Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/20214
DOI URL: http://dx.doi.org/10.21474/IJAR01/20214

REVIEW ARTICLE

BACTERIOPHAGE & IT'S THERAPY: AN OVERVIEW

Chepala Uma Maheswari[1], Maniyar Babyaiesha[1] and Indu Sharma[2]

1. Department of Microbiology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India).
2. Department of Biotechnology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India).

Manuscript Info

Manuscript History
Received: 12 November 2024
Final Accepted: 16 December 2024
Published: January 2025

Key words:-
Bacteriophages, Bacterial Infections, Phage Therapy

Abstract

This review provides a concise overview of the comprehensive exploration of bacteriophages and their applications in phage therapy. Beginning with a historical perspective, the narrative traces the evolution of phage therapy from its early roots to contemporary innovations. The advantages, including high specificity, safety, and adaptability, are highlighted, along with inherent limitations such as specificity requirements and regulatory challenges. The review emphasizes the significance of novel concepts, life cycle insights, and standardized procedures in enhancing the efficacy and responsible implementation of phage therapy. Addressing resistance management and the pursuit of the perfect bacteriophage, the abstract underscores the ongoing research and strategic approaches that position phage therapy as a dynamic and vital component in the ever-evolving realm of antibacterial treatments.

Introduction:-

The treatment of bacterial infections using bacteriophages has begun even before the clinical use of antibiotic drug which is almost 20 years before the 1st antibiotic drug was discovered. But in the 1940s, as broad-spectrum antibiotics were introduced the creations of phage treatments has been kept on hold. Some countries like the Eastern Europe and Soviet Union have continued developing phage therapy. However, with the global spread of bacterial illnesses resistant to drugs, nations such as those in Western Europe and America have begun to re-engage in phage therapy [1].

Bacteriophages or Phages are viruses that enter bacteria and multiply there. The general life cycle of a Bacteriophage is: 1. The bacteriophage first attaches to the surface of a vulnerable bacterial cell's surface. This attachment is particular and is mediated by interactions between viral proteins (situated on the strands pf the phage's tail or spikes) and the bacterial cell wall's receptors. Different phages have different host specificity based on these interactions. 2. The phage inserts its genetic material (DNA or RNA) into the bacterial cell once it has connected. Enzymes released by certain phages break down a portion of the bacterial cell wall, creating an opening through which the phage injects its genetic material. In the case of phages with an icosahedral head and a tail, the tail acts like a syringe to deliver the genetic material into the bacterial cell [2]. 3. The phage genetic material takes over the host's cellular machinery inside the bacterial cell. To create viral RNA and proteins, the viral DNA is copied and transcribed. 4. Complete phage particles are formed by the assembly of freshly generated viral components, such as

Corresponding Author:- Indu Sharma
Address:- Department of Biotechnology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India).

structural proteins and genetic material. 5. Once assembly is complete, the bacteriophage induces the host cell to lyse (burst) by producing enzymes that degrade the bacterial cell wall. This leads to the release of a large number of new phage particles, which can go on to infect other bacterial cells in the vicinity [3].

Phages stand as the most prolific biological entities on Earth, surpassing an estimated $10^{30.}$ The extensive abundance and variety of phages in the natural environment serve as a readily accessible pool for selecting phages with diverse applications, spanning from antibacterial therapy to tasks such as decontamination, infection prevention, identification, and diagnosis. In clinical laboratories, phage typing currently has proven valuable for discerning species and subtypes of bacteria, exemplified in the identification of strains like Salmonella, Bacillus anthracis, Staphylococcus, and various Brucella species [4].

Advantages

(a) Due to the distinct mechanisms employed by lytic phages in bacterial eradication compared to antibiotics, they prove effective against multidrug-resistant (MDR) pathogens. Consequently, they often complement antibiotics, leading to frequent synergies in combination therapies. (b) The inherent specificity of phage therapeutics, where a phage typically targets only a specific bacterial species or subgroup, results in a narrow spectrum of activity. This characteristic is expected to have minimal impact on normal microflora, in contrast to the broader effects of antibiotics [5]. (c) Phages gather exactly at the site of infection because they multiply on the target bacterium directly. The pathogen cell at the injection site are effectively eliminated thanks to their self-replicating nature. (d) The ubiquity of phages in the environment, coupled with their ease of isolation, facilitates the straightforward selection of new phages active against resistant bacterial strains. This simplicity makes the process of discovering new antimicrobials efficient and cost-effective. (e) Phages can encode enzymes capable of degrading biofilms associated with challenging infections. Phage walls break dissolve biofilms, allowing other antimicrobials to get through and penetrate this barrier [6]. (f) In thousands of human cases throughout the former Soviet Union and Eastern Europe, phage therapy has proven safe with very few negative effects documented. (g) Phage cocktails, which combine phages with distinct specificities, can target several bacterial diseases or treat a pathogen's diversity, thereby preventing the formation of resistance. (h) Phages can adapt to newly emerging resistant strains of the host bacterial through coevolution with their bacterium hosts. (i) Phages have the potential to be less expensive to produce than other antibacterials due to their ability to replicate themselves on a bacterial host. (j) Phages may be able to live steadily in living things. (k) Phages often show low immunogenicity, which reduces the likelihood of unfavourable immune responses such heightened inflammatory reactions or phage inefficacy as a result of strong antibody neutralization [7].

Constraints

(a) The requirement for high specificity stems from the limited host range or lytic spectrum that many phages display. This specificity implies that a single phage may not suffice to deal with the several strains that make up a bacterial infection. (b) Phage therapy tactics get more complex when different phage combinations are required to treat the same bacterial disease in different geographical regions due to the presence of certain bacterial pathogens. (c) Through changes to their phage receptor(s) or other mechanisms, bacterial strains can become resistant to phages, which could decrease the efficacy of treatment, including during therapy. (d) Phage cocktailing requires that phage collections be updated on a regular basis in order to handle newly discovered resistant varieties. This may involve continuous discovery efforts or adaptation/engineering of phages [8]. (e) The collection, maintenance, and utilization of extensive banks of diverse phages can pose challenges in terms of safety testing and navigating the regulatory pathway for obtaining approval for therapeutic applications. This complexity may contribute to increased difficulties and expenses. (f) Expanding the therapeutic spectrum of phage cocktails by multiplying their components can complicate and escalate the phage therapy manufacture on a large scale. (g) It's unclear how the human immune system reacts to phages utilized in therapy, raising the potential for impeded efficacy or undesired immune responses, which adds a layer of uncertainty to phage-based treatments [9].

