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Review Article

Phage and Phage Products Now the Main Weapon For Control Multidrug Resistant Bacteria

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ABSTRACT

In the recent years have seen an increase in the prevalence of MDR bacteria, which pose major dangers to the general public's health. In fact, some strains are now practically immune to all of the widely used antibiotics. For example Methicillin-resistant *Staphylococcus aureus* (MRSA), which *S. aureus* has started to acquire antibiotic resistancet hat produced penicillinase, is a well-known example. A list of antibiotic-resistant infections for which new, potent drugs are urgently needed was released by the World Health Organisation (WHO). Additionally, the gram-negative pathogen *Klebsiella pneumoniae* is a clinically significant cause of sepsis, pneumonia, and other antibiotic-resistant infections in hospitals and the community. The development of *Klebsiella pneumoniae* strains that are resistant to carbapenem has been aided by the rising usage of antibiotics.

Bacteriophages are viruses that eradicate bacteria also known as "phages". Bacteriophages depend on host bacteria to grow since they lack a metabolism of their own. They are conceivably the most widespread and oldest known species on Earth, with some estimations placing their age at 3 billion years old. Phages are a typical component of the microflora of all fresh, unprocessed foods, and they play a critical role in maintaining microbial equilibrium in every ecosystem where bacteria thrive. Recently, interest in employing bacteriophages for various practical purposes has increased, with possibly the most effort going towards using them to increase food safety. Animal model development is still essential, though invertebrate and vertebrate models can be used to test the efficacy of therapies more swiftly and affordably than human trials while still being ethical. In the future, novel models might be taken into consideration, like the zebrafish, which is becoming more prominent for researching host-pathogen interactions. Bacteriophage cocktails, which scientists have started utilizing, enable more targeted treatment while also combating the formation of resistance.

Keywords: *Klebsiella pneumonia*; Bacteriophage; Methicillin-resistant; Microflora

INTRODUCTION

Bacteria that are resistant to three or more kinds of antimicrobial medications are referred to as Multidrug-Resistant (MDR) bacteria [1]. The three primary types of MDR bacteria are classified as gram-positive, gram-negative, and other (acid-stain). The medical literature uses a variety of definitions for the terms Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pan Drug-Resistant (PDR) bacteria to describe the various patterns of resistance identified in healthcare-associated, antimicrobial-resistant bacteria [1].

The European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) collaborated to bring together a group of international experts to develop a standardized international terminology to describe acquired resistance profiles in the bacteria *Staphylococcus aureus*, *Enterococcus* spp., Enterobacteriaceae (other than *Salmonella* and *Shigella*), *Pseudomonas aeruginosa*, and *Acinetobacter*

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spp. For each bacterium, epidemiologically significant antimicrobial categories were created [2].

These bacteria use a variety of modifications to prevent or lessen the harm caused by antimicrobials. Multidrug resistance is particularly prevalent today in new infections like *Acinetobacter baumannii* as well as well-known pathogens like *Staphylococcus aureus* and *Mycobacterium* TB [3].

Multi-drug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell [4]. This accumulation occurs typically on Resistance (R) plasmids. Second, multi-drug resistance may also occur by the increased expression of genes that code for multi-drug efflux pumps, extruding a wide range of drugs.

The use of antibiotics has significantly increased as a result of greater access to modern treatment. The development of novel antibiotics is currently being outpaced by the evolution of antimicrobial resistance factors due to the widespread usage of antibiotics. Many additional antibiotics were discovered and put into commercial manufacturing after the 1928 discovery of penicillin. Today, we assume that antibiotic therapy can treat any infectious condition. The production of antibiotics is estimated to be 100,000 tons per year worldwide, and their use has had a significant impact on the survival of bacteria on Earth. The problem of multi-drug resistance has seen an increase in the number of pathogen strains that are resistant to one or more antibiotics, and in some cases, multiple antibiotics and chemotherapeutic drugs [5].

MRSA is typically resistant to aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides. Although such strains are still uncommon, transferable vancomycin resistance is currently relatively widespread in *Enterococcus* and made its way to MRSA in 2002.These strains are also resistant to disinfectants, and MRSA can be a significant contributor to illnesses picked up in hospitals. Vancomycin, a previously used antibiotic, has been revived to treat MRSA infections.

The healthcare industry continues to be challenged by the issue of antibiotic resistance. To tackle this issue, new antibiotics and therapeutic approaches are required. Chemical leads for new medications are being produced as a result of improvements in the discovery of new sources of antibiotic natural products and expanding antibiotic chemical diversity. Inhibitors of microbial virulence and resistance processes are orthogonal approaches that are also producing new compounds that can prolong the shelf life of existing antibiotics. The issues posed by antibiotic resistance in the twenty-first century can be overcome thanks to this novel chemistry and our improving understanding of its mechanisms, causes, and dissemination [6].

Bacteriophages assist in preserving bacterial homeostasis, which regulates bacterial growth, in the natural world. But owing to modern technology, we can now comprehend and make use of this resource from nature. Phages have no effect on commensals in the gastrointestinal system, the accompanying bacterial flora in the environment, or desirable microorganisms in meals [7].

Phages are normal commensals of people and animals and do not alter the organoleptic (*i.e.*, taste, structure, color, and odour) of food products. They also do not have an adverse effect on the environment. The abundance of bacteria in marine and freshwater habitats causes phage counts to routinely reach 10^7/mL and occasionally to exceed this value 300-fold [8]. There are very few truly sterile foods. As a result, phages are probably prevalent because bacteria are present in the majority of food that is consumed [9].

Phages structure are amino acids and nucleic acids, which they break down into a helical tail, a short collar, and a polyhedral head. Head: The head is made up of 2000 capsomeres that each contain double-stranded DNA. Tail: The tail is made up of a hollow inner tube that is encircled by a contractile sheath that has 24 annular rings [10].

