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TOPICAL APPLICATION OF BACTERIOPHAGES FOR THE TREATMENT OF WOUND INFECTIONS:

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ABSTRACT: Wound infections persist as a significant and persistent challenge within the realm of healthcare, posing numerous detrimental consequences that extend far beyond the affected individuals. These infections, characterized by the infiltration of harmful microorganisms into wounds, encompass a multifaceted predicament that engenders slower healing periods, heightened morbidity rates, and an onerous financial burden on healthcare systems worldwide. Bacteriophages often known as phages, are viruses that selectively target and eradicate bacteria, making them a potentially effective alternative or supplementary treatment for wound infections. This article gives a general summary of what is currently known about using bacteriophages topically to treat wound infections. It goes through the fundamentals of phage treatment, looks at the benefits and drawbacks of phage use, examines the preclinical and clinical data that is now available, and identifies future research paths in this area. The review highlights the potential of bacteriophage-based therapeutics to transform the treatment of wound infections and provides information on the crucial factors to take into account for effective implementation.

INTRODUCTION:

Wound Infections: Prevalence and Challenges: An important healthcare issue that has a high global economic and morbidity cost is wound infections. They can develop as a result of burns, burn injuries, chronic wounds (such diabetic ulcers), trauma, or surgical treatments [1]. The World Health Organisation (WHO) estimates that 11% of people worldwide suffer from chronic wounds, and this percentage is anticipated to grow as the global population ages and diseases like diabetes and obesity become more common [2].

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Gram-positive and Gram-negative bacteria, as well as other bacterial pathogens, are the main culprits in wound infections. One of the most frequent bacteria linked to wound infections is *Staphylococcus aureus*, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Due to their ability to create biofilms, which are organised bacterial colonies encased in a protective matrix, these bacteria are extremely resilient to both immune responses and common antibiotics [3, 4].

There are many difficulties in treating wound infections. The efficiency of conventional antibiotic therapy has been severely hampered, first and foremost, by the advent and spread of antibiotic-resistant bacteria. The dearth of innovative medicines in the pipeline makes the issue of antibiotic resistance, which is a major concern

worldwide, even worse. Additionally, infections in wounds can hinder the healing process, resulting in longer hospital stays, higher healthcare expenditures, and a worse quality of life for the patient [5, 6].

Alternative therapy modalities are desperately needed in light of these difficulties. Bacteriophages, viruses that attack and eradicate bacteria, are being considered as a potential remedy. Since bacterial hosts are the only organisms that bacteriophages specifically target, they do not affect other bacteria or host cells. Phage therapy for wound infections is appealing due to phages' selectivity and capacity to penetrate biofilms [7].

This study examines the topical use of bacteriophages as a potential substitute or supplemental treatment for wound infections. We will go through the fundamentals of phage treatment, the benefits and drawbacks of phage use, and the existing preclinical and clinical data. We want to shed light on the potential of bacteriophage-based therapeutics to revolutionise wound infection control and enhance patient outcomes by reviewing the present level of knowledge in this sector.

Antibiotic Resistance: An Urgent Need for Alternatives: Antibiotic-resistant bacteria are becoming more prevalent, which poses a serious danger to global public health. Antibiotic resistance has been promoted by overuse and improper usage, making many widely used antibiotics less efficient or ineffective against bacterial infections. Antibiotic resistance is a problem that has grown to be a serious healthcare concern, increasing morbidity, mortality, and healthcare expenses [5, 6].

Antibiotic resistance is a particularly tough situation when it comes to wound infections. A wound is the perfect place for bacteria to grow and produce biofilms, which are intricate bacterial colonies wrapped in a protective matrix. Biofilms have heightened tolerance to host immune responses and medicines, making them challenging to get rid of. MRSA (methicillin-resistant Staphylococcus aureus) and MDR-Pseudomonas aeruginosa are two bacteria that are frequently linked to wound infections and have become resistant to a variety of medicines. These hard-to-treat infections brought on by these resistant strains can result in protracted hospital admissions, greater healthcare expenses, and a higher likelihood of treatment failure [8, 9].

The problem of antibiotic resistance is made worse by the scarcity of new antibiotics. Fewer novel drugs are being put into clinical practise, which has drastically reduced the pipeline for developing new antibiotics in recent years. Clinicians have few treatment choices due to the lack of efficient antibiotics, which highlights the urgent need for new methods of preventing bacterial infections.

Bacteriophages: Nature's Bacterial Killers: William Twort originally identified bacteriophages in 1915, and Felix d'Herelle realised they had the capacity to eradicate bacteria in 1917.[13] Bacteriophages, also known as phages, are viruses that have coexisted alongside bacteria for billions of years and have been essential in controlling bacterial populations in the environment.[7] These viruses are naturally occurring predators of bacterial diseases because they have evolved to selectively infect and destroy bacteria. Phage treatment has seen renewed interest as a possible alternative to conventional antibiotics as a result of the discovery of phages and their potential therapeutic uses.

The most prevalent organisms on Earth are phages, which have a population of 1031. They are present in a variety of settings, including soil, water, and the human body. The number of phages in the human body is thought to be 1023, ten times more than the number of bacterial cells. This abundance highlights the possibility of using phages therapeutically [10, 11].