Classification

Bacteriophages, or simply phages are viruses that enter and multiply inside of bacterial, and they are essential in molding bacterial populations additionally influencing microbial ecosystems. These viruses are classified based on various characteristics, including their morphology, nucleic acid content, life cycle, and the host bacteria they target (Table 1). One common classification system categorizes bacteriophages into two main groups: the tailed phages and the tailless phages [10].

Table 1:- Classification of Bacteriophages.

Family	Nucleic acid	Characteristic	Role
Myoviridae	Linear dsDNA	Contractile tail, non-enveloped	Infecting the bacteria.
Siphoviridae	Linear dsDNA	Long non-contractile tail, non-enveloped	Serves as a conduit for viral DNA traffic.
Podoviridae	Linear dsDNA	Short non-contractile tail, non-enveloped	Helps with the translocation of the genome into the cell
Tectiviridae	Linear dsDNA	Isometric, non-enveloped	Attach directly to the host cell peptidoglycan structure.
Corticoviridae	Circular dsDNA	Isometric, non-enveloped	Infects gram-negative marine bacteria from the genus Pseudoalteromonas.
Lipothrixviridae	Linear dsDNA	Rod-shaped, Enveloped	Binding to cellular pili-like appendages.
Plasmaviridae	Circular dsDNA	Pleomorphic, Enveloped	Infect wall-less bacteria of the class Mollicutes and are released by budding through the cell membrane without causing host cell lysis.
Rudiviridae	Linear dsDNA	Rod-shaped, Enveloped	Act as a template for site-selective and spatially controlled chemical modification.
Fuselloviridae	Circular dsDNA	Lemon shaped, non-enveloped	Serves for adsorption to the host membrane.
Inoviridae	Circular ssDNA	Filamentous, non-enveloped	Mobilize DNA in the microbial world, and thus play a role in the evolution of microorganisms.
Microviridae	Circular ssDNA	Isometric, non-enveloped	Inhibition of host cell DNA replication and superinfection exclusion.
Leviviridae	Linear ssDNA	Isometric, non-enveloped	Infecting various gram-negative bacteria by adsorption to their pilus structures.
Cystoviridae	Segmented dsDNA	Spherical, Enveloped	Responsible for genome packaging, replication, and transcription.

Tailed phages are characterized by a distinctive structure with an icosahedral head and a tail. This group is further split up into three families: Myoviridae, which have long contractile tails; Siphoviridae, which possess long non-contractile tails; and Podoviridae, distinguished by short tails [11].

On the other hand, tailless phages, also known as polyhedral phages, lack the characteristic tails seen in their tailed counterparts. This group includes the families Microviridae and Inoviridae. Microviridae are small, icosahedral phages with a single-stranded DNA genome, while Inoviridae, also known as filamentous phages, have a filamentous shape and a single-stranded DNA genome [12].

Another classification criterion considers the nature of the nucleic acid within the phage. Bacteriophages can have either DNA or RNA genomes, and they may be further categorized based on whether their genetic material is single-stranded or double-stranded [13].

In summary, the classification of bacteriophages involves considering their morphological features, including the presence or absence of tails, as well as the nature and structure of their nucleic acid. This classification system helps researchers understand the diversity of bacteriophages and their interactions with bacterial hosts, assisting in the development of disciplines like biotechnology and phage therapy [14].

Novel concepts of phage therapy

Phage therapy, an innovative approach to combat bacterial infections, encompasses several novel concepts reshaping the landscape of antibacterial treatments [15].

One key concept is the personalized nature of phage therapy. Unlike traditional antibiotics with broad-spectrum activity, phages can be selected based on the specific bacterial strains causing an infection. This personalized approach allows for precision in targeting pathogens while minimizing disruption to the host's natural microbiota. The ability to tailor phage treatments to the unique characteristics of each infection enhances their effectiveness [16].

Another pivotal concept is the use of phage cocktails. These cocktails consist of multiple phages, offering a broader spectrum of activity against diverse bacterial strains. Phage cocktails are particularly beneficial in addressing infections caused by multiple bacterial species or strains that may evolve resistance during treatment. The dynamic nature of phage therapy allows for the continual adaptation of cocktails, incorporating new phages to counter evolving bacterial threats [17].

Bioengineering of phages represents an exciting concept in phage therapy. Researchers are exploring ways to modify phages for therapeutic purposes, enhancing their natural capabilities. This includes improving host range, stability, and safety. Bioengineered phages hold the potential to overcome some limitations associated with natural phages, providing a more effective and tailored treatment approach [18].

Advancements in phage pharmacology contribute to the success of phage therapy. Optimizing administration routes and dosages ensures effective phage delivery at the site of infection. Techniques such as encapsulating phages in nanoparticles or incorporating them into gels enable targeted delivery, addressing challenges related to phage stability and distribution within the body. These innovations enhance the overall pharmacokinetics of phages, improving their therapeutic potential [19].

Furthermore, regulatory aspects and ethical considerations are crucial in the implementation of phage therapy. Creating standardized phage protocols, characterization, manufacturing safety and efficacy of phage-based treatments. Ethical considerations include the necessity for informed consent, transparency in treatment plans, and the responsible use of phages to mitigate the risk of unintended consequences [20].

In conclusion, the novel concepts in phage therapy, including personalized targeting, phage cocktails, bioengineering, advancements in phage pharmacology, and considerations of regulation and ethics, collectively represent a paradigm shift in the field of antibacterial treatments. Phage therapy holds great promise as a versatile, specific, and adaptable strategy against bacterial infections, offering new hope in the face of antibiotic resistance and the challenges posed by evolving pathogens [21].

