibiotic-associated diarrhoea, pseudomembranous colitis caused by *Clostridium difficile* and sometimes long-term metabolic (diabetes and obesity) and immunological disorders (asthma) [31]. In contrast to the antibiotics, phage therapy has fewer side effects on the gut microbiome, and it is efficient in removing pathogens such as *Shigella sonnei* and uropathogenic *E. coli* [32,33].

3.3. Biofilm penetration

Antibiotics have limited access to biofilm-based bacterial infections [34]. Phages, on the other hand, have enzymes (e.g. EPS depolymerase) present on the outer side of the capsid that degrade the extracellular polymeric substances (EPS). The degradation of the biofilms helps the phages to penetrate the biofilms and have access to the bacterial cells in the EPS matrix [35]. Antibiotics cannot penetrate the dense biofilms and require very high doses to do so. Even at high concentrations, complete eradication is not achieved, and regrowth may occur after treatment [36,37]. Usage of high concentrations of antibiotics leads to tissue toxicity [35]. Biofilms formed by *L. monocytogenes*, *Staphylococcus epidermidis and P. aeruginosa* on the medical devices have been eradicated using phage therapy [38] as shown in Figure 2.

3.4. Bacterial resistance

Bacteria can develop resistance against phages, like they do with antibiotics; however, phages can replicate continuously and change as a living entity that co-exists with the bacterial host. The mutation rate is faster in phages than in bacteria; thus, bacteria have fewer chances to develop phage resistance [39].

In addition, nature contains vast phage resources, and a specific phage can be found in the environment for its corresponding pathogen. Thus, phage therapy has been proven to be a faster and more economical alternative to antibiotic therapy [40].

4. Efficacy of phage therapy

Clinical trials

During earlier years, phage therapy was used to treat medical conditions like typhoid fever, dysentery, urinary tract infections, skin and surgical wound infections [42]. In the 1930s, phage therapy became less of a topic of discussion in Western nations due to criticism stemming from inadequate methodological designs, controls, and uniform manufacture and characterisation of the phage preparations, as well as the discovery of antibiotics [43].

Nonetheless, a few Eastern European nations, primarily Poland and Georgia (the former USSR), kept using phages. The reports' limited geographic scope was likely due to the utilisation of the native language. After reviewing the Georgian and Polish research, it was discovered that phage therapy was effective in treating the illnesses mentioned above [44]. At the end of the 20th century, some encouraging work presented the use of phage therapy involving animal models.

The 1st phase I randomised placebo-controlled trial conducted in the US was published in 2009 by Rhoads et al. [45]. In 42 patients with persistent venous leg ulcers, the trial examined the safety of a phage cocktail that targets *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*. There were no treatment-related adverse effects indicated by the results. The purpose of the study was not to identify any favourable results [46].

Another randomised trial was conducted in the UK that studied the efficacy of a solution containing 6 bacteriophages in patients suffering from chronic otitis caused by

Pseudomonas aeruginosa. The patients reported lower intensity symptoms such as itching, wetness and unpleasant odour. The colony of *Pseudomonas aeruginosa* also decreased, and no adverse reactions were recorded [47].

In the second case, of randomised open study evaluated 9 patients at the Burn Wound Centre of Queen Astrid Military Hospital, Brussels, Belgium. Patients were treated with BFC-1 phage cocktail containing 3 lytic phages: a *Myovirus*, a *Podovirus* against *P. aeruginos*a and a *Myovirus* against *Staphylococcus aureus*. A single spray application was done on a large, burned section, and a portion of the wound was kept as a control. Although the rate was lower than the control arm, which received 1% sulfadiazine silver emulsion cream, it demonstrated a significant decrease in the pathogen load on the wound infections. For the first time, phage therapy was tested using good clinical practices (GCP) and good manufacturing practices (GMP) without any negative side effects [48].

4.1. Oral administration

Research on the oral administration of phages has been conducted. Switzerland developed a T-4-like phage preparation in 2005 to treat diarrheal illness in healthy persons by targeting *E. coli* [49] and in Bangladesh in 2012 [50]. According to self-report, physical examination, and laboratory testing of hepatic, renal, or haematological function, there were no negative effects observed from the oral administration of phages. Healthy people's guts did not become more amplified by phages, and the initial doses of the phages were found in their faeces. The blood did not contain any phages or phage-specific antibodies, and the phage preparation did not change the gut microbiome's composition [50].

4.2. Intravenous administration

There have been studies regarding intravenous phage therapy in humans. The evidence reporting its use was reviewed by Speck and Smithyman [51]. Their focus was primarily on the severe infections, such as bacteremia and typhoid fever caused by *S. aureus*, with all the data present from the past 80 years. A study that collectively treated approximately. 1,000 patients with intravenous therapy were successfully treated without any side effects was referenced to them. Even if there were any side effects (shock or serum sickness-like conditions), they were mainly due to the contamination of the early phage preparations. They concluded that the intravenous administration of phage therapy had a strong possibility of being safe and effective [51].

Individual case studies of the compassionate use of intravenous phage therapy were used to assess its continued usage. The first effective instance of intravenous phage therapy for a systemic multidrug-resistant illness in the United States was reported by Schooley et al. The patient, a 68-year-old male with diabetes, had necrotising pancreatitis due to an MDR

A. baumannii infection. Phage therapy was started after the patient's condition deteriorated and the antibiotic treatment didn't work. Because the phages from the phage libraries were already pre-established against the bacterial pathogen (*Acinetobacter baumannii*), the treatment was readily available. To prevent phage resistance, the phage cocktail had to be administered sequentially. The 68-year-old patient managed the phage therapy effectively [52].

And another case was reported by Chan et al. [53] where an MDR *P. aeruginosa* graft infection was discovered in a 76-year-old patient who had undergone Darcon graft aortic arch replacement surgery. Subsurface chest wall debridement and intravenous ceftazidime were used in the patient's treatment; however, the outcomes were insufficient. Then, ceftazidime and phage OMKO1 were applied simultaneously, once intracavitary, as part of phage treatment. Without any re-infection, the *P. aeruginos*a infection was reduced, and the patient accepted the treatment. But as the bacterial infection subsided, more issues appeared in those who had aortic grafts.

Phase I phage therapy trials and their progression to phases II and III will surely increase in the near future [28].