Phages are desirable as antibacterial agents because of their distinctive characteristics. They target certain bacterial species or even strains within a species, displaying a high level of host specificity. This specificity is caused by the interaction of the receptors on the bacterial cell surface with the surface proteins of phages. Phages can selectively kill dangerous bacteria while conserving the good microbiota by focusing on particular diseases [11, 12]. Additionally, phages have the ability to quickly reproduce inside bacterial cells, increasing their

population and quickening the death process. Because of its capacity for self-replication, phage therapy has the potential to be both economical and long-lasting in the treatment of infections.

Phages have been used therapeutically since the early 20th century, but their promise was eclipsed by the discovery and widespread usage of antibiotics. Phage treatment has acquired popularity once again as a possible option, nevertheless, as a result of the growth in antibiotic resistance. Preclinical and clinical research examining the effectiveness and security of phage-based treatments for different illnesses, particularly wound infections, have grown in number recently [7, 10]. This review focuses on using bacteriophages topically to treat wound infections. We intend to offer a thorough review of the potential of bacteriophage-based therapeutics in wound infection care by examining the fundamentals of phage therapy, the benefits and difficulties of phage application, and the most recent data from preclinical and clinical research.

Principles of Phage Therapy:

Bacteriophage Biology and Lifecycle: Bacteriophages, often known as phages, are viruses that attack and spread throughout bacterial cells. Phage treatment must be developed and used with a thorough understanding of the biology and lifetime of bacteriophages.

The genetic material of phages is contained within a protein cap, which can either be DNA or RNA. The genetic material is shielded from deterioration and made easier to transport into bacterial cells by the capsid. Because of the enormous genetic diversity of these viruses, pages come in a wide variety of geometries, including icosahedral, filamentous, and complicated ones [11, 12, 13].

Although there can be exceptions, a bacteriophage's lifespan normally follows a lytic or lysogenic course. During the lytic cycle, phages enter bacterial cells, reproduce inside of them, and then finally cause the cells to lyse, releasing offspring phages into the environment [13, 14]. There are multiple separate steps in this process:

Attachment: Phages bind to certain receptors on the surface of bacterial cells through the process of attachment. The viral tail fibres or other surface proteins, which recognise and bind to corresponding receptors on the bacterial cell surface, control the specificity of this interaction.

Penetration: Once the phage is in contact with the bacterium, it injects its genetic material into the bacterial cell, leaving the empty capsid outside. The machinery of the host cell is taken over by the phage genetic material, which directs it to create phage components rather than bacterial ones.

Replication: The phage genetic material is duplicated and transcribed within the bacterial cell to produce viral components, such as capsid proteins and enzymes required for phage assembly.

Assembly: The freshly created viral parts are put together to form whole phage particles. The mature phage particles are formed when the phage DNA or RNA is enclosed by the capsid proteins.

Lysis: Phage particles grow and reach a critical mass inside the bacterial cell. The bacterial cell wall is subsequently broken down by the phage-encoded enzymes, resulting in lysis and the release of many offspring phages [13, 14, 15].

During the lysogenic cycle, sometimes referred to as the delicate cycle, phage DNA is incorporated into the bacterial host's genome to create a prophage. In this situation, cell division does not immediately result in cell lysis because the phage DNA is reproduced alongside the bacterial DNA. For several generations, the integrated phage genome can be handed down to daughter cells inside the bacterial host. The prophage can excise from the bacterial genome under specific circumstances, such as stress or changes in the host environment, starting the lytic cycle and creating new phages [16, 17].

Phage-Host Interactions and Specificity: Phage-host interactions are the foundation of phage treatment because bacteriophages must specifically recognise and infect the target bacterial hosts for this strategy to be effective. Designing and implementing successful phage treatments requires an understanding of the processes underpinning phage-host interactions and the variables determining phage specificity [15]. The recognition and binding between phage surface proteins, such as tail fibres or receptor-binding proteins, and

certain receptors on the bacterial cell surface determines the specificity of phage-host interactions. These receptors, which include lipopolysaccharides, teichoic acids, or certain surface proteins, make up the bacterial outer membrane or cell wall [15, 16]. Phages have developed the high specificity recognition and binding to these bacterial surface receptors. The initial attachment of the phage to the bacterial cell depends on the binding affinity between phage surface proteins and bacterial receptors. Different phages can target specific bacterial species, strains, or even individual cells within a bacterial population due to their diverse levels of specificity.

Several Factors Influence the Specificity of Phage-host Interactions:

Receptor Diversity: Surface receptor presence or composition might differ between bacterial species and strains, which can alter phage susceptibility. This variety enables the selective targeting of particular bacterial populations and adds to the specificity of phage-host interactions.

Receptor-binding Proteins found in Phages: These proteins determine the specificity of phages for particular receptors. The structural flexibility of these proteins enables phages to adapt to various receptor variations found in a bacterial species or strain.

Host Range Determinants: Phages include host range determinants, which are genetic components that either allow or prevent them from infecting particular bacterial hosts. Genes that code for proteins that bind to receptors or other elements that are important in recognising and infecting hosts can be among these determinants.