LITERATURE REVIEW

Bacteriophage

Bacteriophages, which means "bacteria eaters" in Greek, were identified about a century ago. Both Felix d'Herrelle and Edward Twort published separate accounts of isolating filterable organisms that could kill bacterial cultures and create little cleared zones on bacterial lawns. Although they shared the credit for the discovery, it was d'Herrelle who gave the organisms the name "bacteriophages" and who spent his entire life studying bacteriophages, including its potential as a form of treatment. Natural bacteriophages, also known as phages, are the most prevalent microbes in our environment and are widely distributed in food and drink. Since each phage is specific for a particular bacterium, they cannot attach to other bacteria. They do not effected on human, animals, or plants. Humans have routinely been exposed to phages at high quantities through food, drink, and the environment for countless millennia without suffering any negative effects. There can be up to 1 billion phages/mL in aquatic habitats and 100 million phages/g in some food products.

Bacteriophage structure

All bacteriophages genetic material, either in the form of DNA or RNA. A protein structure encloses these nucleic acids. In order to connect to the surface of the bacteria they assault, bacteriophages also contain tails and other supporting structures.

Mode of phage action inside the host

These phage/host systems have adapted over millions of years to work together and its ideal structure as shown at Figure 1. Also, every bacterium already has its own complement of specialized phages with which to compete, and a non-specialized newcomer phage would be ineffective [11].

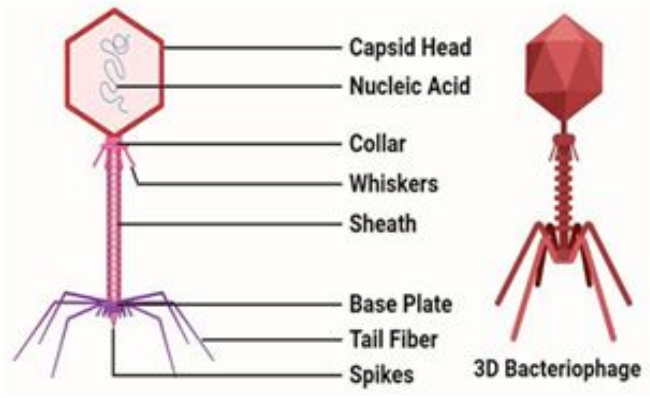

Figure1: Micrograph showing the structure of phage.

There are different cycle of phage entry according to phage nature: In general; the first attachment can be reversed because the cell might not be alive at that moment, making it useless to attach. The phage DNA circularizes after the second attachment stage, which is irreversible, and the bacterium begins to produce phage proteins. The entire host cell is sequestered by these proteins, which force the organism to only create new phages. Following progeny phage assembly, two distinct phage proteins cause cell lysis, releasing daughter phages that are prepared to begin the subsequent cycle as shown at Figure 2. Each phase of the process necessitates compatibility between the phage and host systems, which accounts for the exceptionally high specificity of phages for a particular host.

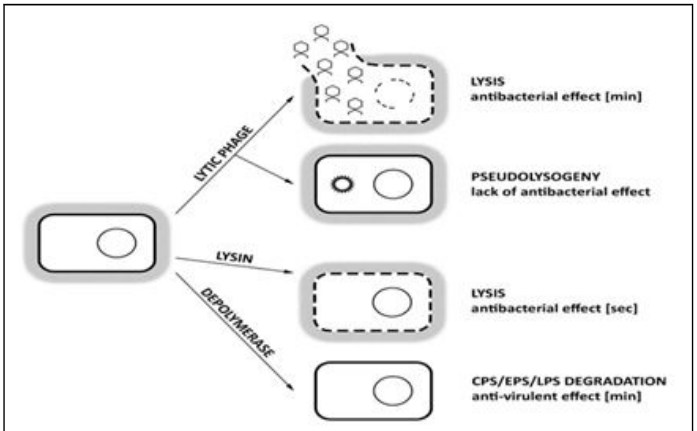

Figure 2: Micrograph showing different cycle of phage entry according to phage nature.

Temperate phage: Integrate their genome into the host chromosome or occasionally keep it as a plasmid to be passed to daughter cells during cell division or horizontally across the bacterial community, temperate phages are not used for therapeutic purposes. They could go through lysogenization or a conventional lytic cycle [12].

When host circumstances are compromised, maybe as a result of a lack of nutrition, temperate phages enter the lytic life cycle, at which point prophages become active [13].

At this point, the reproductive cycle, which causes the bacterial cell to lyse. The virus replicates continuously throughout the lysogenic life cycle as the bacterium grows, and it is present in all bacterial offspring. One such phage with a lysogenic cycle is phage lambda from *E. coli* is a bacterial virus, or bacteriophage, that infects the bacterial species *Escherichia coli* (*E. coli*).

The wild type of this virus has a temperate life cycle that allows it to either reside within the genome of its host through lysogeny or enter into a lytic phase, during which it kills and lyses the cell to produce offspring. Lambda strains, mutated at specific sites, are unable to lysogenize cells; instead, they grow and enter the lytic cycle after superinfecting an already lysogenized cell [14].

The lambda phage lacks a contractile tail, it is unable to 'push' its DNA through a bacterial cell membrane in the course of an infection. It has evolved the tip of its tail to interact with a particular pore to allow entry of its DNA to the hosts, therefore it must instead use an already-existing pathway to infiltrate the host cell [15] Figure 3 shown the step of lambda phage entry.

For conclusion the step of lambda phage entry as example of temperate phage:

- Bacteriophage Lambda's J protein in the tail tip binds to an cell. The J protein interacts with an *E. coli* porin molecule called the maltose outer membrane porin (produced by the gene), which is a component of the maltose operon [16].
- The outer membrane is used to inject the linear phage genome.
- The mannose permease complex processes the DNA in the 12-base G-C-rich cohesive "sticky ends" of the cos sites, which are used to quickly circularise the inner membrane (encoded by the manXYZ genes) [17].
- Host DNA ligase ligates the ends of the viral single-strand DNA. Most people are unaware that the first direct nucleotide sequencing of a biological DNA focused on the 12 bp lambda cohesive ends [18,19].

