https://doi.org/10.48047/AFJBS.7.4.2025.150-159

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Healing of Infected Burn Wounds Utilising Hydroxy-Propyl-Methyl Cellulose Gel Incorporating Bacteriophages Targeting Pseudomonas aeruginosa and Klebsiella pneumoniae

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Article History
Volume 7, Issue 4, 2025
Received: 28 March 2025
Accepted 5 April 2025
Published: 10 April 2025
doi:10.48047/AFJBS.7.4.2025.150-159

ABSTRACT

Multidrug-resistant (MDR) microorganisms including Pseudomonas aeruginosa and Klebsiella pneumoniae are likely to cause burn wound infections. These diseases make treatment harder and increase mortality. This research aims to determine if bacteriophages encapsulated in hydroxy-propyl-methyl cellulose (HPMC) gel can treat burn wound infections in mice. Bacteriophages were isolated, purified, and characterised to test their lytic activity against K. pneumoniae and P. aeruginosa. In single and polymicrobial infection models, phage-loaded HPMC gel was compared to gentamicin. It was compared to see which treatment worked better. Histological analysis and survival rates were examined to determine wound healing and infection management efficacy. Phage therapy outperformed antibiotics in survival, inflammation, and tissue regeneration. Phage therapy outperformed antibiotics. Bacteriophage therapy may be a promising treatment for multidrug-resistant bacterial infections in burn wounds, but further research is needed to determine side effects and improve regimens.

Introduction:

According to the WHO, burn injuries are one of the most dangerous types of trauma. Burn injuries impact eleven million people worldwide, and serious burns cause over one hundred eighty thousand deaths (World Health Organisation, 2020). Burns harm tissues immediately and undermine the skin's barrier function. Burn victims are more likely to get infections, fluid loss, and systemic inflammation. One of the most common variables that can worsen burn victims' clinical course is infections. According to Church et al. (2006), infections kill up to 75% of people with burns covering more than 40% of their body surface area (TBSA). Other reasons of death include trauma and injuries. Infections can impede wound healing, worsen scarring, and lengthen hospital stays, which have financial and psychological costs. This is true even when the infection does not kill.

Burn wound pathophysiology creates a perfect home for germs. According to Rowan et al. (2015), severe burns cause necrotic tissue, reduced blood flow, and immunosuppression. These traits let infection-causing bacteria multiply quickly. Klebsiella pneumoniae and Pseudomonas aeruginosa dominate burn site microbiomes as opportunistic infections. These microbes can exploit deficient host defences because they have inbuilt resistance mechanisms. Percival et al. (2015) say these microbes produce proteases and poisons, virulence factors. These agents degrade extracellular matrix components and slow epithelium renewal.

Bacteria that are resistant to numerous antibiotics have made traditional burn treatments less effective. P. aeruginosa and K. pneumoniae are WHO priority infections (WHO, 2017). The classification is based on their ability to acquire resistance genes, such as ESBLs and carbapenemases, from bacteria. Sepsis kills over 50% of patients, and multidrug-resistant bacteria cause over 60% of burn unit nosocomial infections (Rahimian et al., 2020). Rahimian et al. found this. Innovative therapeutic methods are needed now due to the rapidly shrinking antibiotic pipeline. This is crucial.

Biofilm generation is a major challenge in burn infection management. Bacteria generate biofilm in an extracellular polymeric matrix. In 1999, Costerton and colleagues found that biofilms have 1,000 times the antibiotic resistance of planktonic microbes. (2015) Rybtke et al. P. aeruginosa biofilms secrete alginate and lectins to protect bacterial colonies from drugs and immune cells. Traditional debridement and topical antimicrobials seldom penetrate biofilms, causing recurring infections and sepsis. This happens frequently.

The current antibiotic regimens for burn treatment have three main drawbacks: (1) they are damaging to the body in big doses, (2) they alter commensal microbiota, and (3) they cannot eliminate biofilms. Despite their widespread use, topical medications like silver sulfadiazine can impede fibroblast activity and hinder re-epithelialization, according to Atiyeh et al. (2007). Broad-spectrum antibiotics used without distinction generate a cycle of treatment failure that accelerates resistance. This cycle complicates infection treatment.