Co-evolutionary Dynamics: Bacteria and phages have been involved in a co-evolutionary arms race that has resulted in the development of bacterial resistance mechanisms and a variety of phage methods to get past bacterial defences. The specificity and effectiveness of phage-host interactions are shaped by this continual co-evolution [15, 16, 17].

Mechanisms of Bacteriophage Mediated Bacterial Killing: Bacteriophages, or phages, employ various mechanisms to kill bacterial cells effectively. Understanding these mechanisms is crucial for developing and applying phage therapy as an antimicrobial strategy [18, 19].

Lytic Enzymes: Lytic enzymes, such endolysins and holins, are frequently produced by phages and are essential for the lysis of bacterial cells. The bacterial cell wall is the target of endolysins, which break down peptidoglycans and lead to cell lysis. Proteins called holins create openings in the bacterial cell membrane that let endolysins escape into the periplasm and facilitate cell lysis. The bacterial cell is quickly eliminated as a result of the combined action of endolysins and holins [20].

DNA/RNA Degradation: Nucleases produced by some phages can break down bacterial DNA or RNA. These nucleases have the ability to damage crucial genetic material, impeding critical cellular functions and ultimately leading to bacterial cell death.

Superinfection Exclusion: Phages have mechanisms to stop superinfection, which is when many phages infect a bacteria at once. Phage genes can encode superinfection exclusion mechanisms that prevent the replication or invasion of new phages into a bacterial cell that is already infected. This process makes sure that the phage infection cycle is successfully completed without the intervention of rival phages.

Programmed Cell Death: In some circumstances, phage infection can cause the processes that lead to programmed cell death in bacteria. Phage infection causes a phenomena known as phage-induced cell lysis or phage-induced cell death (PICD), which is the activation of certain bacterial cell death mechanisms. Phages' ability to kill more effectively can be increased by PICD, which can also lessen bacterial resistance to phage infection.

Immune System Evasion: To protect themselves from phage infection, bacteria have a variety of immune systems, including CRISPR-Cas and restriction-modification systems. In response, phages have developed methods to go around or overpower these immune systems, enabling effective bacterial invasion and eradication. Inhibiting the CRISPR-Cas system, for instance, certain phages express anti-CRISPR proteins, while others alter their DNA to resist restriction enzymes.

Formulation and Delivery of Phage Therapeutics: The creation and use of phage treatments are essential to the effectiveness and success of phage treatment. The creation of suitable formulations and delivery methods ensures phage stability, targeted dispersion, and optimal activity at the site of infection.

Formulation [21, 22]:

Buffer and pH Optimisation: When creating phage formulations, it's important to choose the right buffer systems and pH levels to preserve the stability and activity of the phage. The kind of phage, the storage environment, and compatibility with other formulation components all have a role in the buffer choice. (Payne *et al.,* 2000; Golshahi *et al.,* 2018)

Additives and Stabilisers: To improve phage stability and stop deterioration during storage and administration, certain additives and stabilisers can be added to phage formulations. These might be proteins (like bovine serum albumin), carbohydrates (like trehalose or sucrose), or cryoprotectants (like glycerol). (Payne *et al.,* 2000; Golshahi *et al.,* 2018)

Lyophilization (Freeze-drying): It is a popular technique for long-term phage preservation. Phage formulations are freeze-dried, removing water while maintaining the vitality and stability of the phages. Phage formulations that have been lyophilized can be reconstituted before use, giving them a longer shelf life and making shipping simpler. (Payne *et al.,* 2000; Golshahi *et al.,* 2018).

Delivery:

Topical Application: Phage formulations are directly applied to the site of infection during topical administration. This method is frequently applied to treat wound infections. To establish good contact with the infected region, phage solutions or gels can be administered using syringes, spray devices, or dressings. (Rhoads and others, 2009; Jault and others, 2019) [23, 24].

Aerosolization: The injection of phages to treat respiratory illnesses is made possible via aerosol delivery. To efficiently enter the lungs, phage particles are disseminated in a nebulizer or inhaler device. (Jault *et al.,* 2019; Golshahi *et al.,* 2018) [21, 24].

Oral Delivery: When phages are administered orally, they are administered as liquids, capsules, or tablets. This approach enables for simple and non-invasive delivery and is particularly helpful for gastrointestinal illnesses. (Jault *et al.,* 2019; Golshahi *et al.,* 2018).

Intravenous (IV) Delivery: Phage formulations are injected into the circulation during intravenous administration. Phages can penetrate systemic illnesses thanks to this delivery technique. The elimination of endotoxins, for example, necessitates careful consideration of phage stability and safety. (Jault *et al.,* 2019; Golshahi *et al.,* 2018).

Mix Approaches: To increase the efficacy of phage treatment, in some circumstances a mix of delivery techniques, such as topical application and systemic administration, may be used. (Jault *et al.,* 2019; Golshahi *et al.,* 2018) [21, 24].

Advantages of Topical Phage Application:

Targeted Antimicrobial Activity: The potential of topical phage treatment to deliver tailored antimicrobial action against particular bacterial infections is one of its main benefits. The treatment of wound infections can benefit from this focused approach in a number of ways.

Selective Bacterial Killing: Bacteriophages are very precise in their interactions with bacterial hosts, which results in selective bacterial killing. They are able to selectively infect and kill the intended bacterial pathogens while leaving helpful bacteria and host cells intact because they are able to recognise and connect to particular receptors on the surface of target bacteria. This selectivity minimises the potential of collateral harm to healthy tissues and lessens the disturbance of the natural microbiome. (2011) Abedon *et al*.; Jault *et al* [24, 26].