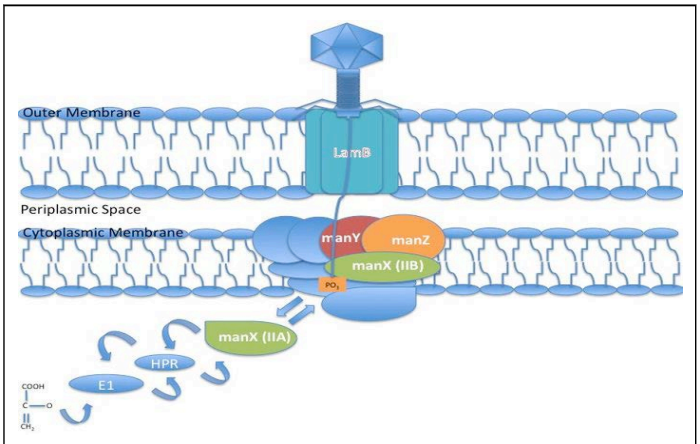

Figure 3: Micrograph showing mode of action Lambda phage DNA injection into the cell membrane using Mannose PTS permease (a sugar transporting system) as a mechanism of entry into the cytoplasm.

Lytic phage

Adsorption: One bacteriophage at a time attaches to the surface of a bacterial host during adsorption. By attaching its tail to receptors on the surface of the target bacteria, many bacteriophages only target one particular kind of bacteria is due to the precise interactions between the tail and receptors.

Penetration: The bacteriophage on the surface of the bacterial cell enters its cell membrane during penetration. It accomplishes this by dissolving the bacterial cell wall utilizing certain enzymes. After then, the bacteriophage fuses its genetic material with the bacterial cell.

Transcription: The bacteriophage utilizes the machinery present in each particular bacterial cell during transcription. It reproduces its genetic material and other necessary proteins using the bacterial cell.

Biosynthesis: The phage DNA replicates inside the cell, synthesizing new phage DNA and proteins

Maturation: The replicated material assembles into fully formed viral phages (each made up of a head, a tail and tail fibers [20]. Figure 4 shown the step of lytic phage entry.

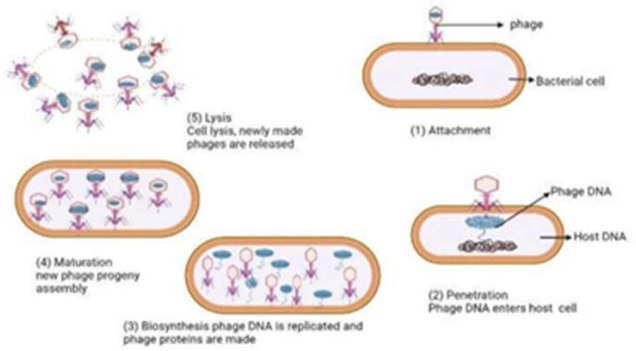

Figure 4: Micrograph showing the life cycle of phage.

Lytic phages solely employ endolysins: Rapture the bacterial cytoplasm I by holins, allowing endolysins to access the glycoproteins therein. Endolysins can only reach bacterial murein at a specific moment controlled by holins, which synchronises the holin-endolysin system to the late stage of viral replication. Cells are lysed and mature lytic phage progeny are released *via* the synergistic holin-endolysin system as shown in Figure 5. Depolymerases are related to the virion particle and are used by lytic phages, which only use VALs, to break down bacterial cell walls at the start of an infection. Depolymerases break down polysaccharide molecules like capsule, Lipopolysaccharide (LPS), or biofilm matrix, whereas VALs are in charge of PG degradation necessary for phage genetic material injection into the infected host cell.

lytic phages can encode holins and endolysins, which break down bacterial cell walls and membranes, making them a possible new weapon in the war against bacterial diseases a. Due to this characteristic, they are effective against bacteria that are both susceptible to and resistant to antibiotics.

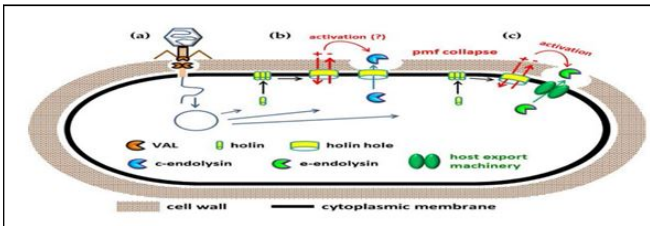

Figure 5: Micrograph showing phage entry mechanism by endolysins.

DISCUSSION

Lytic phage solely injection phage's genetic material by tails

The stages of infection which requires injection of the phage's genetic material into the host cell. For this the phage must penetrate the bacterial cell. Evidence has suggested that attachment and penetration are coordinated by the baseplate in tailed phage. Phage tails vary widely through nature, but the most sophisticated have a tube for delivering genetic material surrounded by a contractile sheath. The sheath contracts like a coiled spring and then on release drives the tube into the bacterial cell. In T4 phage, the entire baseplate-tail-tube complex consists of around one million atoms, making up 145 chains of 15 different proteins. The empty phage cell which is left outside the bacterium is called the ghost or doughnut.

Lytic phage synthesizes early proteins endonucleases and exonucleases that degrade the host genome

Once inside, the phage synthesizes early proteins including endonucleases and exonucleases that degrade the host genome. They are then able to use the host cell's machinery to synthesize proteins and produce progeny. The nucleotides released may be recycled by the phage for the replication of their own progeny (e.g., T7 phage) or excreted from the host cell (e.g., T5 phage). Small chemical modifications (in the case of T4 phage, chemical modification of the viral cytidines) to the phage genome allow its genetic material to be differentiated from the host genome and prevents self-degradation during this process. Other early proteins include those required for replication of the phage genome. RNA is not broken down, so the phage may also produce inhibitors that prevent host RNA polymerase from interfering with viral polymerases during later infection as shown in Figure 6. The newly synthesized phage genome produces late proteins including the capsid subunits and tail. This process can occur within minutes of the bacteria becoming infected.