4.3. Therapeutic applications of Phage therapy in agriculture, veterinary and environmental

Phage treatment is being investigated in agriculture and veterinary medicine to reduce antibiotic use in livestock and poultry operations [125]. Antibiotic use in these industries has accelerated the emergence of antibiotic resistance. Bacteria in animals can develop resistance, which can then be transmitted to people via the food chain. Phage treatment is a potential method for managing bacterial infections in animals, leading to enhanced health and reduced antibiotic usage [126].

Phages are used to target dangerous bacteria such as *Salmonella* and *Escherichia coli* in poultry and livestock, lowering illnesses without the need for medicines. In veterinary medicine, phages are used to cure illnesses in cattle and companion animals, minimising the need for antibiotics and thereby lowering overall antimicrobial consumption, which is thought to be a major component in the development and spread of AMR. Phage treatment has been used to manage *Salmonella* and *Campylobacter* infections in poultry, *E. coli* infections in cattle, and *Staphylococcus aureus* infections in dairy cows. These uses increase livestock health and production while lowering the danger of antibiotic-resistant bacteria transmission to people via meat, dairy, and other animal products [127, 128, 129].

Phage treatments are currently used on crops such as tomatoes, peppers, and potatoes to combat bacterial pathogens like *Xanthomonas* and *Erwinia*, which cause illnesses like bacterial spot and soft rot. Similarly, phage sprays are used to address a deadly bacterial illness caused by *Erwinia amylovora*, which damages apples and pear crops [134]. Phage therapy in agriculture addresses the demand for antibiotic-free and sustainable agricultural practices, providing a natural and tailored approach to illness management in animals [130,131,132]. Phage therapy's application in agricultural and veterinary medicine has substantial consequences for food safety and public health. Controlling bacterial infections in animals without medicines can prevent the spread of antibiotic-resistant microorganisms, reducing the burden of resistance in human populations [133]. Phages may also be used in the environment to disinfect hospital surfaces and remediate effluent. Environmental phages may play a key part in One Health's response to AMR by allowing us to better understand, control, and limit the global and local development, selection, and transmission of antibiotic-resistant bacteria and their genes [134].

5. Current strategies for phage therapy

5.1. Conventional phage therapy

"Conventional phage therapy" refers to the use of bacteriophages as the only therapeutic agents administered to patients at the time of bacterial infection. It also includes mono- and polyphage therapy, which differ in that they employ either a single phage or a combination of phages [54].

The use of a single phage to treat bacterial infections is known as monophage treatment (Figure 3). It is primarily used on animal models during the design and phage preparation, followed by testing. The therapy is focused on MDR infections caused by pathogens such as *A. baumannii* (carbapenem-resistant) [55,56] and *E. faecium* septicemia (vancomycin-resistant) [57]. The development of resistance in bacteria against bacteriophages limits the success of Monophage therapy. Although phages (in contrast to antibiotic therapy) can defeat bacterial resistance, this is insufficient for the clinical use of monophage therapy [58,59].

To overcome the limitations of monophage therapy, polyphage therapy is also used. Polyphage therapy or phage cocktails can target single bacterial strains, multiple bacterial strains (belonging to a single bacterial species or multiple species). However, the synthesis and purification of these mixtures take longer, are more complicated, and may trigger immunological reactions [54]. These limitations are addressed by evaluating each phage's effectiveness individually in a cocktail and eliminating any weak or ineffectively active phages [60]. Phage cocktails are accessible in Georgia and

Russia and can be evaluated for the treatment of bacterial illnesses without a prescription. The two most popular phage cocktails are Intestiphage (II) and Pyophage (PYO) [61].

Pyophage consists of phages directed against *P. aeruginosa, S. aureus, Proteus vulgaris, and Streptococcus pyogenes* and the Intestiphage is directed against approximately 23 different enteric bacteria [61].

5.2. Phage-derived proteins

Phages encode several enzymes during their life cycle that can destroy bacterial cells, such as virion-associated peptidoglycan hydrolases (VAPGH), endolysins, and polysaccharide depolymerases. Virion-associated peptidoglycan hydrolases (VAPGH), which are found on the phage base plate, are used by the phages to hydrolyse the bacterial cell wall after they have absorbed onto its peptidoglycan. After the breakdown of the cell wall, the phage genetic material is injected into the bacterial cell [62]. These enzymes of stable and specific in nature and can act as a perfect antimicrobial if studied and applied in clinical treatment [28].

Another class of phage-encoded proteins that can lyse the bacterial cell, named endolysin (lysin), was discovered by Jacob et al. around 1958. They can directly destroy the peptidoglycan cell wall [63]. S. aureus bacteriophage phi MR11 encoded lysin can quickly lyse the bacterial cell completely, including many tested *S. aureus* strains, vancomycin-resistant *S. aureus* (VRSA) and methicillin-resistant *S. aureus* (MRSA) [64,65,66].

Apart from VAPGH and endolysins, depolymerases are also encoded by the bacteriophages [69,70]. They hydrolyse polysaccharide compounds of bacteria, such as capsule, lipopolysaccharide (LPS) or extracellular polysaccharides of biofilms [71,72]. Bacteria may withstand the effects of phagocytes, disinfectants, and antibiotics with the aid of bacterial capsules and biofilms. Therefore, by using depolymerases to dissolve these pathogen-protective barriers, it is possible to significantly boost the therapeutic effect of bacterial infection. A polysaccharide depolymerase (Dpo48) from the *A. baumannii* phage IME200, for example, was found to be able to degrade the bacterial capsule and to have the same activity spectrum as its parent phage [73].

5.3. Bio-engineered phages

Phage treatment is one area of life science research and application that has benefited substantially from genetic engineering technologies [74]. Phage genetic engineering can improve therapeutic properties through a range of processes, including phage capsid modification, exogenous gene delivery, and a wide host range. One popular way to get a wide range of spectra is to engineer the genes that encode receptor-binding proteins (RBPs) in the phage's tail fibres and spikes [74].

Mahichi et al. used homologous recombination to incorporate the long fibre genes of phage IP008 into the *E. coli* T2 phage, thereby expanding its host range [75]. The resulting recombinant phage displayed both the potent lytic activity of phage T2 and a wider host range of phage IP008 [75]. Bacterial biofilms were also targeted by modified E. coli T7 phages expressing dispersin B enzyme (Lu and Collins). Upon infection, the enzyme degraded key components of biofilm. The reduction in biofilm cell count by 100-fold was observed by the application of the engineered phage [76]. Kim et al. [77] found that by incorporating polyethene glycol (PEG) into the phage, its circulation time in blood could be increased.

Antibiotic-specificity to counter MDR bacterial pathogens can be enhanced using bioengineered phages. Lytic phages were attached with chloramphenicol molecules [78], and the targeted bacterial cells received the antibiotic directly.