Combating Antibiotic Resistance: The control of wound infection is significantly hampered by the presence of antibiotic-resistant bacteria. Applying phage topically helps get over the restrictions put on by antibiotic resistance. Phages target bacterial surface receptors that are less prone to develop changes leading to resistance in order to infect and kill bacteria, even antibiotic-resistant strains. Because of this, phages provide a possible

substitute for conventional antibiotics in wound infections [27, 28].

Penetration of Biofilms: Biofilms, structured communities of bacteria encased in a protective matrix, contribute to the persistence and resistance of wound infections. Topical phages have demonstrated the ability to penetrate and disrupt biofilms, enabling better access to bacterial cells embedded within the biofilm structure. Phages can degrade the extracellular matrix and lyse bacterial cells within the biofilm, enhancing treatment efficacy and reducing infection recurrence [29].

Self-Replication at the Infection Site: Bacteriophages can replicate within bacterial cells, amplifying their population at the site of infection. As the bacterial population grows, the phage population increases accordingly, ensuring a continuous supply of phages to target and kill bacteria. This self-replication capacity contributes to the sustained antimicrobial effect of topical phage application and reduces the need for repeated or high-dose treatments. (Jault *et al.,* 2019; Abedon *et al.,* 2011) [26, 27].

Potential for Personalized Treatment: Topical phage therapy allows for a personalized approach to infection management. Phages can be isolated and selected based on the specific bacterial strains causing the wound infection. This tailored approach enables the customization of phage therapy to the individual patient, considering the unique bacterial pathogens in their wound [27, 28].

Broad Host Range and Specificity: Another significant advantage of topical phage application is the broad host range and specificity of bacteriophages. These features contribute to the effectiveness and versatility of phage therapy in treating wound infections [28, 30].

Wide Host Range: Bacteriophages may infect and destroy a variety of bacterial species due to their wide host range. Phages can target a variety of pathogens that could be present in wound infections thanks to their wide host range. Phages are useful in complicated wound infections involving polymicrobial communities because they may simultaneously target numerous bacterial strains or species, unlike antibiotics, which frequently have restricted action spectra.

High Specificity: Phages target bacteria at the strain level with great specificity while having a wide spectrum of potential hosts. Phages are able to selectively infect and kill particular strains or variations within a bacterial species by recognising particular surface receptors on the bacterial cell surface. This selectivity minimises the disruption of helpful microorganisms and lowers the chance of side effects that are not intended.

Co-evolutionary Adaptations: Over the course of millions of years, bacteria and bacteriophages have co-evolved, creating a dynamic arms race that has changed their interactions. While bacteria have evolved defences and resistance mechanisms against phages, phages have created unique techniques to circumvent them. The formation of highly precise phage-host interactions as a result of this continual co-evolution ensures effective bacterial killing while lowering the likelihood of resistance development.

Phage Isolation and Selection: A sizable pool of possible therapeutic candidates may be obtained by isolating phages from a variety of environmental sources, including soil, water, or sewage. Selection of phages with high specificity and effectiveness against target bacterial strains is made possible by the isolation and screening methods. Genetic Engineering and Modification: New genetic engineering techniques enable the modification of phages to improve their specificity and efficacy. Phages can be genetically modified to express extra antimicrobial components like lysins or enzymes that target certain bacterial pathogenicity factors. These changes can address particular difficulties related to wound infections and significantly improve the therapeutic potential of phages [31, 32, 33].

Biofilm Eradication Potential: Topical phage application offers a unique advantage in eradicating biofilms, complex bacterial communities encased in a protective matrix. Biofilms contribute to the persistence and resistance of wound infections. Here, we delve into how phages can effectively target and eradicate biofilms [29, 31].

Penetration of Biofilm Matrix: Biofilms exhibit increased antibiotic resistance and host immune responses due to their protective extracellular matrix.

However, phages have shown the ability to penetrate and degrade this matrix, allowing them to access and target bacterial cells within the biofilm. Phage-encoded enzymes, such as depolymerase or polysaccharide depolymerase, can degrade the matrix components, disrupting the biofilm structure and facilitating access to bacterial cells [29].

Specificity for Biofilm-Forming Bacteria: Phages can be selected or engineered to target biofilm-forming bacteria specifically. Biofilm-associated bacteria often exhibit unique surface features or molecular signals that can serve as targets for phage attachment and infection. By tailoring phages to recognize these specific characteristics, they can selectively target and kill biofilm-forming bacteria without affecting non-biofilm-forming counterparts.

Synergistic Action with Enzymes: Phages can work synergistically with phage-encoded or exogenous enzymes to enhance biofilm eradication. Enzymes such as dispersin, DNases, or proteases can be combined with phages to degrade the extracellular matrix, release bacterial cells, and increase phage access to the biofilm. This cooperative action enhances the effectiveness of phage therapy in biofilm environments [32, 33].

Quorum Sensing Disruption: Some phages can disrupt quorum sensing, a communication mechanism bacteria employ within biofilms to coordinate their behavior and enhance biofilm formation.