Depending on how urgently new antibiotics are needed to treat each pathogen, the pathogens have been grouped into three priority categories: Critical, high, and medium. Bacteria that have developed resistance to numerous medicines, including the carbapenems, a last-resort antibiotic class, are pathogens with a significant need for new antibiotics. Pathogens in the second and third groups contain additional bacteria that are developing an increasing level of antibiotic resistance and are responsible for more widespread illnesses including gonorrhoea and salmonellosis.

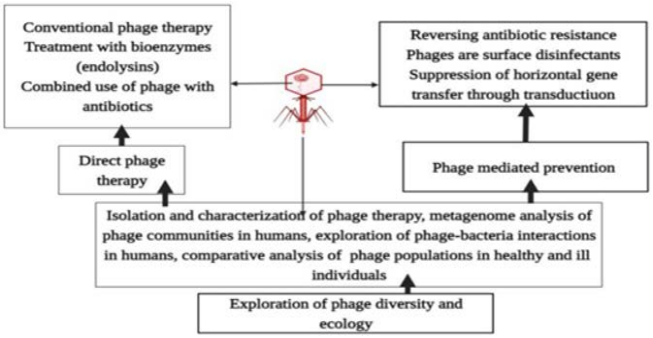

Figure 6: Micrograph showing the lytic phage stage therapy.

Examples gram-positive MDR bacteria

***Staphylococcus aureus*:** Known as the golden cluster seed, is a spherical bacteria that is frequently present in the skin, intestines, vagina, nose, and throat of humans. It is a pathogen of higher concern because it can produce a wide range of diseases that can be fatal and because it can quickly adapt to various environmental circumstances. Due of the rapid rate at which *S. aureus* develops resistance to standard antimicrobial drugs, these characteristics have made it harder to treat *S. aureus* infections.

Multiple antibiotic resistance is a major health concern in the treatment of staphylococcal infections, especially infections of Methicillin-Resistant *S. aureus* (MRSA) which occurs due to the extensive use of antimicrobial agents, coupled with the transmission of an appreciable proportion of the organism by person-to-person contacts. Hence, effective control of antibiotic use and prevention of the transmission of these strains are essential to eradicate this infectious organism. The importance of faecal-oral transmission in humans is demonstrated by the fact that the gut is an important habitat for parasites and bacteria that can be spread through objects contaminated with faeces. Staphylococci in stools have been identified as a significant pathogen causing antibiotic-associated diarrhoea in people.

Early investigations compared, categorised, and categorised *S. aureus* phages based on how they responded to polyclonal antiserum, which can prevent phage infection. phages were divided into six serogroups based on the results of the phage neutralisation assays. There are 11 distinct staphylococcal serogroups (A–H and J–L) that have been identified with additional staphylococcal phage isolates and sera. It was discovered that group E, J, and K phages had a preference for coagulase-negative staphylococci and were non-virulent to *S. aureus*. Serogroups A, B, and F might be used to categorise the majority of the temperate phages that infect *S. aureus*.

There are different types of phage:

Phages belonged to the Herelleviridae family and the *Silviavirus* genus: The coverage and the identity percentages between the genomes varied from 85% to 94% and from 97%to 99%, respectively. TEM analysis showed the straight contractile tail and icosahedral head as shown in Figure 7. Similarly, KSAP11 narrow neck characteristic of phages presenting a Myoviridae morphology.

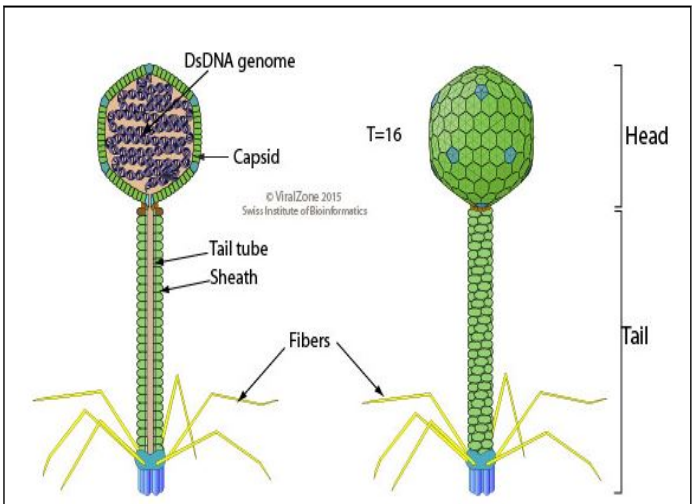

Figure 7: Morphology of Silviavirus showing arrow indicates contract tail with icosahedral head.

The presence of a long contractile tail as shown in Figure 8. This phage has an average head diameter of 93 nm, a tail with a length of 210 nm, and a width of 22 nm, which is close to Remus. Specifically, these features correspond to those of other reported Herelleviridae S. *aureus* phages.

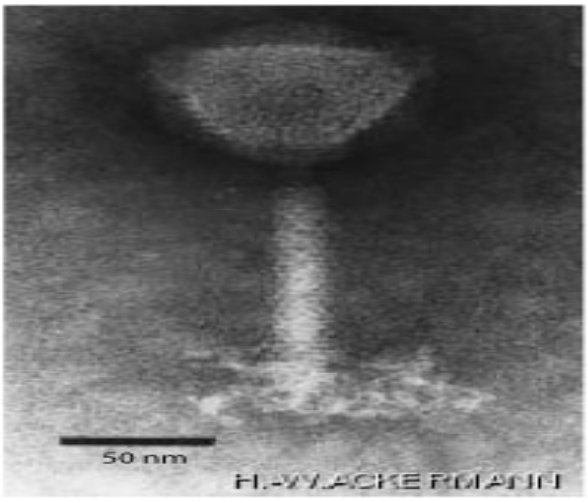

Figure 8: Morphology of Herellevirida phage under TEM bar indicates 50 n.