Life cycles

Bacteriophages, or phages, exhibit two distinct life cycles, known as the lysogenic cycle and lytic cycle. During the lytic cycle, the phage first adheres to the surface of the host bacterium, injects its genetic material, and then uses the bacterial apparatus to synthesize phage components – a process known as biosynthesis. Subsequently, these

components are assembled into new virions during the maturation phase. The host bacterium is then lysed, leading to the release of newly formed phages. This cycle's direct and detrimental effect on host bacterium is what makes it unique [22].

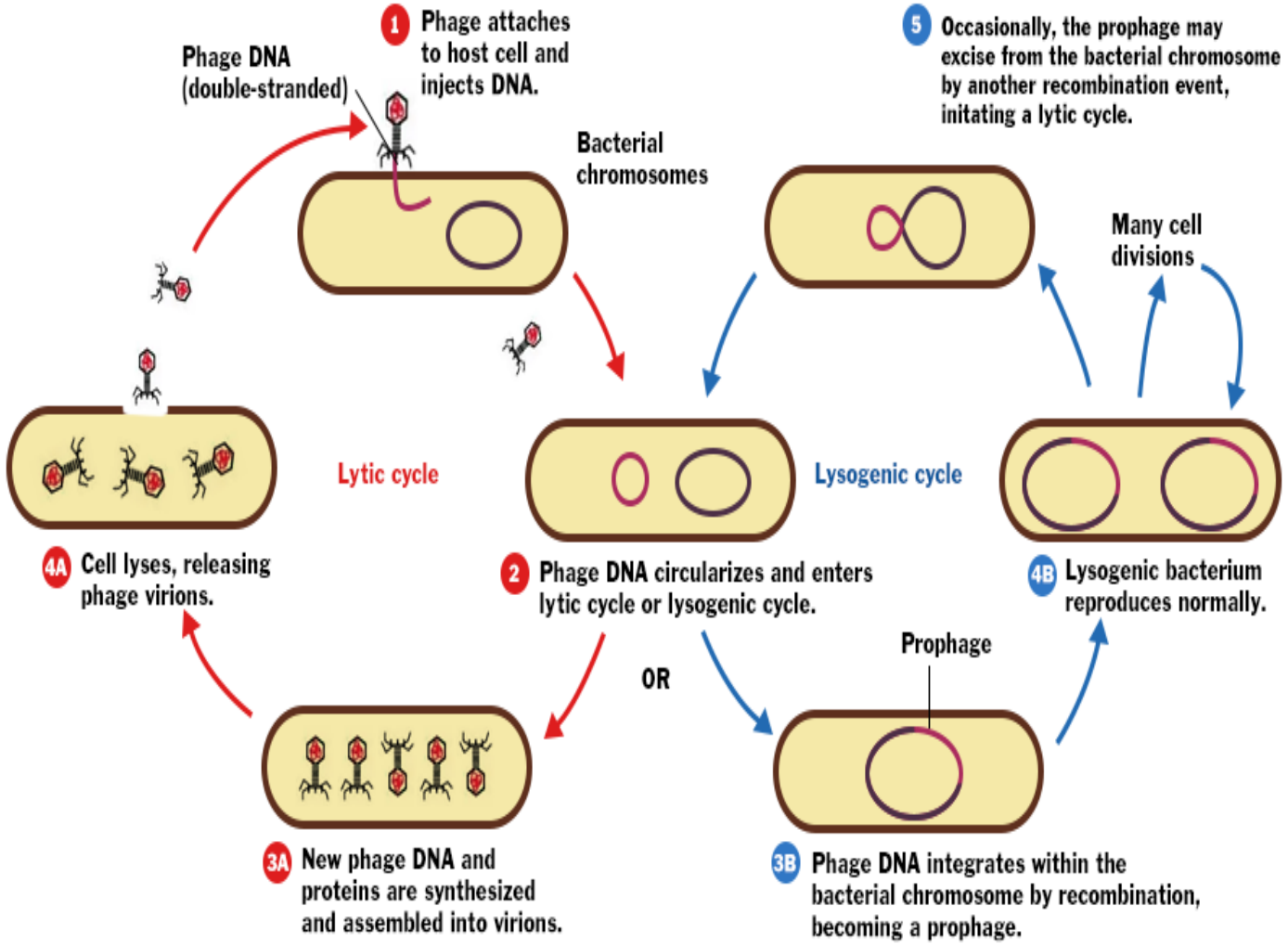

Figure 1A:- Showcasing the lytic cycle and lysogenic cycle.

Conversely, the lysogenic cycle involves a more intricate process. Its starts with the phage attaching to and penetrating the bacterial cell, just as the lytic cycle. Nevertheless, the phage's genetic material merges with the bacterial chromosome to form a pro-phage during the lysogenic cycle [23]. During the cell division, the prophage replicates along with the bacterial DNA. Because of its integrated state, the phage can live inside the host bacterium for a long time without endangering it right away. Under certain environmental conditions, such as specific triggers, the prophage may undergo induction, causing it to go from lysogenic to the lytic cycle. This transition involves the biosynthesis of new phage components, maturation, and eventual release through host cell lysis [24].

In summary, the host bacteria is immediately destroyed by the lytic cycle, but the lysogenic cycle entails a more latent and integrated relationship between the phage and its host, with the potential for later activation into the lytic cycle. These cycles represent fundamental strategies employed by bacteriophages in their interactions with bacterial hosts [25].

Stabilization and Formulation

Like other protein-based macromolecules, bacteriophages can lose their functioning in unfavourable situations due to protein misfolding, aggregation, and denaturation. Previous research has demonstrated how sensitive bacteria are too many conditions, including salinity, pH, temperature and organic solvents [26]. Different methods have been

developed for the long-term preservation of free phages, and they indicate that, for the most part, phages that are already at room temperature may be kept at 4 degrees Celsius for lengthy periods of time with very small titer decreases. However, survivability varies widely among bacteriophages, and instances of titer depletion over short durations, even at 4°C, have been reported. Freezing at -80°C provides additional preservation, and when rapid deterioration occurs, the use of addictives such as gelatin, magnesium ions, and glycerol can improve stability results [27].