Because of this, the drug's potency in vitro was increased by a factor of roughly 20,000, and any possible negative effects that might have resulted from the drug's interaction with human cells and their surrounding microbiota were also eradicated. Incorporating dominant sensitivity genes via genetic engineering can also be utilised to reverse antibiotic resistance in bacterial cells [79].

5.4. Phage-antibiotic synergy

Phage-antibiotic synergy (PAS) is a phenomenon in which sub-inhibitory concentrations of antibiotics can stimulate the propagation of phages and enhance their ability to infect and cleave bacterial cells and increase their phage progeny [80,81,82]. The sub-lethal concentration of antibiotics can speed up the replication rate of phages and increase the size of phage plaques [80,82]. PAS was proposed by Comeau et al. in 2007 [82].

Due to the antibiotic's inability to pass through all the layers of biofilm, bacteria can confer antibiotic resistance. To treat biofilm *Klebsiella pneumoniae*, the antibiotic ciprofloxacin is combined with the lytic phage depolymerase instead of being used alone [83]. The phage and antibiotics can improve the removal of bacterial biofilms [83]. The phage enzyme depolymerase degrades the exopolysaccharides of the biofilms, making it easier for the antibiotics to penetrate the biofilm and attack bacteria [84].

Shlezinger et al. found that the combination of vancomycin-phage EFLK1 (anti-Enterococcus faecalis phage) was more effective during the treatment against vancomycin-resistant *E. faecalis* (VRE) as compared to phage or antibiotic alone [85]. Apart from the anti-bacterial effect, the combination of phage and antibiotics can also reduce the chances of bacterial resistance [86]. It was found out recently that the UTI caused by drug-resistant K. pneumonia can be cured by the combination of sulfamethoxazole-trimethoprim and the phage cocktail. Apart from curing the UTI, it also inhibits the occurrence of bacterial resistance [87]. In the same way, the antibacterial effect can be achieved by using the combination of phage-derived enzymes and other drugs.

A diagrammatic representation of the three phage therapy approaches mentioned above, viz., phage-antibiotic synergy, bio-engineered phages and phage-derived proteins, is shown in Figure 4.

5.5. CRISPR-Cas system

To overcome the growing challenge of antibiotic resistance, bacteriophages have been genetically engineered to deliver CRISPR/Cas9 systems directly into bacterial cells (Figure 5). This allows for precise targeting and disruption of specific bacterial genes, including those responsible for virulence or antibiotic resistance, thereby restoring the effectiveness of conventional antibiotics. Instead of

relying on plasmid-based delivery systems, the CRISPR/Cas9 mechanism is integrated directly into the phage genome, improving its stability, packaging, and delivery efficiency. Additionally, virulence genes from the bacterial host used in phage production are removed to prevent contamination with toxins, enhancing the biosafety of the final therapeutic product. The host range of these engineered phages is further broadened by incorporating tail fibre proteins from phages known to infect a wider variety of bacterial strains. Building on this innovation, combination therapy using both antibiotics and CRISPR-equipped phages presents a powerful strategy, especially in targeting persistent infections involving biofilms. Biofilms, which are complex bacterial communities embedded in a protective matrix, commonly develop around medical devices such as catheters, pacemakers, and artificial joints, making them highly resistant to antibiotic penetration. The synergy between antibiotics and engineered phages enhances the ability to penetrate these biofilms, disrupt bacterial defences, and achieve more effective infection control, particularly in clinical settings where device-associated infections are prevalent [123].

5.6. Personalised Phage Therapy

Personalised phage therapy includes selecting or designing phages that are particular to the bacterial strain infecting the patient. Advancements in genetic and diagnostic technologies enable rapid and reliable identification of bacterial pathogens, making this procedure more effective. The first step in individualised phage therapy is isolating and identifying the bacterial strain causing infection [124]. After identifying the bacterium, phages from a library are screened to determine their effectiveness against the specific strain [125]. If there are no relevant phages in existing libraries, they can be isolated from environmental samples or created to target specific pathogens. Next-generation sequencing (NGS) and other sequencing technological improvements have substantially eased the rapid identification of bacterial pathogens, allowing for faster and more accurate matching of phages to illnesses [126].

6. Phage therapy and host immune system

The studies about the interactions between the phages and the host immune system are very important before carrying out the treatment because phage therapy may carry the risk of certain immunological reactions. An immune response against bacteriophages is largely dependent on the location of the bacterial infection and the injection site of the therapeutic phages [88].

Everyday encounters with phages occur between humans and animals, and there is compelling evidence that both human and animal serum contains anti-phage antibodies [89]. When phages are administered orally to treat bacterial infections caused by *Staphylococcus*, *Klebsiella*, *Escherichia*, and *Pseudomonas*, an immune response is induced. Nevertheless, there is no proof of immunological issues following a high phage ingestion rate [90].

But it's not the same with intravenous administrations because internal organs and blood organs are not the natural environment for phages. The intravenous administration of phages strongly stimulates innate and adaptive immunity [91]. In the case of the absence of any specific bacterial pathogen present for the phages, the phagocytic cells actively prey on the phages and remove them from the blood and internal organs [92]. In addition to this, there is internalisation of phages inside the reticuloendothelial cells of the liver and spleen, followed by their elimination [92]. The phagocytosis of phages by Kupffer cells (specialised macrophages located in the liver) is four times faster than spleen macrophages [90,92]. Various studies have shown that there is a decrease in the number of mature neutrophils and an increase in neutrophil precursors in the peripheral blood [93]. These results give an indication that phage administration can generate an innate immune response, which increases the efficiency of removing bacterial pathogens [94].

Phage-neutralising antibodies (also known as neutralising antibodies) are also produced during phage therapy, which may reduce the effectiveness of phages to target their corresponding bacterial pathogen [95,96,97,46]. Now, what defines phage-neutralising antibodies is their ability to bind to those epitopes present on the phages that are essential in binding with the bacterial cells and kill them [98]. There are many factors on which the concentration of the antibodies depends, especially the route of phage administration (topical and oral administration lead to a small increase in antibodies) and the dosage protocol [11]. According to the studies, lower efficacy of phage therapy is probably due to phage-neutralising antibodies [89]. Although an increase in phage-targeting antibodies is observed during phage therapy, there is an increase in non-neutralising antibodies and enhanced immune response after subsequent doses [59,99].