Several phages from the order Caudovirales: Obligatorily lytic on *S. aureus* and cannot transfer bacterial DNA have a complex virion structure comprising a head and long contractile tail. Some, less abundant and comprising only 14 isolates of sequenced genomes have a short tail the structure clear at Figure 9. They follow the Picovirinae subfamily of family Podoviridae. Regardless of being found in different topographical areas, phages that infect *S. aureus* are much alike, and have been interrelated to the *Rosenblumvirus* genus, subfamily Picovirinae.

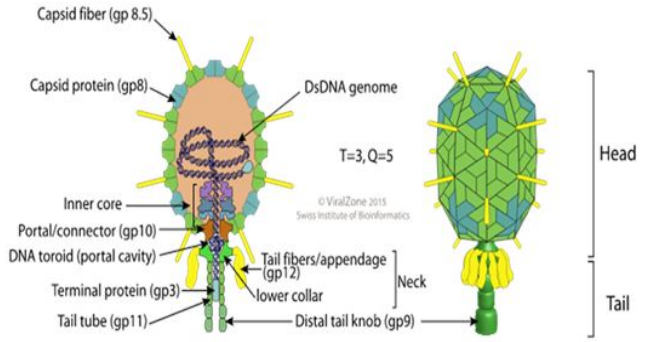

Figure 9: Morphology of Picovirinae phage.

***Staphylococcus* phage P954, *Staphylococcus* phage ROSA, *Staphylococcus* phage PT1028 and *Staphylococcus* phage P954 and *Staphylococcus* phage ROSA:** These phages of *Staphylococcus aureus* with dsDNA genomes of about 40 kbp. They show close relationships to a number of other phages with similar sized genomes isolated from the same host. Although the morphology of P954 and ROSA have not been reported, related viruses show a morphotype with isometric heads and long non-contractile tails as shown in Figure 10. These phages and their relatives are probably members of the Siphoviridae. *Staphylococcus* phage PT1028 is a phage of Staphylococcus aureus with an unknown morphology and a dsDNA genome of around 20 kbp that shows little relationship to any other phage.

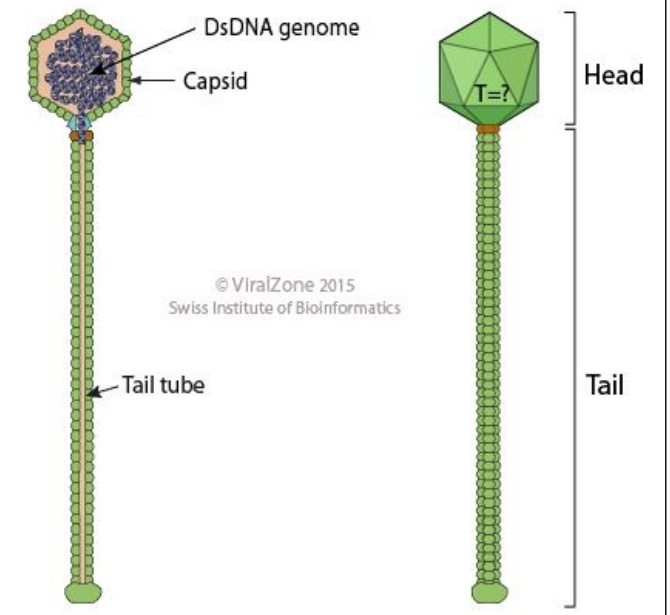

Figure 10: Morphology of *Staphylococcus* phage P954 phage.

Romulus and remus: These phage classified as members of the family Myoviridae. Both phages possess an isometric head with a diameter of 90 nm and a contractile tail with a length of 204 nm and a width of 17 nm as shown Figure 11. These dimensions correspond to those of other twort like viruses infecting S. *aureus*. Consequently, phage SA11, closely related to Romulus and Remus, was presumably erroneously described as a siphovirus.

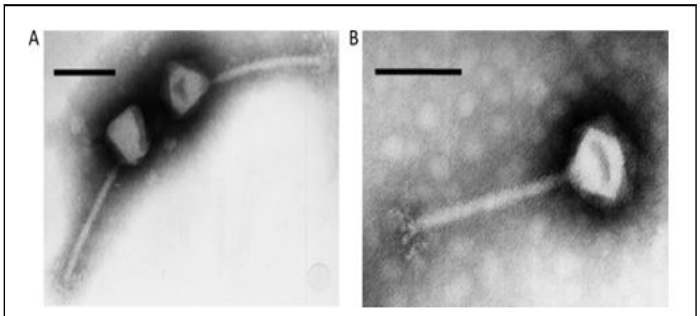

Figure 11: Morphology of Romulus and Remus. Shown are transmission electron microscopic images of myoviruses Romulus (A) and Remus (B) negatively stained with 2% (wt/vol).

Phages infecting *S. aureus* genus Twortlikevirus: appear to be genotypically and proteomically as shown in Figure 12 related and have been their double-stranded genomes contain 127,188 bp to 140,194 bp, display a G+C content of 30.04 to 30.60%, and contain 183 to 217 open reading frames (ORFs).

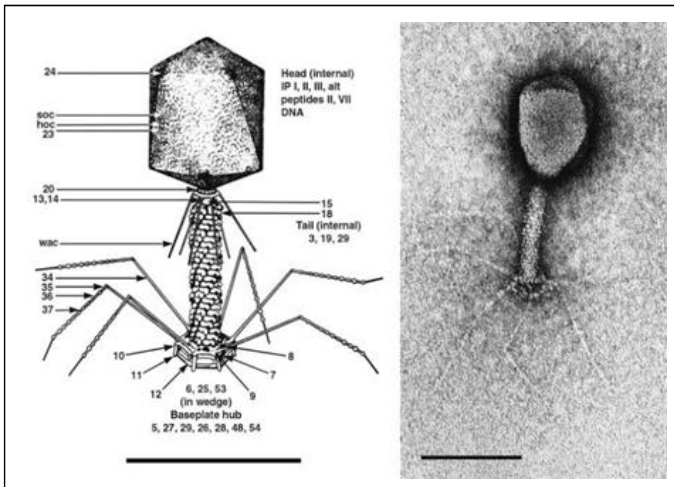

Figure12: Morphology of Myoviruses under transmission electron microscopic images of Myoviruses.