When preparing bacteriophage formulations for therapeutic delivery, there are more obstacles to overcome than when keeping free phage lysates in the lab. Phage formulations may be stored in harsh conditions, as opposed to long-term storage under ideal circumstances, contingent on the use. For example, in the setting of gastrointestinal illnesses, a therapeutic phage cocktail needs to endure and operate in an extremely acidic environment, which can be too serves for phages that aren't prepared [28]. Phages in non-liquid, dry formulations are generally more stable over the long run, but they are still susceptible to heat and other stressors that might lower the titer. Moreover, bacteriophage degradation may arise from the essential formulation production processes of freeze-drying and spray-drying. These factors highlight how crucial it is to create stable phage formulations, with an emphasis on evaluating phage transport to target bacteria, figuring out how stable a formulation is under different circumstances, and improving phage survival throughout the formulation production process [29].

Phage formulations are frequently created using standard techniques that entail encapsulation. This general phrase includes a number of methods, including electrospinning, liposome encapsulation, freeze-drying, spray-drying, and emulsification. By coating or encasing bacteriophages in stabilizing chemicals, these techniques provide protection from the outside world. To target bacterial cells, encapsulated phages need to be freed from the substance. [30].

Phage's interactions with mammalian cells

Phages and tissues of the mucosa

Phages interact with mucosal surface immunity of the host, a crucial immunological and physiological barrier found in various animals, including the digestive system and respiratory system of humans. This surface plays a dual role, protecting populations of commensal microorganisms and preventing the growth of bacterial pathogens. Composed mainly among the much glycoproteins that the subsurface epithelium secretes, the mucosal surface provides both structure and nutrients, influencing microbiota composition and favoring commensal symbionts [31]. Studies indicate that mucosal surfaces, like those in the gut, often harbor bacterial colonies that are both more prevalent than those in surrounding, partly due to mucin degradation by gut microbes and host epithelium secretions that influence the commensal microbiota in a certain way. These secretions from the hosts include several antimicrobials, including RegIII and alpha-defensin. In contrast, in response to invasion bacterial species that are harmful, the epithelium may enhance antimicrobial agent production, hypersecrete mucin, or alter mucin glycosylation patterns to increase the physical clearance of invasive bacterial species and thwart microbial adhesion [32].

These layers of mucous serve as hosts to huge and varied phage communities, as depicted in Figure 2A. Phage communities associated with mucus exhibit significant enrichment in contrast to the non-mucosal surroundings. According to studies conducted on a variety of mucosal surfaces in fish, humans, mice, and corals, mucus has an average 4.4-fold higher phage count than bacterial cells. Phage adherence is thought to be responsible for this increased abundance; phages bind mucin glycoproteins weakly using immunoglobulin-like (Ig-like) protein domains on their capsids [33]. The Ig-like fold, which is found in T-cell receptors and antibodies and is extensively distributed in nature, is vital to the human adaptive immune system's ability to mediate critical binding contacts. These Ig-like domains are found in around 25% of the genomes of sequenced Caudovirales and are usually present on the surface of the virus. It is thought that these architecturally visible Ig-like domains help phages adsorb their bacterial host in the environment, even though they are frequently unnecessary for phage growth in lab settings. It is claimed that phages with Ig-like domains that efficiently bind to the mucus layer will go through positive selection in the mucosa. This gives rise to the idea of the bacteriophage adherence to mucus (BAM) model, which is a phage-mediated non-host-derived layer of immunity. [34].