In addition to the humoral response, the cellular response also plays a major role, resulting in the inactivation of phages. Strong hypersensitivity reactions resulted from the subcutaneous injection of MS-2 phage in a guinea pig, as shown in a study conducted by Langbeheim[100]. However, the cellular response has little role to play when it comes to phage inactivation [101].

Amidst all this, immunosuppressive activity exerted by phages has been observed through some studies [102]. Overall, the results indicate that it is very important to test the immunological response of each phage, especially when considering intravenous therapy. However, previous clinical and animal studies did not result in severe immunological reactions during phage treatment [91].

7. Barriers and limitations to Phage therapy

Phage therapy is currently thought to be superior to antibiotic therapy. Nevertheless, further research is required to fully understand the issues of phage therapy. Before phage therapy is used clinically, a few issues must be resolved [84].

7.1. Narrow host range

The specificity of the phages towards bacterial host is both a pro and a con in phage therapy. It works effectively against a specific bacterium, but with specificity comes a narrow spectrum. This narrow range limits the bacteriophage's effectiveness in killing a variety of bacteria. To counter this specificity issue, phages can be employed in combination with other phages (called phage cocktails), bio-engineered phages and products derived from the lytic phages (phage-derived enzymes) are used. They increase the range of specificity of phages towards a variety of bacteria [104,105].

7.2. Phage resistance

Phage resistance is a common occurrence in phage therapy, where the bacterial cells develop resistance to phages they were once sensitive to. This may occur due to the modification of bacterial cell surface receptors, which include lipopolysaccharides (LPS), outer membrane proteins, capsules, flagella, pili or quorum-sensing effect during the co-evolution between phage and bacteria [106,107,108]. In addition, bacterial cells have evolved various other defence mechanisms that can inhibit the different stages of phage cycles. These include the intracellular restriction modification system, abortive infection system (that blocks phage replication, transcription and translation), phage exclusion, CRISPR-Cas system, etc. [109,110]. To counter phage resistance, various strategies have been proposed, such as the usage of phage cocktails (with different host ranges), phage-antibiotic combinations, etc.

7.3. Delivery in the eukaryotic system

Since bacteriophages infect bacteria, it's easier for the phages to penetrate bacterial cells, but that is not the same with respect to the eukaryotic system. Given that the bacteriophages need a bacterial carrier to enter the cells directly, this could be a treatment-limiting factor in eukaryotes [107,109]. When employing pharmacological therapy, porous nanoparticles are used to transport the medications into the cells. Nevertheless, because of their asymmetrical and larger structure in comparison to the pore size of the nanoparticles used to load them, phages cannot be loaded in the same way [104,109]. This can be avoided by delivering phages and their derivatives to the infection site using various techniques. To increase the effectiveness of phage transport to the infected locations, for example, phages are encapsulated in liposomes [110,111].

7.4. Transduction

The ability of a phage to transfer DNA from one bacterium to another is known as transduction. During phage therapy, there are chances of transfer of genes that are responsible for pathogenicity and virulence to other bacteria. This may lead to the development of resistance in bacteria towards phages, which reduces the efficiency of phage therapy [112,113]. However, to tackle this issue, those phages are used that do not use the host’s machinery to synthesise the DNA or those phages that cannot pack extra host DNA [114].

7.5. Endotoxins

Endotoxin release is a common event after bacterial cell lysis. Bacterial cell lysis carried out by phage may result in the release of certain bacterial substances, such as endotoxins (LPS) may be released from Gram-negative bacteria that act as super-antigens and cause inflammation, which may lead to multi-organ failure [115,116]. For instance, endotoxin-independent anti-inflammatory responses are induced by the phages of S. aureus and P. aeruginosa [114]. Since the demand for an alternative method for the treatment of drug-resistant infections is increasing with each passing day, the issue around the safety of phage preparations and administration should be urgently addressed.

7.6. Phage storage and handling

Most phages used in therapy are freshly prepared water suspensions. The processing of phages with stable and well-defined medicinal characteristics is not well understood. Phages lack stability, which, along with high affinity, safety, solubility, and specificity, is one of the best qualities of a medicine [118,119]. Because of the nature of their proteins, they are somewhat stable in solution. Phage preparations must be stored in a cool environment due to their structural fragility, which makes long-term storage challenging. Phages in an aqueous solution can be stabilised by adding stability enhancers, processing them into a different formulation, spray-drying, lyophilising, or adding them to ointments, microparticles, or biodegradable polymer matrices [120,121].

8. Future of Phage Therapy

In contrast to other antibacterial medicines like antibiotics, phages can exhibit a wider range of mechanisms of action and, in many situations, be safer. The difficulty for phage therapy lies in maximising these advantageous qualities while considering present regulatory procedures and potential phage integration into the current economic frameworks that drive the distribution and application of antibacterial medicines. Phage therapy seems to be thriving right now, especially in areas with more lenient regulations and less payment to phage-product producers (such as Poland and the former Soviet Union). Regulatory bodies, like the FDA in the US and the EMA in Europe, must collaborate with researchers and doctors to create recommendations that balance rigorous safety and efficacy testing with flexibility to accommodate phage variability and specificity [137].

As more carefully monitored phage therapy research is published by mainstream Western scientists and companies, we anticipate that phage therapy will demonstrate growing promise for usage as an antibiotic to combat illnesses. This could potentially increase the demand for phage therapy [122].

Expanding phage libraries should also be one of the priorities to cover a broader spectrum of bacteria, and is ultimately important for the widespread application of phage therapy. The discovery and characterisation of new phages extracted from environmental sources such as soil, water and sewage has been made easier through high-throughput screening methods and metagenomics approaches [138, 139, 140] and increases the diversity of therapeutic phages that are available for clinical use [135].

Raising public awareness about phage therapy is a crucial step in educating people and building confidence among them regarding this new emerging treatment. Unlike antibiotics, phage therapy is relatively new and unknown to the vast majority of people, excluding scientific and medical communities [141, 142, 143]. Educating healthcare practitioners about the benefits, mechanisms, and applications of phage treatment is crucial. Medical conferences, continuing education programs, and incorporating phage therapy into medical school curricula can all help support these activities. Raising public awareness of phage therapy through media, patient advocacy groups, and public health organisations helps foster trust in these therapies. To ensure patients and clinicians are well-informed about phage therapy, it's crucial to communicate both its potential benefits and limitations [135].

Phage therapy, an alternate, narrow-spectrum antibacterial treatment, may become more important over longer periods of time due to the risks associated with disturbance of the human microbiome caused using relatively broad-spectrum antibacterial drugs [123].