Anti-*S. aureus* lytic Caudovirales bacteriophages: These anti-S. *aureus* phages belong to various families and genera, including Herelleviridae (Kayvirus, Silviavirus genera) and Podoviridae, with Silviavirusphages having the broadest activity spectrum.

Gram-negative MDR bacteria

***Klebsiella pneumonia*:** The strong propensity of *Klebsiella pneumoniae* to develop sophisticated resistance characteristics makes it an opportunistic pathogen that is exceedingly challenging to treat. Notably, 22%-72% of hospitalised and immunocompromised patients die from Multidrug Resistant (MDR)-*Klebsiella pneumoniae* (KP) infections. There is still a need to research and create an effective strategy against MDR-KP infections, even when new medications or combined antibiotic therapy show some success.

Phages that have effect on *Klebsiella pneumoniae* (KP) infections

vB_Kpn_ZC2 phage: Phage ZCKP2 with an icosahedral head, a filamentous, cross-banded, and non-contractile tail; these

morphological findings are characteristics of Siphovirus as shown in Figure 13. The phage proportions were measured on virions and the head diameter is ~65 nm, and the tail length is ~160 nm.

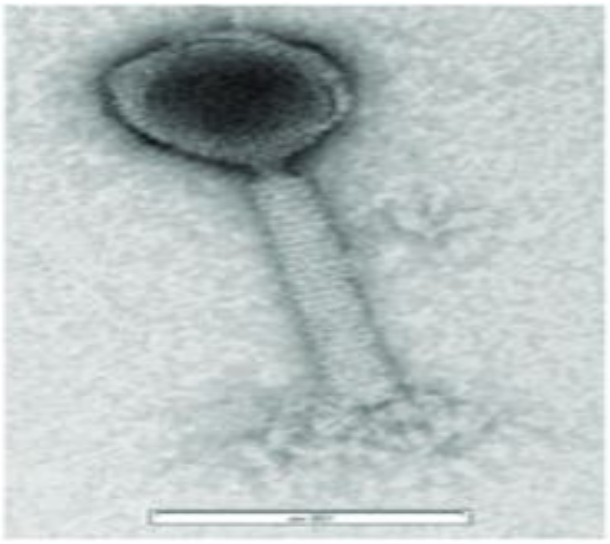

Figure 13: Transmission electron microscopic image of phage ZCKP2.

Phage KPO1K2: Morphologically KPO1K2 possessed icosahedral head with pentagonal nature with apex to apex head diameter of about 39 nm. Presence of short noncontractile tail (10 nm) suggested its inclusion into family Podoviridae as shown in Figure 14 with a designation of T7-like lytic bacteriophage.

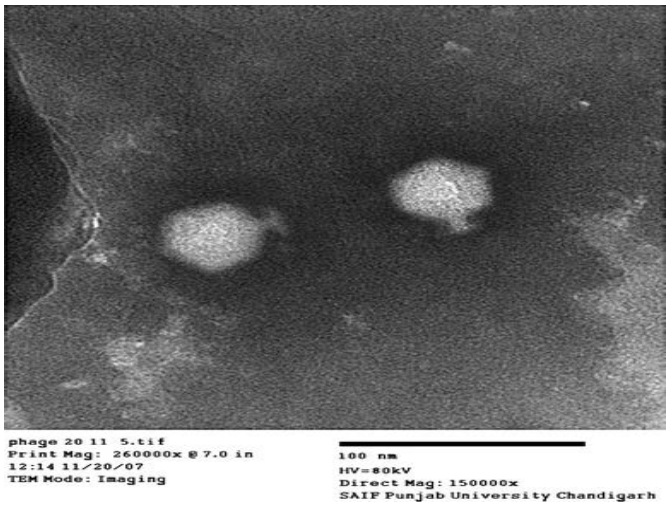

Figure 14: Transmission electron microscopic image of phage KPO1K2.

Isolated from effluent water and isolated to prevent *K. pneumoniae*. This phage, which belonged to the Podoviridae family and has T7-like as shown in Figure 15 characteristics, was demonstrated to be stable throughout a broad pH range of 4-11. The spot test revealed that 28% (7/28) of *K. pneumoniae* had been infected by the phage.

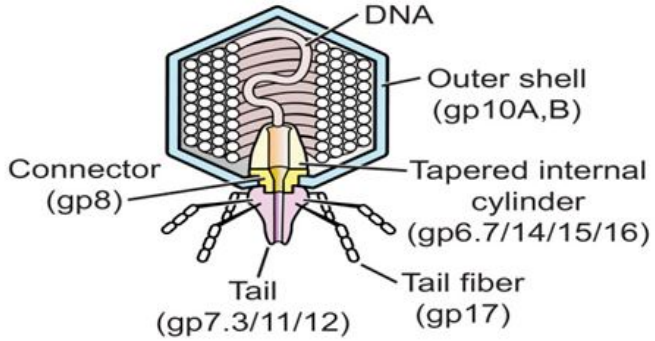

Figure 15: Morphology of *K. pneumoniae*, which belonged to the Podoviridae family and has T7-like characteristics.

Phage vB_KpnP_IME279

Isolated from hospital urine sample was lysed using an isolate from hospital sewage. This phage, which is a member of the Podoviridae has an icosahedral capsid of ~57 nm in diameter and a short, non-contractile tail. Stable throughout a large pH range of 3 to 11, as well as a temperature range of 40°C to 60°C. The lytic properties of vB_KpnP_IME279 against various tested *K. pneumoniae* should be noted as shown in Figure 16. Additionally, phage genome research demonstrates that the under investigation phage does not contain a toxin gene, providing a safety assurance for the phage's potential clinical use in the future.