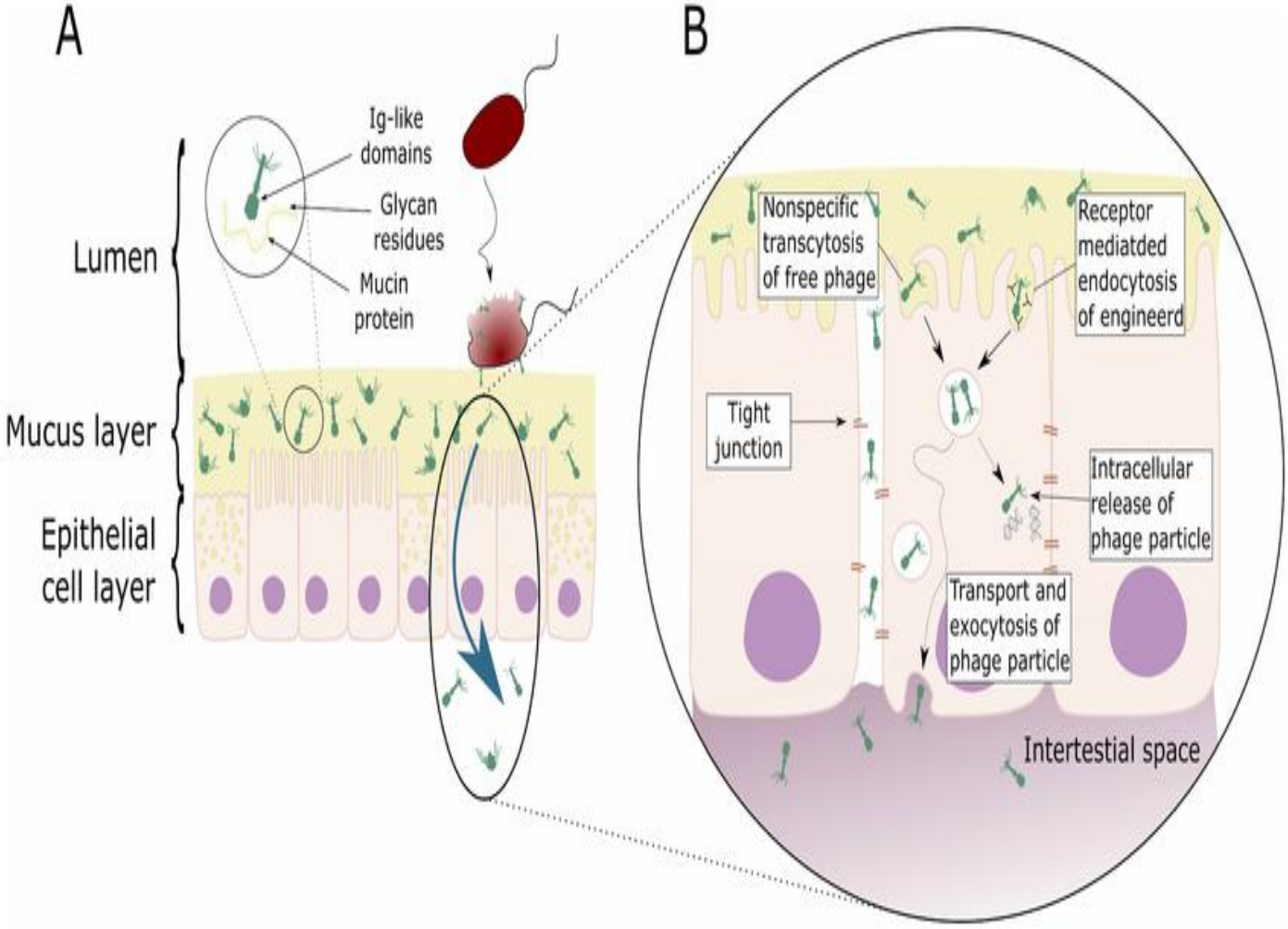

Figure 2 A:- Depiction of mucosal layers that serve as hosts for phage communities.

Phages, besides directly influencing bacterial populations, can indirectly impact bacterial host colonization of animal cells. Neisseria meningitidis in this instance furthermore, filamentous phage (MDA), the phage enhances colonization of host cells by increasing bacterial binding to epithelial cells. The filamentous phage acts as a linker between bacteria, promoting their aggregation and colonization. This specificity to epithelial cells suggests a potential mutual benefit, where the phage, instead of lysing bacteria, provides additional binding sites, elevating the colonization frequency. This dynamic interaction challenges traditional views of phages as mere bacterial predators, hinting at a more complex relationship between phages and bacteria [35].

Phage Transcytosis

The epithelium of cells below mucosal surfaces structures an additional obstacle, separating the densely colonized mucosa from sterile body regions. Phages, prevalent in the epithelial mucus layer, interact with these layers. Bacterial translocation, where commensal bacteria move from the intestine to lymph nodes and internal organs across the mucosal epithelium, is crucial in disorders. While bacterial translocation is well understood, the translocation of bacterial viruses is not fully grasped [36].

M13 phages, employed as control vectors in phage display, showed limited internalization by enterocytes and endothelial cells both in vivo and in vitro. Chloroquine, an inhibitor of clathrin-dependent endocytosis, blocked the in vitro uptake of M13 phages, indicating a receptor-mediated pathway for internalization. This suggests that the specific interactions between phages and the epithelium, requiring binding to membrane receptors, may play a key role in phage uptake [37].

Studies on the administering non-engineered phages orally in vivo have shown both effective and ineffective systemic dissemination. While natural movement of phages is feasible to go from gut to circulation, various factors, including the host's physiological status and phage characteristics, may regulate this process. The most important component seems to be the dose, although phage particle physical characteristics like size and shape may also affect their capacity to enter mammalian bodies. A higher dose firmly given the likelihood of finding orally applied phages in circulation or tissues. Furthermore, variations in the phage's capacity to replicate on gut bacteria may restrict its systemic distribution following oral administration [38].

Orally administered phages need to effectively cross the mucosal barrier and bypass the cellular epithelium for systemic distribution. Recent studies demonstrate that phages achieve this through a non-specific transcytosis mechanism, primarily from the apical to the basal across various epithelial cell types. This process involves diverse phage types and morphologies, with about 10% of cells in the epithelium endocytosing phage particulates. Phage particles undergo exocytosis at the basal cell layer after passing via the Golgi apparatus after being endocytosed. This mechanistic insight explains the structural presence of phages in the anatomy of humans without causing disease. However, conflicting observations raise questions about the impact of phages near the cell nucleus on cellular function and whether RNA produced from phages triggers biological reactions [39].

Phage resistance during Phage therapy

The application of phages as medicinal agents presents difficult issues regarding the range of hosts, the mode of administration, pharmacokinetics/pharmacodynamics, and managing resistance risk. Unlike antibiotics, phages can self-replicate at the infection site, resembling predator-prey population dynamics. Theoretical predictions propose that combining phage therapy with host defenses can prevent bacterial overgrowth and the development of variations resistant to phage. Studies in a P. aeruginosa pneumonia mouse model highlighted the essential role of neutrophil-phage synergism in disease resolution [40]. Two general models for managing resistance involve phage cocktails and personalized phage therapy. Phage cocktails broaden host range and reduce resistance emergence, while personalized therapy adapts single phages to patient conditions, offering flexibility and efficiency. Despite documented cases of phage resistance during therapy, careful monitoring and adaptation of phage composition, along with potential synergies with host defenses or antibiotics, can contribute to successful outcomes in certain infections [41].

Standard operation procedure for Phage therapy

The standard operating procedure (SOP) for phage therapy includes a number of systematic actions to guarantee the secure and efficient application of bacteriophages as therapeutic agents. The first step is the isolation and identification of bacteriophages with specificity for the target pathogenic bacteria. This may involve screening phage libraries, isolating phages from environmental sources, or selecting from pre-existing phage collections. Following identification, the phages undergo comprehensive characterization, including assessments of lytic activity, host range, and stability [42].

The formulation of a phage cocktail is a crucial next step, where multiple phages are combined to broaden the host range and reduce the risk of resistance. The formulation process considers factors such as stability under storage conditions, appropriate dosage, and the selected route of administration. Patient-specific considerations play a key role, involving the isolation of the pathogenic bacteria from the patient and conducting sensitivity testing to ensure the most effective phage selection. This personalized approach tailors the phage therapy to the specific microbial profile of each patient [43].