9. Conclusion

Phage therapy, as an alternative to antibiotics, appears to be a promising treatment option for bacterial infections, particularly those caused by multidrug-resistant bacteria, according to the literature currently available on the application of phages and proteins derived from them. Nevertheless, before phage therapy is widely used, more research is required to fully understand the interaction between the phage, microbiome, and human host. This is evident from conflicting recent results regarding the immunomodulatory effects, the host range, and the possibility of horizontal gene transfer. These factors, along with the ease of phage lysin production, purification, and storage, make them potentially significantly more effective therapeutic agents than antibodies.

10. References

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List of figures

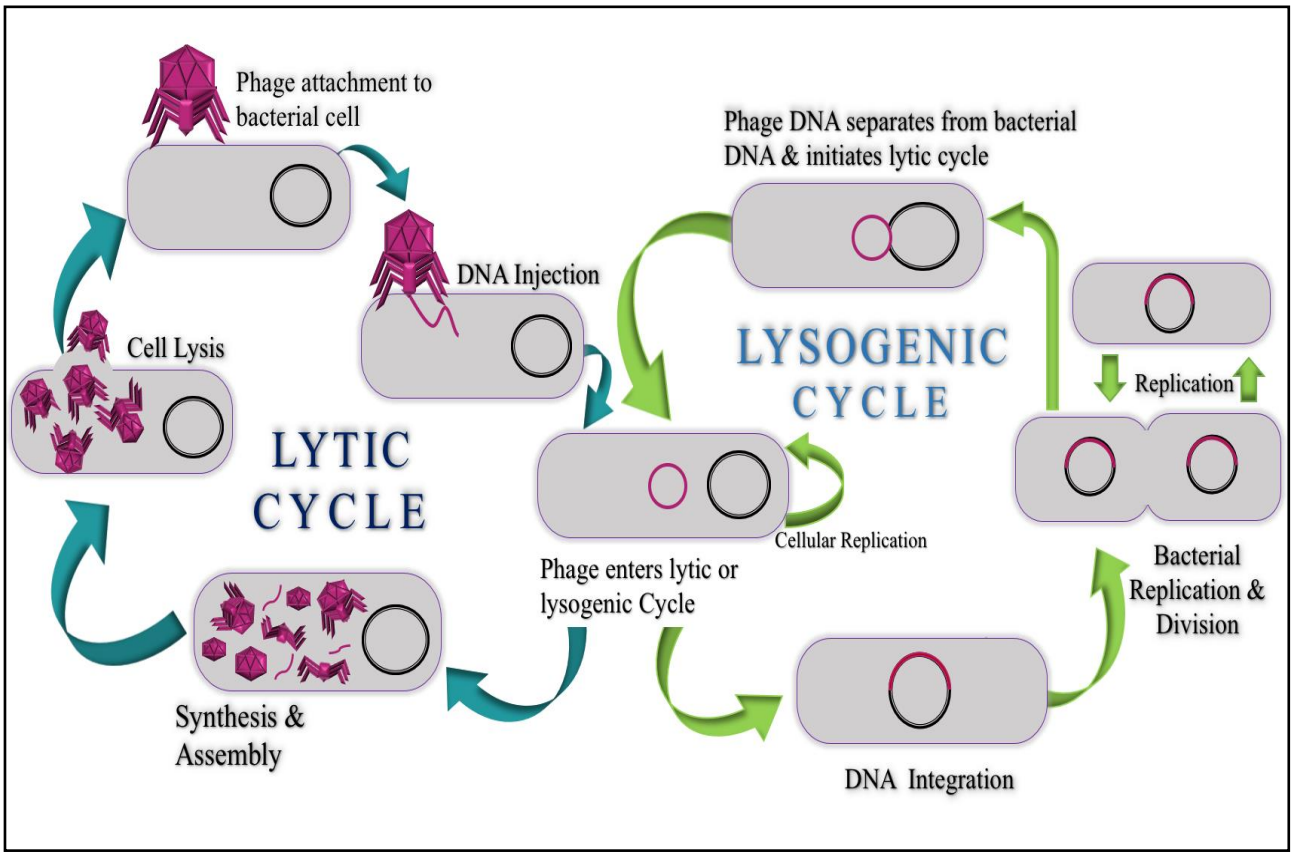

Figure 1. Bacteriophage life cycle. Left: Lytic cycle of phage replication. The phage adsorbs on the bacterial surface via receptors, followed by penetration, replication of phage DNA and protein production and finally assembly of new phage particles and ultimately host cell lysis. Right: Lysogenic cycle. After injecting its DNA into the bacterial cell, the phage integrates its DNA into the host genetic material (referred to as prophage), which multiplies with the host DNA for several generations.