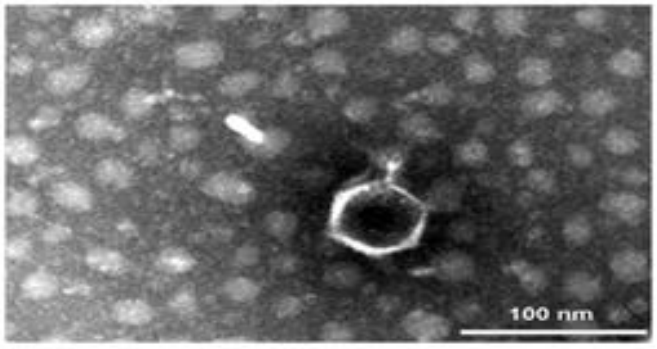

Figure 16: Morphology of phage vB_KpnP_IME279 as revealed by transmission electron micrographs. Phage vB_KpnP_IME279 has an icosahedral capsid of ~57 nm in diameter and a short, non-contractile tail. Scale bar represents 100 nm.

Phage 0507-KN2-1

The morphological characteristics of 0507-KN2-1 resembled Myoviridae family members, which have an icosahedral head and a contractile tail isolated for a new type of capsular isolates of *K. pneumoniae* (KN2). The genomic investigation of this phage, which was assigned to the Myoviridae phage family, revealed a potential polysaccharide depolymerase encoded by the 3738-bp gene. This protein was synthesised in recombinant form, and its enzymatic activity and capsular polysaccharide specificity were confirmed through analysis as shown in Figure 17. Then, in spot testing, this pure depolymerase caused *K. pneumoniae* strains to be decapsulated. The ability of this protein to cause digestion-like marks on *K. pneumoniae* strains with

different types of capsules or KN2 capsular polysaccharide mutants was noteworthy.

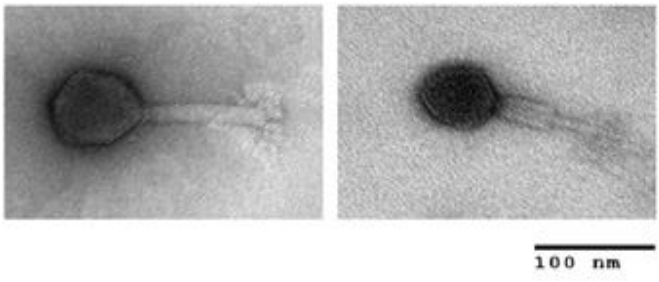

Figure17: Morphology TEM images of 0507-KN2-1 taken at 150 000 6 magnification. Scale bars for both pictures, 100 nm.

***Klebsiella* phage PG14:** The phage's icosahedral head and tail, which also had tail fibres, were visible in a Transmission Electron Microscopy (TEM) image of the organism, showing that it is a member of the order Caudovirales as shown in Figure 18 and family Siphoviridae.

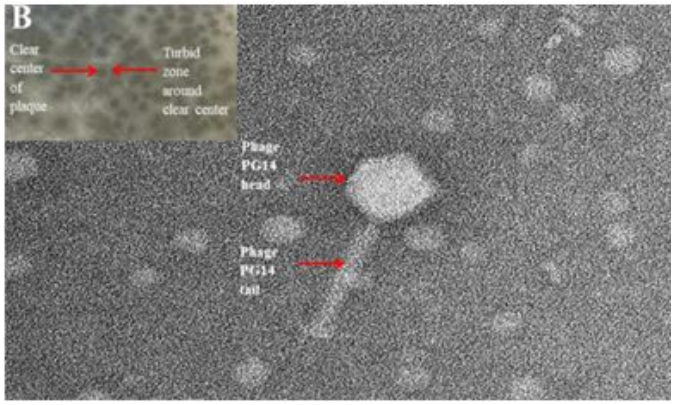

Figure 18: Morphology TEM images of *Klebsiella* phage PG14 taken at 150 000 6 magnifications. Scale bars for both pictures, 100 nm.

Phage therapy our weapon for fight pathogenic bacteria

One of the safer choices for treating disorders brought on by bacteria that are both vulnerable to and resistant to antibiotics is phage therapy. Despite the fact that phage therapy was initially utilised a century ago, it has recently gained popularity once more as a result of the rapidly rising rates of bacterial antibiotic resistance that have resulted in high rates of morbidity, mortality, and monetary expense. In addition to prescription antibiotics, it entails treating bacterial infections with live, lytic, bioengineered, and phage-encoded biological products. Importantly, phages will exit the body within seven days after an infection has been treated. While focusing on certain bacterial strains, they barely disrupt the microbial balance in humans.

Compared to regular antibiotic therapy, phage therapy provides a number of benefits:

- Phage isolation is quick, rather easy, and affordable.
- Phage resistance spreads roughly 10 times more slowly than antibiotic resistance.
- Phage tend to continue reproducing until the population density of the host bacterium has been greatly reduced.
- Phage stay infectious in extremely hostile environmental circumstances. These characteristics suggest that phage therapy —in contrast to standard chemical antibiotics—may require fewer or limited administrations while functioning as well as or better than conventional therapies.

Most of the problems made in these early phage therapy researches can be attributed to ignorance of the biology of phages. Rudimentary purification and preservation methods resulted in low titers of active phage and contamination from bacterial antigens, and phages that were ineffective against the target bacteria were used as a treatment. The transmission of the phage to the infection site was made more challenging by the medical limitations of the day. For example, it was just recently learned that the patient's innate immune response contributes to the removal of active phage and decreases the efficacy of phage therapy.

Phage against medically serious pathogenic sample

Phage therapy against a variety of pathogens that are therapeutically significant has recently been investigated using animal models as shown Figure 19. Oral phage therapy prevented 66.7% of mice from dying when they were exposed to gut-derived sepsis caused by *P. aeruginosa*, compared to 0% in the control group.