By interfering with quorum sensing signaling molecules, phages can disrupt biofilm stability and promote the dispersal of bacterial cells from the biofilm structure, making them susceptible to phage-mediated killing.

Continuous Phage Replication: Phages have the capacity for self-replication within bacterial cells. When phages infect biofilm-associated bacteria, they can replicate and release new phage particles, creating a continuous supply of phages to target and kill bacterial cells within the biofilm.

This ongoing replication process contributes to topical phage therapy's sustained antimicrobial effect and biofilm eradication potential [32, 33].

Natural and Self-Replicating Agents: Topical phage application offers the advantage of utilizing natural and self-replicating agents to treat wound infections. These characteristics provide several benefits regarding phage therapy's safety, efficacy, and sustainability [28, 30, 34].

Naturally Occurring Agents: Bacteriophages are viruses that infect and eradicate bacteria. They are prevalent in many settings, including soil, water, and the microbiota of humans. Over millions of years, phages and bacteria co-evolved, creating a natural predator-prey relationship. They are appealing as therapeutic agents because of their natural origin and affinity for bacteria, which can provide focused antibacterial action without adversely harming host cells or the good microbiota.

Self-Replication: Bacteriophages possess a special capacity for self-replication inside bacterial cells. A phage multiplies its population at the site of infection by using the bacterial machinery to create multiple offspring phages once it has infected a target bacterium. This self-replication property makes that there is always a supply of phages available to hunt for and eradicate bacterial infections, increasing the efficiency and longevity of phage treatment.

Preventing the Development of Resistance: Bacterial resistance to phages can exist, although it typically develops more gradually than resistance to antibiotics. Phages are able to quickly develop and adapt to changes in the surface receptors of target bacteria, retaining their efficacy. Additionally, by continuously creating phages within bacterial cells, which counteracts bacterial resistance mechanisms, more resistant bacterial strains are likely to be successfully infected and killed.

Self-Dosing Capability: Phages' capacity for self-replication also serves as a self-dosing mechanism. To ensure that the phages can keep up with bacterial growth, the phage population grows proportionately as the bacterial population does. This self-dosing property can lead to persistent antimicrobial effectiveness without frequent administration or large phage dosages, helping to

maintain the balance between phage and bacterial populations.

Lower Environmental Impact: When compared to traditional antimicrobial medicines, phage treatment may have a lower environmental impact. Phages are biodegradable, naturally occurring organisms that don't last long in the environment. Their focused and self-restraining behaviour reduces the possibility of ecological disturbance or the emergence of broad resistance.

Potential Synergy with Conventional Therapies: Topical phage administration may work in conjunction with traditional treatments like antibiotics and wound care techniques. This combination can improve the overall efficacy of care and offer further advantages for treating wound infections [28].

By focusing on germs that are resistant to common antibiotics, phage treatment can combat antibiotic resistance. Antibiotic-resistant bacteria have been eliminated when phages and antibiotics are used together. By dissolving biofilms, raising bacterial sensitivity, and offering a different method of bacterial death, phages can increase the efficacy of antibiotics. This broadens the spectrum of effective treatment choices [29]. Antimicrobial substances, especially antibiotics, are notoriously resistant to biofilms. By focusing on and eliminating bacteria embedded in biofilms, topical phage treatment can enhance standard therapy. Phages can enter the biofilm matrix, cause structural disruption, and liberate bacterial cells, making biofilm-associated bacteria more susceptible to antibiotics and other antimicrobial treatments [35]. Phages and antibiotics can work together to lessen the need for high dosages or lengthy antibiotic regimens. Lower antibiotic concentrations particularly may be adequate to produce the required therapeutic effect by employing phages to target and destroy bacteria. This decrease in antibiotic use can lessen the emergence of antibiotic resistance and lessen its negative consequences. There have been claims that phage treatment helps wounds heal faster. Phages have the ability to speed up healing, induce tissue regeneration, and alter the host immunological response. When phages are present at the infection site, bacterial pathogens can be more easily removed, lowering inflammation and fostering an environment that is conducive to wound healing [35, 36].

Challenges and Limitations:

Phage Resistance Development: Phage resistance development is a significant challenge associated with phage therapy. Bacteria can evolve mechanisms to evade phage predation, leading to reduced effectiveness of phage treatment. Understanding the factors contributing to phage resistance is crucial for successfully implementing phage therapy [37-42].

Bacterial Surface Receptor Mutations: Phages typically recognize and bind to specific surface receptors on bacterial cells for infection. Bacteria can acquire mutations in these receptors, altering their structure and preventing phage attachment. These mutations can lead to phage resistance by reducing or abolishing phage binding, thereby hindering the initial step of phage infection. (Labrie *et al.,* 2010; Alemayehu *et al.,* 2012)

Restriction-Modification Systems: Bacteria possess restriction-modification systems as a defense against foreign DNA, including phages. These systems recognize and degrade phage DNA, limiting phage replication. Bacterial strains with active restriction-modification systems can resist phage infection, as the phage DNA is degraded before initiating the infection cycle. (Labrie *et al.,* 2010; Alemayehu *et al.,* 2012).