Dosage and administration routes are with caution determined depending on elements like the location of infection, the overall health status of the patient, and the characteristics of the pathogenic bacteria. Continuous monitoring and evaluation are integral components of the SOP, involving regular assessments of the patient's response to treatment, monitoring bacterial load, and adjusting the phage cocktail if necessary. The potential interactions with the patient's immune system and any emergence of phage-resistant strains are closely observed [44].

The SOP also addresses documentation, emphasizing thorough record-keeping of the entire process. This includes detailed information on phage isolation, characterization, formulation, patient-specific considerations, treatment outcomes, and any observed complications. Regular review and updates to the SOP are essential to ensure that the phage therapy protocol remains aligned with the latest scientific knowledge and best practices [45].

Desirable traits in a perfect Bacteriophage

Phages with antimicrobial potential should possess a range of desirable traits to effectively combat bacterial infections while minimizing potential drawbacks. Firstly, high host specificity is a crucial trait, ensuring that the phage selectively targets the pathogenic bacteria without affecting beneficial or commensal bacteria. This specificity reduces the risk of disrupting the host microbiota and, consequently, mitigates unintended consequences on the patient's health. Additionally, a broad host range within a phage population or a well-designed phage cocktail is advantageous, allowing for the treatment of diverse bacterial strains and reducing the likelihood of bacterial resistance [46].

Rapid and efficient lytic activity is another desirable trait. Phages should be able to take hold of and spread throughout their bacterial hosts swiftly, resulting in the timely destruction of the targeted bacteria. This trait is particularly important for achieving a prompt therapeutic response, especially in acute infections. Moreover, phages should exhibit stability under diverse environmental conditions, including variations in temperature and pH, to ensure their efficacy during storage and administration [47].

The avoidance of lysogeny, where phages integrate their genetic material into the bacterial chromosome and potentially contribute to bacterial virulence, is a crucial trait for therapeutic phages. Lytic phages, which lead to the direct destruction of the bacterial host upon replication, are preferred to minimize any potential adverse effects on the patient. Furthermore, the absence of undesirable genes, such as those encoding toxins, enhances the safety profile of therapeutic phages [48].

Phages with a low likelihood of provoking an immune response in the host organism are advantageous. This trait helps to ensure that repeated phage administration remains effective without triggering significant immune reactions, which could limit the phage's therapeutic potential. Additionally, the ability of phages to resist bacterial resistance mechanisms, such as CRISPR-Cas systems or other bacterial defense mechanisms, is desirable for sustained efficacy in the face of evolving bacterial threats [49].

Lastly, scalability and ease of production are important practical considerations. Phages with traits that facilitate cost-effective large-scale production, purification, and formulation into therapeutic preparations are more likely to be viable candidates for widespread clinical use. Overall, a combination of these desirable traits contributes to the effectiveness, safety, and practicality of phages as antimicrobial agents in the context of phage therapy [50].

Conclusion:-

In conclusion, bacteriophages, with their historical significance and innovative applications in phage therapy, present a promising solution to combat bacterial infections. While their advantages include high specificity, safety, and adaptability, challenges such as specificity requirements and regulatory complexities must be addressed. Novel concepts, life cycle insights, and standardized procedures enhance the efficacy and responsible use of phage therapy. As we navigate the complexities of resistance management and seek the perfect bacteriophage, ongoing research, and strategic implementation position phage therapy as a dynamic and vital player in the evolving landscape of antibacterial treatments

References:-

[1] D. M. Lin, B. Koskella, and H. C. Lin, “Phage therapy: An alternative to antibiotics in the age of multi-drug resistance,” World J Gastrointest Pharmacol Ther, vol. 8, no. 3, p. 162, Aug. 2017, doi: 10.4292/WJGPT.V8.I3.162.

[2] A. Y. Hassan, J. T. Lin, N. Ricker, and H. Anany, “The Age of Phage: Friend or Foe in the New Dawn of Therapeutic and Biocontrol Applications?,” Pharmaceuticals 2021, Vol. 14, Page 199, vol. 14, no. 3, p. 199, Feb. 2021, doi: 10.3390/PH14030199.

[3] A. J. Cann, “Replication of Viruses,” Encyclopedia of Virology, p. 406, Jan. 2008, doi: 10.1016/B978-012374410-4.00486-6.

[4] S. Batinovic et al., “Bacteriophages in natural and artificial environments,” Pathogens, vol. 8, no. 3, Sep. 2019, doi: 10.3390/PATHOGENS8030100.

[5] Z. Golkar, O. Bagasra, and D. Gene Pace, “Bacteriophage therapy: A potential solution for the antibiotic resistance crisis,” J Infect Dev Ctries, vol. 8, no. 2, pp. 129–136, Feb. 2014, doi: 10.3855/JIDC.3573.