A single dose of phage given concurrently with the administration of *Clostridium difficile* was sufficient to prevent infection in a hamster model of the condition. Phage treatments administered after infection saved 11 out of 12 mice, whereas control animals given *C. difficile* and clindamycin perished within 96 hours. Additionally, using a hamster model, phage combinations dramatically inhibited *C. difficile* growth *in vitro* and constrained proliferation *in vivo*. Intraperitoneal administration of a single phage strain was sufficient to rescue 100% of mice in bacteremia models using vancomycin-resistant *E. faecium*, extended spectrum β-lactamase producing *E. coli*, and imipenem-resistant *P. aeruginosa*.

Phage cocktails have also been used to treat antibiotic-resistant *P. aeruginosa* infections of the skin, lungs, and gastrointestinal tract in animal models. Additional animal studies show similarly promising results for multidrug-resistant *E. coli* O25:H4-ST131, *Vibrio parahaemolyticus*, *S. aureus*, and *A. baumanii*. There is even an indication that phage are capable of restoring antibiotic sensitivity in antibiotic-resistant bacteria, as in the case of multidrug-resistant *P. aeruginosa*.

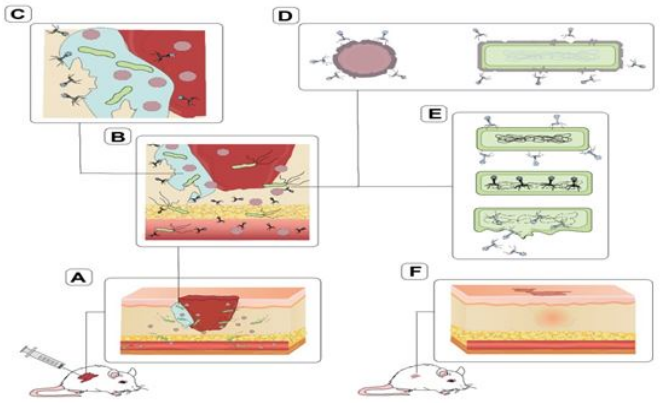

Figure 19: Phage treatment employs a variety of strategies to stop bacterial infection. (A) Bacterial pathogens have taken up residence in the wound. (B) Bacteriophages shield people from developing septicemia from bacterial infections. (C) Bacteriophages remove the wound-produced bacterial biofilm, which is one of the main contributors to antibiotic resistance. (D) By removing virulence factors like capsules, bacteriophages reduce bacterial invasiveness. (E) Bacteriophages stop wound infections by eliminating their hosts. (F) Applying bacteriophages to different wounds has been shown to have the effect of accelerating the healing process.

The Eliava institute of bacteriophage and the institute of immunology and experimental therapy in Wroclaw, Poland, are two Eastern European institutions where phage therapy trials on humans have been conducted for almost a century. Phage has been widely employed by The Eliava Institute to treat common bacterial infections such *S. aureus*, *E. coli*, *Streptococcus* spp., *P. aeruginosa*, *Proteus* spp., *S. dysenteriae*, *Salmonella* spp., and *Enterococcus* spp. in preclinical and clinical settings.

Effective uses include both preventative and therapeutic surgical and gastroenterological procedures. Topical administration of *S. aureus*-specific phage was adequate for recovery in each patient in a six-patient case series of diabetic foot ulcers that were resistant to antibiotics. In animal models, *P. aeruginosa* infections of the skin, lungs, and digestive system have also been treated with phage cocktails.

The ability of phages induces certain immune responses that result in the generation of specific antibodies against phage antigens is known as immunogenicity, and it is a crucial factor to consider. Phage immunogenicity in humans has received little research and is subject to conflicting opinions. According to substantial clinical findings, the immunogenicity of phages varies greatly depending on the kind, dosage, and method of administration of the phage as well as the immunological status of the host. The effectiveness of phage therapy was typically not definitively correlated with antiphage antibodies.

The dynamics of phage immunogenicity were investigated in a mouse model that was especially designed to target *S. aureus*. Anti-phage antibodies were detected, but they were unable to counteract the phage-antibacterial action over the 21–25 days that phages were present in the blood flow.

A fundamental issue with *S. aureus* phage therapy is the dearth of reliable clinical research for *S. aureus* infections. There haven't been any randomised double-blind trials, in particular. Case reports or small clinical trial series serve as the foundation for the vast majority of already utilised treatments. Additionally, although we are aware of the antibacterial properties of phages in vitro, we lack understanding on these agents' activity *in vivo*, particularly from clinical trials, and more knowledge is required before phages may be employed in healthcare.

K. pneumoniae is known to be the organism most frequently responsible for both community and hospital-acquired pneumonia. The ST11 and ST383 strains are frequently detected in patients with pneumonia. However, *K. pneumoniae* resistance to antibiotics is rapidly increasing. In the fight against MDR bacteria, phages have shown positive clinical results and efficacy as supplemental or alternative treatments to antibiotics.

Two phages (pKp11 and pKp383) that specifically targeted ST11 and ST383 MDR *K. pneumoniae* were discovered due to their wide host range, robust lytic activity, and remarkable environmental adaptation. Early-stage pneumonia in mice was successfully treated with both phages, and cocktails of the two phages were more effective at reducing bacterial loads, inflammatory markers, and pathogenic loss.

CONCLUSION

Multidrug-Resistant (MDR) bacteria are those that are resistant to three or more types of antimicrobial drugs. Due to the widespread use of antibiotics, the evolution of antimicrobial resistance factors is now outpacing the development of novel medications. Phage therapy was first used a century ago, but it has recently come back into fashion as a result of the sharp increase in antibiotic resistance among bacteria, which has led to high rates of morbidity, death, and monetary expense.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHORS' CONTRIBUTIONS

Conceptualization: H.H. and R.A. Resources and data curation: H.H. and R.A. Writing original draft: H.H. and R.A. Writing, review and editing: H.H. and R.A. All authors have read and approved the manuscript.

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