CRISPR-Cas Systems: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems provide adaptive immunity against phages in bacteria. Bacteria incorporate short fragments of phage DNA into their CRISPR arrays, allowing them to recognize and destroy phage DNA upon subsequent encounters. This acquired immunity mechanism can confer resistance to specific phages and limit their effectiveness in phage therapy. (Barrangou *et al.,* 2007; Dedrick *et al.,* 2019).

Superinfection Exclusion: Some bacteria can resist phage infection by activating super infection exclusion mechanisms. These mechanisms prevent subsequent phage infections by interfering with phage replication or blocking phage DNA injection into the cell.

Super infection exclusion can limit the efficacy of phage therapy, as it restricts the ability of phages to infect and kill bacteria within a population. (Seed *et al.,* 2013; Dedrick *et al.,* 2019).

Phage-Polysaccharide Interactions: Bacterial polysaccharide capsules can serve as barriers against phage infection. Capsules can hinder phage access to bacterial surface receptors, preventing successful attachment and subsequent infection. Bacteria can modify their capsule structure or produce excess material, reducing phage susceptibility and promoting resistance development. (Forti *et al.,* 2018; Dedrick *et al.,* 2019). To mitigate phage resistance development, several strategies can be employed. These include using phage cocktails that target multiple receptors, engineering phages to broaden their host range, and developing combinatorial treatments involving phages and other antimicrobial agents. These approaches aim to minimize the emergence of resistance by targeting multiple bacterial vulnerabilities. (Lu *et al.,* 2009; Dedrick *et al.,* 2019).

Regulatory Considerations: Phage therapy for the treatment of wound infections faces several regulatory challenges and considerations that need to be addressed for its successful implementation. These considerations include safety, efficacy, quality control, and regulatory approval processes.

Addressing regulatory considerations is vital to facilitate the translation of phage therapy from the research phase to clinical practice. Collaboration between researchers, regulators, and industry stakeholders is crucial to establish appropriate regulatory frameworks that ensure patient safety, product quality, and efficacy while fostering innovation and access to phage-based therapies [43, 45].

Phage Production and Formulation Issues: Phage production and formulation present challenges and limitations that must be addressed to develop and apply phage therapy for wound infections successfully. These issues encompass phage isolation, purification, formulation stability, and scalability [44-46].

Phage Isolation and Characterization: It might be difficult to isolate and characterise phages with the appropriate characteristics. Phages must be isolated using certain bacterial hosts from relevant environmental samples, such as water or sewage. Finding the phage's host range, effectiveness, and safety characteristics is known as characterization. To find the best phages for therapeutic uses, a rigorous screening and selection procedure is needed.

Purification and Concentration: Phage preparations must be purified and concentrated in order to eliminate undesirable components and raise phage concentration. Filtration, centrifugation, and chromatography are purification methods used to remove detritus, host DNA, and bacterial contamination.

To generate high phage titers, concentration techniques like ultrafiltration or ultracentrifugation might be used. These procedures guarantee the efficacy, reliability, and safety of phage preparations.

Formulation Stability: Phage medicines for topical treatment require a stable formulation, which might be difficult. Phages are susceptible to environmental conditions that might impact their viability and activity, such as temperature, pH, and UV light.

To safeguard phages during storage and preserve their stability, formulations must be optimised. The use of protective compounds, such as stabilisers or cryoprotectants, and the creation of suitable storage conditions are some possible strategies.

Scalability and Manufacturing: It might be challenging to scale up phage manufacturing to suit clinical demands. It takes the right host bacteria, growth medium, and bioreactor systems to develop enormous numbers of phages. To guarantee dependable phage production and quality, the procedure needs to be properly managed.

Batch-to-Batch Variability: Due to the biological nature of phages and the manufacturing process, phage preparations might display batch-to-batch variability.

Scaling up also entails resolving issues with cost-effectiveness, repeatability, and regulatory compliance. Phage content, purity, and activity

fluctuation may affect the effectiveness of treatments and adherence to regulations. Phage treatment may be made more consistent and batch-to-batch variability can be reduced by putting into place strong quality control procedures.

Lack of Standardized Protocols and Guidelines: The need for standardized protocols and guidelines challenges the implementation and widespread adoption of phage therapy for wound infections. Standardization ensures consistent and reproducible practices across different research groups and clinical settings. Here are some specific details regarding this challenge [46-47].

Variability in Phage Isolation and Characterization: The isolation and characterization of phages can vary among research groups, leading to inconsistencies in phage therapy protocols. Differences in isolation methods, host bacteria selection, and characterization criteria can affect phage preparations' specificity, efficacy, and safety. Standardized protocols for phage isolation and characterization facilitate comparability and reproducibility of results.

Diverse Treatment Approaches: There needs to be more consensus on the optimal treatment approaches for phage therapy. Factors such as phage dosage, administration routes, treatment duration, and combination therapies with other antimicrobials vary among studies. The absence of standardized guidelines hinders the ability to compare results and determine best practices for phage therapy in wound infections.

Quality Control and Standardization of Phage Preparations: Quality control measures and standardization of phage preparations are essential to ensure safety and efficacy. However, there must be standardized guidelines for quality control testing, including potency assays, purity assessments, and stability evaluations. Harmonized protocols and reference materials would facilitate consistent evaluation and comparison of phage preparations.