[6] I. Ul Haq, W. N. Chaudhry, M. N. Akhtar, S. Andleeb, and I. Qadri, "Bacteriophages and their implications on future biotechnology: A review," Virol J, vol. 9, no. 1, pp. 1–8, Jan. 2012, doi: 10.1186/1743-422X-9-9/FIGURES/2.
[7] P. Ioannou, S. Baliou, and G. Samonis, "Bacteriophages in Infectious Diseases and Beyond—A Narrative Review," Antibiotics 2023, Vol. 12, Page 1012, vol. 12, no. 6, p. 1012, Jun. 2023, doi: 10.3390/ANTIBIOTICS12061012.
[8] K. Fong, C. W. Y. Wong, S. Wang, and P. Delaquis, "How Broad Is Enough: The Host Range of Bacteriophages and Its Impact on the Agri-Food Sector," Phage, vol. 2, no. 2, p. 83, Jun. 2021, doi: 10.1089/PHAGE.2020.0036.
[9] N. Wu and T. Zhu, "Potential of Therapeutic Bacteriophages in Nosocomial Infection Management," Front Microbiol, vol. 12, Jan. 2021, doi: 10.3389/FMICB.2021.638094.
[10] M. Ye et al., "A review of bacteriophage therapy for pathogenic bacteria inactivation in the soil environment," Environ Int, vol. 129, pp. 488–496, Aug. 2019, doi: 10.1016/J.ENVINT.2019.05.062.
[11] Y. Bao, Y. Kapustin, and T. Tatusova, "Virus Classification by Pairwise Sequence Comparison (PASC)," Encyclopedia of Virology, pp. 342–348, Jan. 2008, doi: 10.1016/B978-012374410-4.00710-X.
[12] A. Luque, S. Benler, D. Y. Lee, C. Brown, and S. White, "The Missing Tailed Phages: Prediction of Small Capsid Candidates," Microorganisms, vol. 8, no. 12, pp. 1–18, Dec. 2020, doi: 10.3390/MICROORGANISMS8121944.
[13] S. R. Krishnamurthy, A. B. Janowski, G. Zhao, D. Barouch, and D. Wang, "Hyperexpansion of RNA Bacteriophage Diversity," PLoS Biol, vol. 14, no. 3, p. 1002409, Mar. 2016, doi: 10.1371/JOURNAL.PBIO.1002409.
[14] M. Podlacha et al., "Interactions of Bacteriophages with Animal and Human Organisms—Safety Issues in the Light of Phage Therapy," International Journal of Molecular Sciences 2021, Vol. 22, Page 8937, vol. 22, no. 16, p. 8937, Aug. 2021, doi: 10.3390/IJMS22168937.
[15] B. Zalewska-Piątek and R. Piątek, "Phage Therapy as a Novel Strategy in the Treatment of Urinary Tract Infections Caused by E. Coli," Antibiotics 2020, Vol. 9, Page 304, vol. 9, no. 6, p. 304, Jun. 2020, doi: 10.3390/ANTIBIOTICS9060304.
[16] F. Zeynali kelishomi, S. Khanjani, F. Fardsanei, H. Saghi Sarabi, F. Nikkhahi, and B. Dehghani, "Bacteriophages of Mycobacterium tuberculosis, their diversity, and potential therapeutic uses: a review," BMC Infect Dis, vol. 22, no. 1, pp. 1–14, Dec. 2022, doi: 10.1186/S12879-022-07944-9/FIGURES/3.
[17] B. K. Chan, S. T. Abedon, and C. Loc-Carrillo, "Phage cocktails and the future of phage therapy," Future Microbiol, vol. 8, no. 6, pp. 769–783, Jun. 2013, doi: 10.2217/FMB.13.47/ASSET/IMAGES/LARGE/FIGURE2.JPEG.
[18] L. Cui, S. Veeranarayanan, K. Thitiananpakorn, and D. L. Wannigama, "Bacteriophage Bioengineering: A Transformative Approach for Targeted Drug Discovery and Beyond," Pathogens 2023, Vol. 12, Page 1179, vol. 12, no. 9, p. 1179, Sep. 2023, doi: 10.3390/PATHOGENS12091179.
[19] M. I. Qadir, T. Mobeen, and A. Masood, "Phage therapy: progress in pharmacokinetics," Brazilian Journal of Pharmaceutical Sciences, vol. 54, no. 1, p. e17093, May 2018, doi: 10.1590/S2175-97902018000117093.
[20] P. Hyman, "Phages for Phage Therapy: Isolation, Characterization, and Host Range Breadth," Pharmaceuticals 2019, Vol. 12, Page 35, vol. 12, no. 1, p. 35, Mar. 2019, doi: 10.3390/PH12010035.
[21] G. Muteeb, M. T. Rehman, M. Shahwan, and M. Aatif, "Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review," Pharmaceuticals 2023, Vol. 16, Page 1615, vol. 16, no. 11, p. 1615, Nov. 2023, doi: 10.3390/PH16111615.
[22] Publications service, "On an invisible microbe antagonistic toward dysenteric bacilli: brief note by Mr. F. D'Herelle, presented by Mr. Roux," Res Microbiol, vol. 158, no. 7, pp. 553–554, Sep. 2007, doi: 10.1016/J.RESMIC.2007.07.005.
[23] A. Coffey, L. O'Sullivan, C. Buttimer, O. McAuliffe, and D. Bolton, "Bacteriophage-based tools: Recent advances and novel applications," F1000Res, vol. 5, 2016, doi: 10.12688/F1000RESEARCH.9705.1.
[24] J. Marchi, S. Zborowsky, L. Debarbieux, and J. S. Weitz, "The dynamic interplay of bacteriophage, bacteria and the mammalian host during phage therapy," iScience, vol. 26, no. 2, p. 106004, Feb. 2023, doi: 10.1016/J.ISCI.2023.106004.
[25] M. Zhang, T. Zhang, M. Yu, Y. L. Chen, and M. Jin, "The Life Cycle Transitions of Temperate Phages: Regulating Factors and Potential Ecological Implications," Viruses, vol. 14, no. 9, Sep. 2022, doi: 10.3390/V14091904.