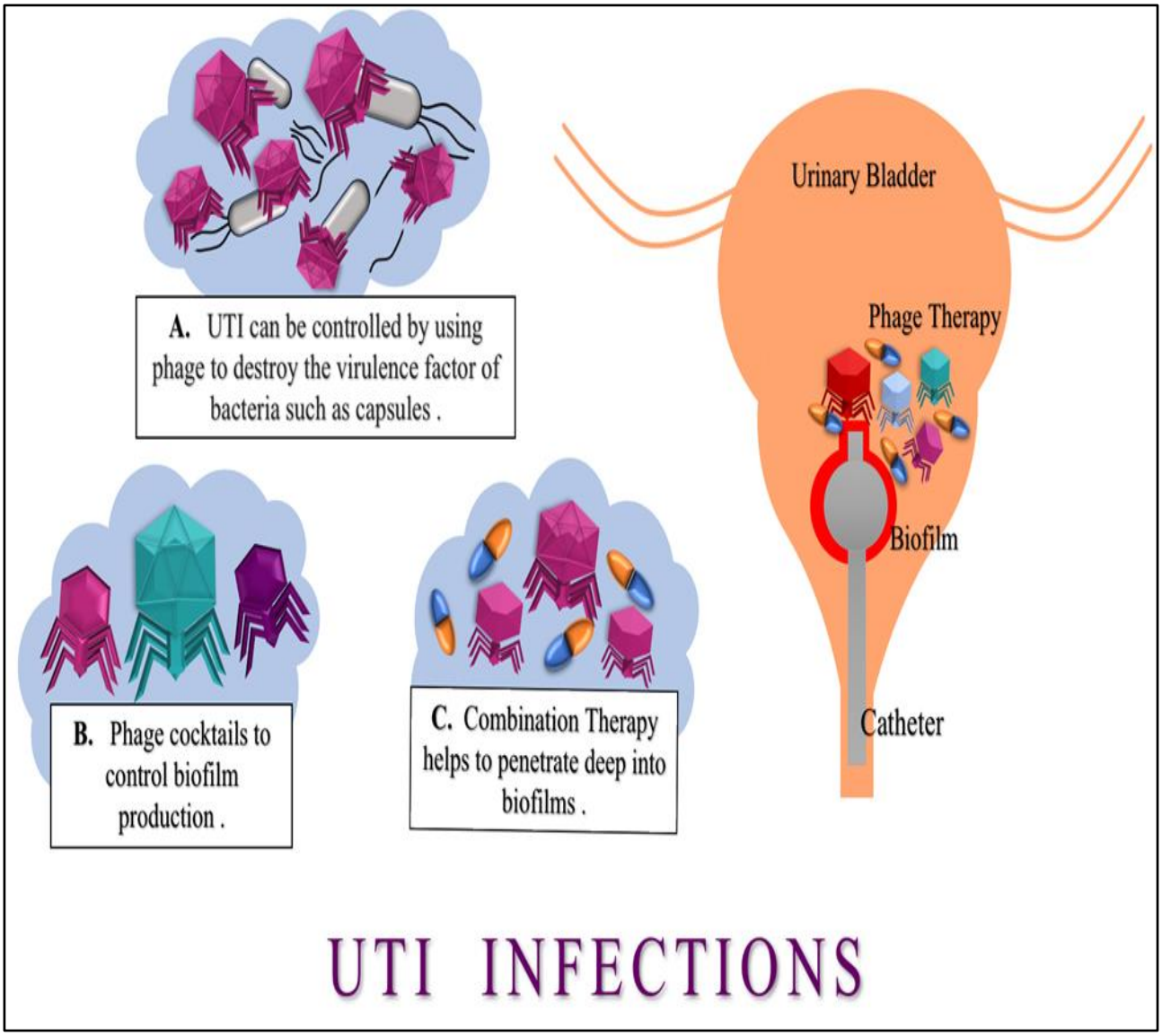

Figure 2-Urinary tract infections (UTI) associated with the catheters and different forms of phage therapy used to inhibit and eliminate the bacterial biofilm. (a) UTI can be controlled by using phages to destroy the virulence factors of bacteria, such as capsules. (b) Using phage cocktails to control multi-species biofilms. (c) Using bacteriophages in combination with antibiotics (combination therapy) for penetrate deeper into the biofilms.

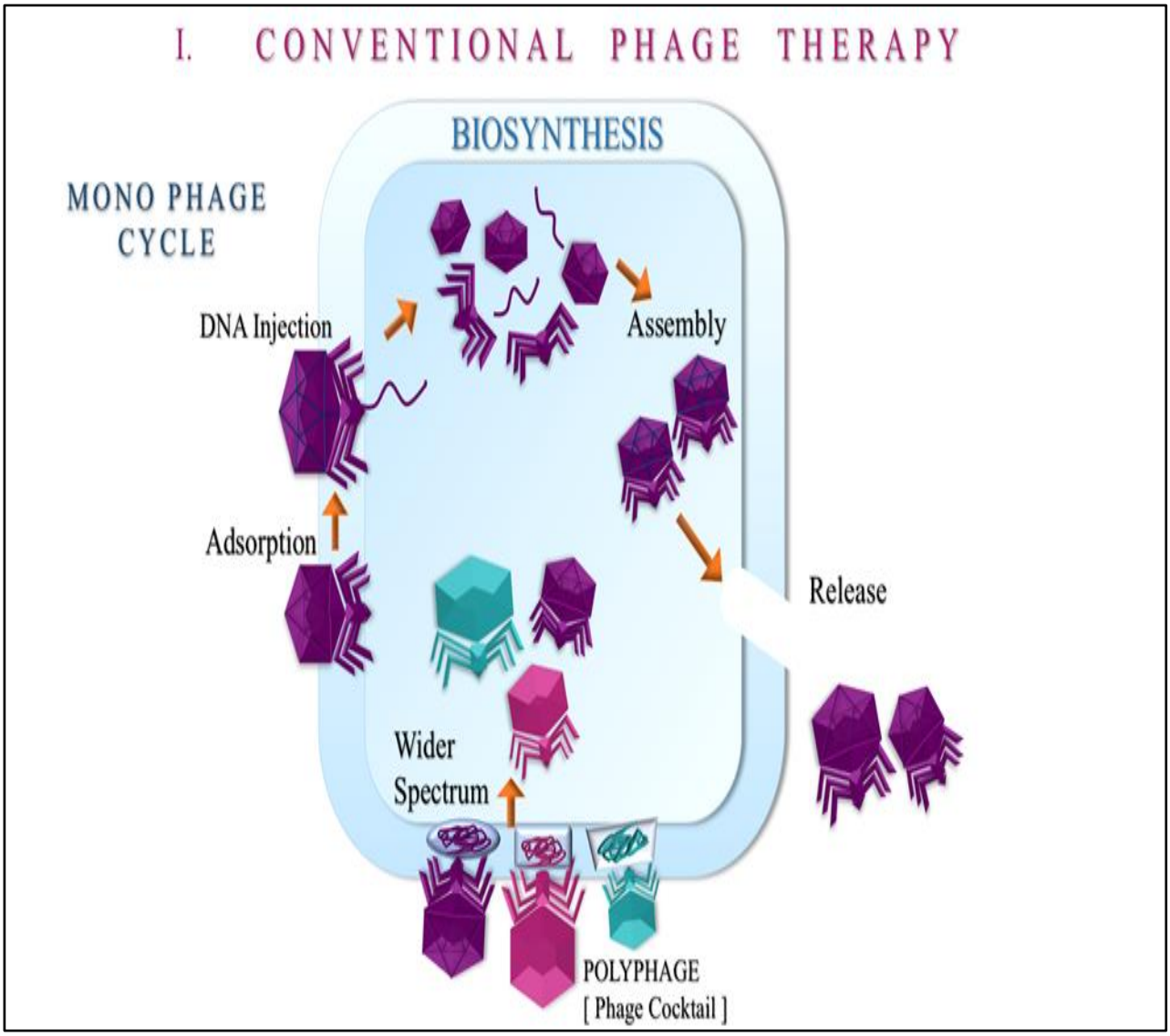

Figure 3. Phage therapy approaches. I) Conventional therapy in which phages are the only therapeutic agents used either in the form of monophage or polyphage therapy.