Clinical Trial Design and Reporting: Clinical trials evaluating phage therapy often need more standardized study designs and reporting guidelines. Variability in trial designs, endpoints, patient populations, and outcome measures makes comparing and pooling data across different studies challenging. Establishing standardized guidelines for conducting and reporting phage therapy clinical trials would enhance the reliability and generalizability of findings.

Regulatory Harmonization: Regulatory approval processes for phage therapy can differ among countries and regions, leading to inconsistencies in requirements and timelines. Harmonizing regulatory guidelines and approval pathways would facilitate the development and commercialization of phage therapies, ensuring consistent safety, efficacy, and quality standards across different jurisdictions.

Preclinical and Clinical Evidence:

***In-vitro* and Animal Model Studies:** Preclinical studies using *in-vitro* experiments and animal models have provided substantial evidence supporting the efficacy of topical phage application for the treatment of wound infections. These studies have demonstrated the ability of phages to specifically target and eliminate bacterial pathogens, disrupt biofilms, and accelerate wound healing. Vitro studies have consistently shown that bacteriophages possess potent bactericidal activity against many bacterial pathogens commonly implicated in wound infections, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Phages can recognize specific surface receptors on bacteria, leading to their attachment and subsequent lysis of bacterial cells. This specificity enables phages to selectively target and kill the infecting bacteria while leaving beneficial bacteria unharmed [48, 49, 50].

Bacterial biofilms, complex communities of bacteria encased in a self-produced extracellular matrix, pose a significant challenge in wound infections due to their increased resistance to conventional antibiotics. Animal model studies have demonstrated the effectiveness of phage therapy in disrupting biofilms and eradicating biofilm-associated bacteria. Phages can penetrate the biofilm matrix, infect the bacterial cells within, and effectively lyse them, thereby reducing the bacterial load and restoring susceptibility to antimicrobial agents. Phage treatment has been demonstrated to accelerate wound healing in animal models of wound infections. Phages can

speed up the removal of bacterial infections from wounds, lower inflammation, and modify the immunological response. Phages facilitate wound healing processes such granulation tissue development, re-epithelialization, and angiogenesis by successfully eradicating the infecting bacteria. Better wound closure and overall wound healing results follow from this [49, 50].

Studies using animal models have repeatedly shown that phage treatment is safe and tolerable. Since they only target bacteria and do not infect or harm mammalian cells, pages are typically regarded as harmless. According to studies, topical treatment of phages does not significantly worsen wound healing or have other negative consequences. Following therapy, phages are quickly eliminated from the body, reducing the possibility of systemic harm [48-50].

Clinical Trials and Case Reports: The clinical effectiveness and safety of topical phage treatments for the treatment of wound infections have been extensively studied in clinical studies and case reports. These research have shown that phage treatment has the ability to successfully manage infections, expedite wound healing, and enhance patient outcomes. The specifics of clinical studies and case reports are as follows [51, 52].

Phage treatment for wound infections has shown good outcomes in clinical studies. As an illustration, the effectiveness and acceptability of a combination of bacteriophages in treating burn wounds infected by Pseudomonas aeruginosa were examined in a randomised, controlled, double-blind phase 1/2 experiment (PhagoBurn). In comparison to the control group, the phage-treated group demonstrated a statistically significant decrease in bacterial load, better wound healing, and a decreased incidence of adverse effects [53].

Numerous case studies have demonstrated the effectiveness of phage treatment in treating wound infections. In these papers, specific patient instances are described in which topical phage administration resulted in full clearance of the infection and wound healing. For instance, a case report described how modified bacteriophages were used to successfully treat a patient with a disseminated, drug-resistant Mycobacterium abscess us infection [54]. After receiving phage treatment, the patient showed quick clinical improvement and full recovery from the illness.

Future Directions and Considerations:

Phage Cocktail Formulations: The creation of phage cocktail formulations will receive a lot of attention in upcoming studies and clinical trials of phage treatment for wound infections. In comparison to single-phage preparations, phage cocktails which include many phages that target several bacterial strains or species offer a number of benefits. Here are some things to think about and where phage cocktail compositions are going in the future.

In comparison to single phage preparations, phage cocktails can target a wider variety of bacterial infections. Cocktails can successfully treat polymicrobial illnesses and cover a larger spectrum of bacterial strains by including numerous phages with various specialties. This is especially important for wound infections since they frequently involve diverse bacterial communities [55].

A mixture of phages can assist prevent bacteria from developing phage resistance. A single phage may cause bacteria to become resistant to it. However, the possibility of successfully locating and destroying the infecting bacteria can be increased by the presence of additional phages with various modes of operation. Phage cocktail therapies that use properly chosen phages can delay the development of resistance and increase the effectiveness of phage treatment.

Phage cocktails have the ability to increase bacterial death through synergistic effects between the phages. Some phages may have many modes of operation or focus on various phases of the bacterial life cycle. Cocktails of phages can boost overall effectiveness and bactericidal activity by mixing phages with complimentary features, leading to better treatment outcomes. Stability and shelf life factors must be taken into account while creating phage mixtures. The stability of the cocktail depends on choosing phages with comparable properties, such as similar environmental needs and compatible growth kinetics. The shelf life of the cocktail can be increased by using the right formulation

procedures, such as lyophilization or encapsulation, to retain phage viability [55-57]. The unique bacterial pathogens involved in wound infections must be carefully taken into account for the rational design and optimisation of phage cocktails. It's critical to comprehend the diversity of bacteria and patterns of antibiotic resistance in clinical settings in order to choose phages with the right host range and specificity. Additionally, optimising the cocktail's phage concentrations and ratios can boost effectiveness and reduce the risk of phage interference.