[26] A. Bhatwa, W. Wang, Y. I. Hassan, N. Abraham, X. Z. Li, and T. Zhou, "Challenges Associated With the Formation of Recombinant Protein Inclusion Bodies in Escherichia coli and Strategies to Address Them for Industrial Applications," Front Bioeng Biotechnol, vol. 9, Feb. 2021, doi: 10.3389/FBIOE.2021.630551.
[27] A. Jurczak-Kurek et al., "Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage," Scientific Reports 2016 6:1, vol. 6, no. 1, pp. 1–17, Oct. 2016, doi: 10.1038/srep34338.
[28] D. Rosner and J. Clark, "Formulations for Bacteriophage Therapy and the Potential Uses of Immobilization," Pharmaceuticals, vol. 14, no. 4, 2021, doi: 10.3390/PH14040359.
[29] B. Loh, V. S. Gondil, P. Manohar, F. M. Khan, H. Yang, and S. Leptihn, "Encapsulation and Delivery of Therapeutic Phages," Appl Environ Microbiol, vol. 87, no. 5, pp. 1–13, Mar. 2021, doi: 10.1128/AEM.01979-20.
[30] K. Moelling, F. Broecker, and C. Willy, "A wake-up call: We need phage therapy now," Viruses, vol. 10, no. 12, Dec. 2018, doi: 10.3390/V10120688.
[31] A. Carroll-Portillo and H. C. Lin, "Bacteriophage and the Innate Immune System: Access and Signaling," Microorganisms 2019, Vol. 7, Page 625, vol. 7, no. 12, p. 625, Nov. 2019, doi: 10.3390/MICROORGANISMS7120625.
[32] P. Paone and P. D. Cani, "Mucus barrier, mucins and gut microbiota: the expected slimy partners?," Gut, vol. 69, no. 12, p. 2232, Dec. 2020, doi: 10.1136/GUTJNL-2020-322260.
[33] M. Kaltenpoth, W. Göttler, G. Herzner, and E. Strohm, "Symbiotic bacteria protect wasp larvae from fungal infestation," Current Biology, vol. 15, no. 5, pp. 475–479, Mar. 2005, doi: 10.1016/J.CUB.2004.12.084.
[34] J. D. Van Belleghem, K. Dąbrowska, M. Vaneechoutte, J. J. Barr, and P. L. Bollyky, "Interactions between Bacteriophage, Bacteria, and the Mammalian Immune System," Viruses, vol. 11, no. 1, Jan. 2019, doi: 10.3390/V11010010.
[35] A. York, "Bacterial pathogenesis: Bacteriophages, the glue that holds bacteria together," Nat Rev Microbiol, vol. 15, no. 9, pp. 514–515, Sep. 2017, doi: 10.1038/nrmicro.2017.96.
[36] A. A. O'callaghan and S. C. Corr, "Establishing Boundaries: The Relationship That Exists between Intestinal Epithelial Cells and Gut-Dwelling Bacteria," Microorganisms 2019, Vol. 7, Page 663, vol. 7, no. 12, p. 663, Dec. 2019, doi: 10.3390/MICROORGANISMS7120663.
[37] A. Kim et al., "Cellular Internalization Mechanism and Intracellular Trafficking of Filamentous M13 Phages Displaying a Cell-Penetrating Transbody and TAT Peptide," PLoS One, vol. 7, no. 12, p. 51813, Dec. 2012, doi: 10.1371/JOURNAL.PONE.0051813.
[38] H. Bao et al., "Transient carriage and low-level colonization of orally administrated lytic and temperate phages in the gut of mice," Food Production, Processing and Nutrition, vol. 2, no. 1, pp. 1–8, Dec. 2020, doi: 10.1186/S43014-020-00029-7/FIGURES/3.
[39] M. C. Bichet et al., "Bacteriophage uptake by mammalian cell layers represents a potential sink that may impact phage therapy," iScience, vol. 24, no. 4, p. 102287, Apr. 2021, doi: 10.1016/J.ISCI.2021.102287.
[40] S. C. Nang et al., "Pharmacokinetics/pharmacodynamics of phage therapy: a major hurdle to clinical translation," Clinical Microbiology and Infection, vol. 29, no. 6, pp. 702–709, Jun. 2023, doi: 10.1016/J.CMI.2023.01.021.
[41] K. M. Chung, X. L. Liau, and S. S. Tang, "Bacteriophages and Their Host Range in Multidrug-Resistant Bacterial Disease Treatment," Pharmaceuticals, vol. 16, no. 10, Oct. 2023, doi: 10.3390/PH16101467.
[42] Z. Cui, X. Guo, T. Feng, and L. Li, "Exploring the whole standard operating procedure for phage therapy in clinical practice," J Transl Med, vol. 17, no. 1, p. 373, Nov. 2019, doi: 10.1186/S12967-019-2120-Z.
[43] R. Flint, D. R. Laucirica, H. K. Chan, B. J. Chang, S. M. Stick, and A. Kicic, "Stability Considerations for Bacteriophages in Liquid Formulations Designed for Nebulization," Cells, vol. 12, no. 16, Aug. 2023, doi: 10.3390/CELLS12162057.
[44] P. Jiao, Y. Jiang, J. Jiao, and L. Zhang, "The pathogenic characteristics and influencing factors of health care-associated infection in elderly care center under the mode of integration of medical care and elderly care service: A cross-sectional study," Medicine, vol. 100, no. 21, p. E26158, May 2021, doi: 10.1097/MD.0000000000026158.
[45] P. Hyman, "Phages for Phage Therapy: Isolation, Characterization, and Host Range Breadth," Pharmaceuticals 2019, Vol. 12, Page 35, vol. 12, no. 1, p. 35, Mar. 2019, doi: 10.3390/PH12010035.
[46] J. H. Rex et al., "Progress in the Fight Against Multidrug-Resistant Bacteria 2005-2016: Modern Noninferiority Trial Designs Enable Antibiotic Development in Advance of Epidemic Bacterial Resistance," Clinical Infectious Diseases, vol. 65, no. 1, pp. 141–146, Jul. 2017, doi: 10.1093/CID/CIX246.

[47] H. Ling, X. Lou, Q. Luo, Z. He, M. Sun, and J. Sun, "Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era," Acta Pharm Sin B, vol. 12, no. 12, pp. 4348–4364, Dec. 2022, doi: 10.1016/J.APSB.2022.05.007.
[48] V. S. Gummalla, Y. Zhang, Y. Te Liao, and V. C. H. Wu, "The Role of Temperate Phages in Bacterial Pathogenicity," Microorganisms 2023, Vol. 11, Page 541, vol. 11, no. 3, p. 541, Feb. 2023, doi: 10.3390/MICROORGANISMS11030541.
[49] R. Capparelli et al., "Bacteriophage therapy of Salmonella enterica: A fresh appraisal of bacteriophage therapy," Journal of Infectious Diseases, vol. 201, no. 1, pp. 52–61, Jan. 2010, doi: 10.1086/648478.
[50] L. Aghebati-Maleki et al., "Phage display as a promising approach for vaccine development," Journal of Biomedical Science 2016 23:1, vol. 23, no. 1, pp. 1–18, Sep. 2016, doi: 10.1186/S12929-016-0285-9.