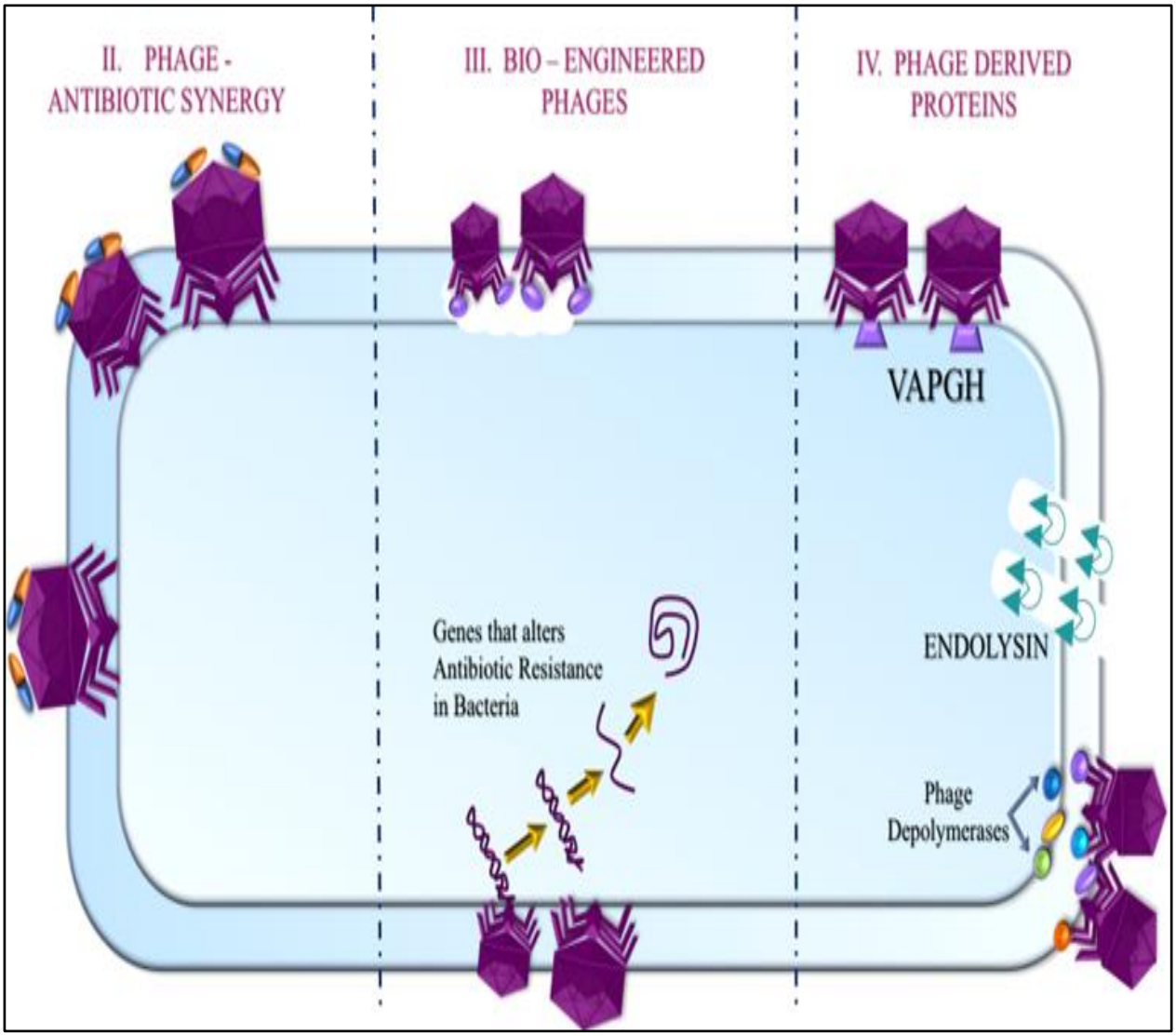

Figure 4. Phage therapy approaches. II) Phage antibiotic synergy is the phenomenon of combining an antibiotic with a phage particle to improve its ability to affect the target bacteria and increase its propagation. III) The use of bioengineered phages improves the antibiotic-phage capsid attachment for its release in targeted bacteria, to deliver genes that reverse antibiotic resistance. IV) Phage-derived enzymes such as depolymerases, holins, endolysins, and VAPGH are used as therapeutic agents.