In order to assess the effectiveness, safety, and applicability of phage cocktails for wound infections, more clinical trials and comparative research are required. These research ought to evaluate how phage cocktail compositions affect therapeutic outcomes including wound healing, infection control, and patient satisfaction. The benefits and possible drawbacks of phage mixtures will be clarified by comparative studies comparing them to single phage preparations and conventional therapies.

Combination Therapies and Bioengineered Phages: The use of bioengineered phages and the investigation of combination treatments are potential future paths in phage therapy for wound infections. These methods improve the efficacy, adaptability, and clinical usefulness of phage treatment.

Phage therapy may have synergistic benefits and better therapeutic results when combined with other treatment techniques, such as antibiotics, antimicrobial peptides, or immune-modulating drugs. The use of phages in conjunction with traditional treatments can improve bacterial clearance, reduce bacterial resistance, and accelerate wound healing. The best combinations, dosage plans, and mechanisms of action for synergistic effects should be investigated in future research [58]. It is possible to increase the therapeutic potential of phages by using bioengineering methods. To increase their bactericidal effectiveness and target certain pathogenic mechanisms of bacteria, phages can be genetically modified to express extra antimicrobial proteins, enzymes, or virulence factors. It is also possible to create bioengineered phages that target resistant bacterial strains by altering the proteins that bind to the phage receptors [59]. Phages can be delivered to the wound site in a controlled and sustained manner by being included into biomaterials or wound dressings. By enhancing phage stability, bioavailability, and localised distribution, this strategy can maximise their therapeutic effectiveness. The development of phage-incorporated materials that retain phage viability and guarantee regulated release throughout time should be the major goal of future research [59, 60].

To create the best possible treatment plans and dosage schedules, it is essential to comprehend the pharmacokinetics and pharmacodynamics of phages *in-vivo*. Future research should look at the distribution, clearance, and durability of topically administered phages in wounds, as well as their pharmacokinetic characteristics. Achieving the best therapeutic results also depends on selecting the right phage dose, frequency of administration, and length of therapy [61].

CONCLUSION:

Recap of Key Findings: We have examined the topical use of bacteriophages to treat wound infections in this review study. We started out by talking about how common wound infections are and how urgently we need alternate treatments owing to antibiotic resistance. The fundamentals of phage treatment, such as bacteriophage biology, phage-host interactions, and the processes of bacteriophage-mediated bacterial death, were then covered in depth. We also spoke about how phage treatments are created and administered.

The benefits of topical phage application were then highlighted, including their inherent capacity for self-replication, broad host range, broad host specificity, and focused antimicrobial efficacy. We also spoke about how phage therapy and conventional therapies may work together.

We also recognised the difficulties and restrictions associated with phage treatment, such as the emergence of phage resistance, regulatory hurdles, and problems with phage manufacturing and formulation. One issue that needs focus is the absence of standardised processes and guidelines. We next looked at in vitro and animal model studies that proved phage therapy's effectiveness,

safety and advantages for wound-healing before moving on to the preclinical and clinical data. Evidence of good results, enhanced wound healing, and patient satisfaction was shown in clinical studies and case reports.

Potential of Bacteriophage-based Therapies: Bacteriophage-based therapeutics have great promise for the treatment of wound infections. Phage-mediated targeted antimicrobial activity enables precise control of bacterial infections and lowers the danger of the emergence of antibiotic resistance. Phages offer a comprehensive approach to wound treatment by disrupting biofilms and accelerating wound healing. Phage therapy has also demonstrated promise when used in conjunction with conventional therapies, delivering synergistic benefits and the potential to lower antibiotic consumption.

Recommendations for Future Research and Implementation: To further advance the field of topical phage therapy for wound infections, several areas of research and implementation should be prioritized:

Standardised Protocols: By establishing standardised procedures and rules for manufacturing, inspecting, and dispensing phage therapies, consistency and repeatability will be guaranteed in a variety of contexts.

Clinical Studies: To assess the safety, effectiveness, and cost-effectiveness of phage treatment, extensive clinical studies with rigorous research designs are required. Comparative research can assist identify patient subgroups that would benefit most and the best treatment methods.

Phage Cocktail Formulations: To increase their efficacy, widen their range of activity, and combat bacterial resistance, further research should concentrate on creating and optimising phage cocktail formulations.

Bioengineered Phages: By investigating bioengineering methods to alter phages and improve their therapeutic qualities, more effective and specialised phage medicines may be created.

Combination Therapies: Phage therapy can be used in conjunction with other therapeutic modalities, such as antibiotics or immune-modulating drugs, to address complicated wound infections. Researching these interactions can help to enhance treatment outcomes.

Regulatory Considerations: Establishing regulatory frameworks that guarantee the safe and effective application of phage treatment requires cooperation between regulatory authorities, researchers, and pharmaceutical corporations.

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