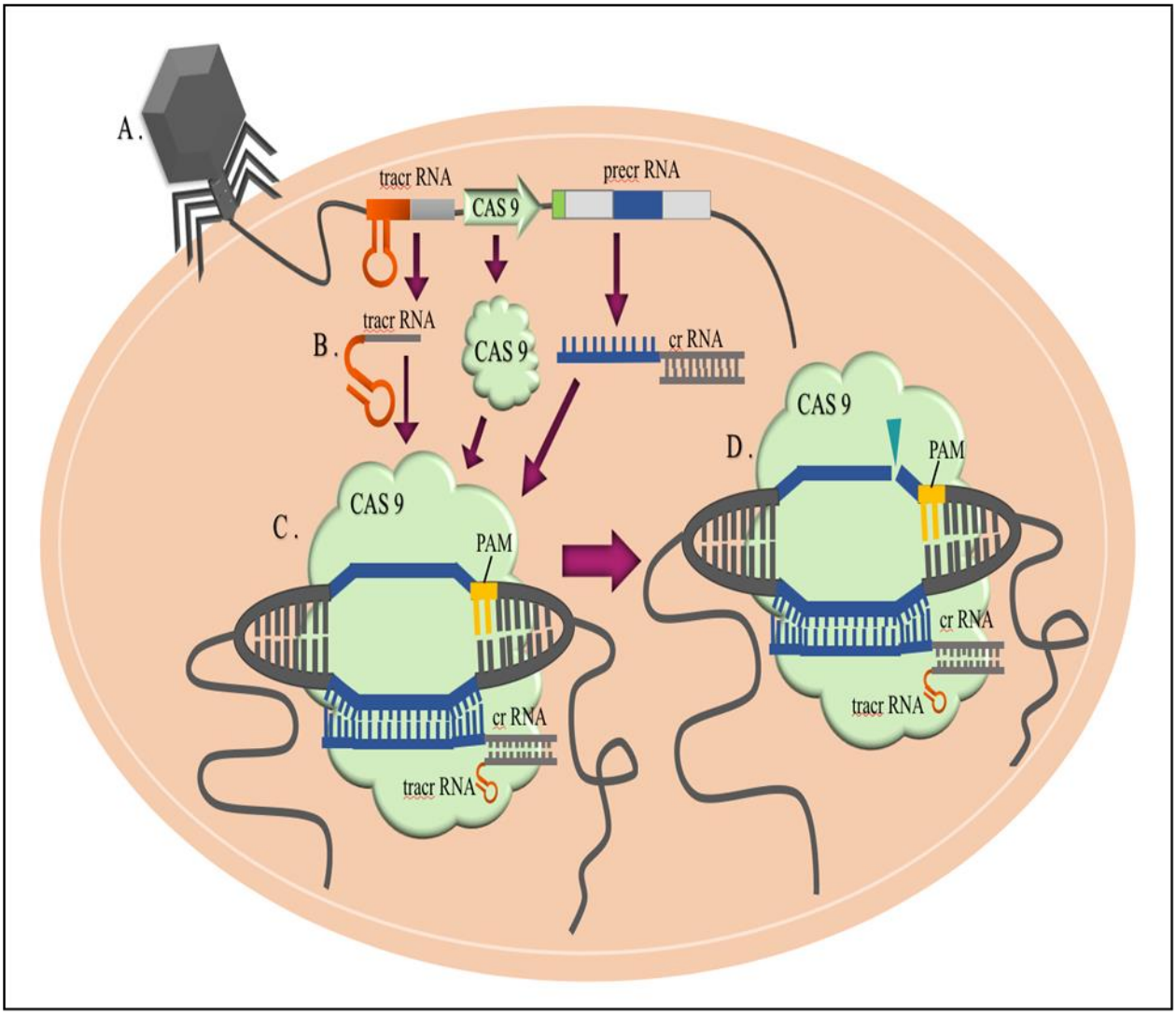

Figure 5. Incorporation of CRISPR-Cas 9 system in phage therapy. A) Injection of phage genome incorporated with CRISPR-Cas 9 system targeting a specific region on the bacterial genome (virulence factors or antibiotic resistance) into the bacterial cell. B) Expression of CRISPR-Cas 9 system by using bacterial cell machinery. C) The Cas 9 proteins bind with the guide RNA, forming a complex that recognises sequences complementary to the guide RNA (antibiotic resistance gene or virulence factor gene). D) As soon as the complex locates the targeted gene, it introduces a double-stranded break at that site (site next to PAM). This break disables the bacterial resistance gene or results in cell death.

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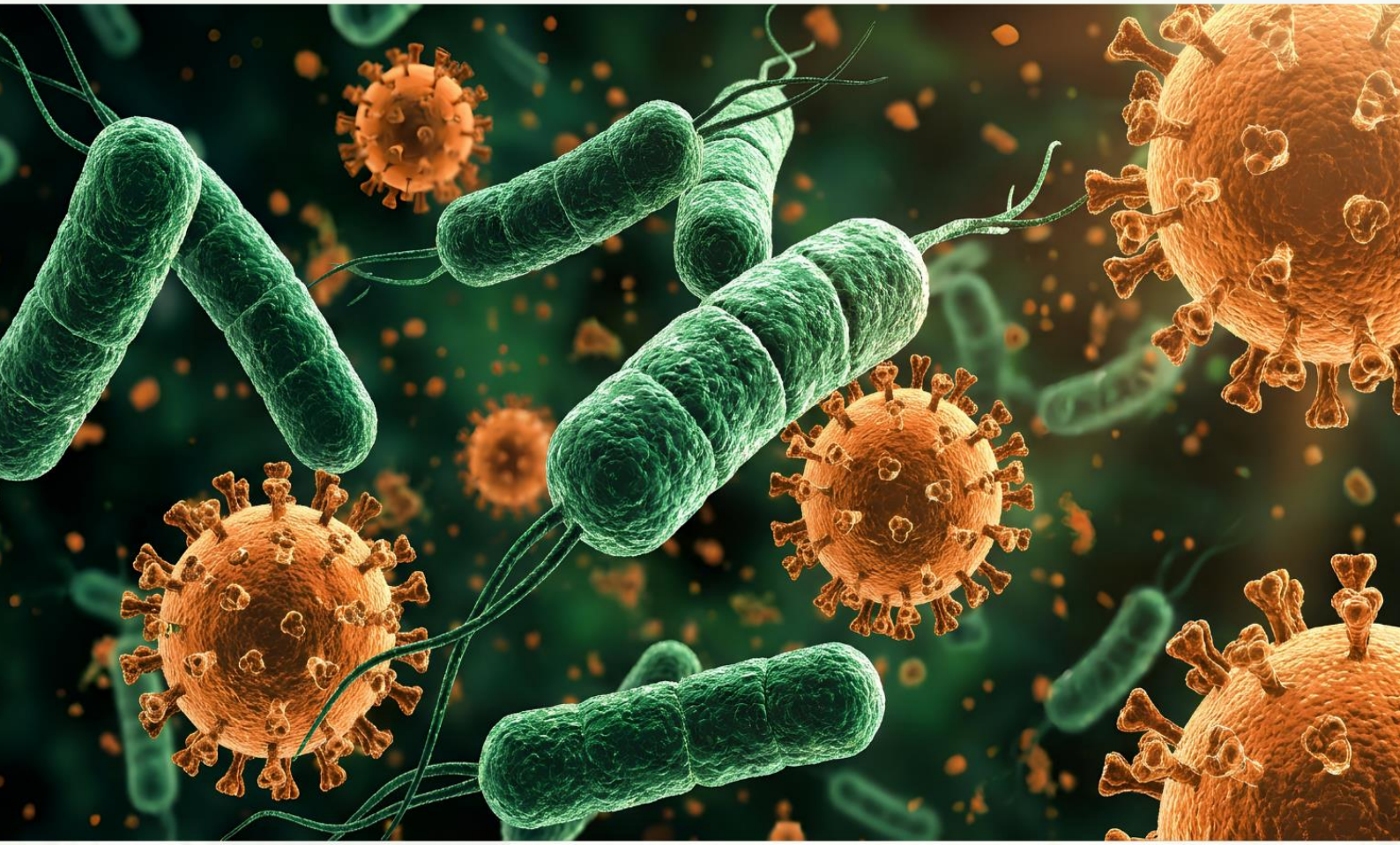

HARNESSING THE POWER OF BACTERIOPHAGES: BRIDGING FUNDAMENTAL BIOLOGY AND CLINICAL INNOVATION TO COMBAT ANTIBIOTIC-RESISTANT INFECTIONS IN THE POST-ANTIBIOTIC ERA.

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