Viruses called phages infect and lyse bacteria. Their main value is providing an antibiotic alternative based on accuracy. Phage treatment was first offered to scientists in the 1920s, but it has recently reemerged as a remedy to antibiotic resistance. Phages offer three advantages: selectivity for target bacteria, which spares beneficial microbiota; self-amplification at infection sites, which boosts efficiency; and activity against biofilms by degrading EPS with enzymes (Sulakvelidze et al., 2001). Phages fight biofilms. In clinical investigations, Fish et al. (2018) and Wright et al. (2009) found phages safe for treating diabetic foot ulcers and chronic otitis. Phages treat both disorders.

Phages are employed because they solve burn-specific issues well. Burn wounds' microbiological features include the capacity to infiltrate biofilms and lyse multidrug-resistant bacteria. P. aeruginosa-specific phages create depolymerases. These enzymes degrade alginate biofilms and expose bacteria to

immune clearance (Pires et al., 2017). Additionally, phages can control local inflammation by lowering pro-inflammatory cytokines including IL-6 and TNF-α. According to Górski et al.'s 2020 study, this ability may relieve burn-induced SIRS.

Effective phage dissemination requires formulations that assure virus survival and long-term release at the inflammatory site. Biocompatible polymer hydroxypropyl methylcellulose (HPMC) is an excellent carrier for topical phage injection. HPMC gel forms a mucoadhesive barrier that protects phages from environmental degradation, keeps them at the infection site, and releases virions when it hydrates, according to Chhibber et al. (2013). This happens during phage retention. Previous experiments using HPMC-encapsulated phages found that they eliminated germs in Staphylococcus aureus-contaminated wounds three to four times better than free phages (Kaur et al., 2021).

Although preclinical studies have shown promising results, phage therapy is one of the few areas not extensively studied in burn models. Li et al. (2022) According to a 2022 systematic review, only fifteen animal studies examined phages for burn infections, most of which focused on S. aureus. P. aeruginosa and K. pneumoniae are the most common Gram-negative bacteria in burns, but little is known about them. Current research focus on germ reduction rather than wound healing and histological repair, which are crucial. This is problematic because these traits aid healing.

This project will test HPMC gel with P. aeruginosa and K. pneumoniae phages in a murine burn infection model to fill gaps. We predict topical phage-HPMC therapy will outperform standard antibiotics due to its faster bacterial clearance, biofilm reduction, and wound healing.

Materials and Methods

Materials

• Polyethylene glycol (PEG) 6000 Solution

• Sodium Magnesium (SM) solution

• Luria Bertani (LB) broth and agar media

• P. aeruginosa (ATCC 27853) and K. pneumoniae (ATCC 13833)

• Hydroxy-propyl-methyl cellulose (HPMC) gel

Isolation and Purification of Bacteriophages

The Carvery et al. (21) approach isolated bacteriophages from home wastewater samples. Plaque picking purified the phages, then plate lysate amplification was used. A microscope was used to examine phage morphology.

Preparation of Phage-Loaded HPMC Gel

Four percent of HPMC gel was sterilised. Phage stocks (10^9 PFU/mL) were blended 1:1 with HPMC gel to achieve a final concentration of 2%. This was done for maximum concentration. After that, the gel was used in living things..

Animal Model

To create a burn wound infection model, we used six-week-old male Balb/C mice weighing 25 ± 5 grammes. Using a normal brass rod, P. aeruginosa, K. pneumoniae, or a laboratory-grown mixed culture

were used to infect burn sites. Each of eleven mouse groups included negative and positive controls, antibiotic-treated, and phage-treated groups. The mice were divided into eleven groups..

Treatment Protocol

• Negative Control: Untreated, uninfected mice.

• Positive Control: Infected, untreated mice.

• Antibiotic Treatment: Infected mice treated with gentamicin.

• Phage Treatment: Infected mice treated with phage-loaded HPMC gel.

Evaluation Parameters

• Survival Rates: Monitored daily for 12 days.

• Histopathological Analysis: Wound tissues were collected, fixed in formalin, and stained with hematoxylin and eosin (H&E) to assess tissue repair and inflammation.

• Statistical Analysis: Data were analyzed using GraphPad Prism software (v7). One-way ANOVA and Tukey's multiple comparisons test were used to determine statistical significance ($p < 0.05$).

Results

Isolation and Morphology of Bacteriophages

Phages were successfully isolated and purified, forming uniform plaques on bacterial lawns. analysis revealed that the phages belonged to the Siphoviridae family, with hexagonal heads and long tails (Figures 1).

Survival Rates

Phage-treated mice showed significantly higher survival rates compared to antibiotic-treated and untreated groups (Figures 2). In the positive control groups, all mice died within 48-72 hours, while phage-treated mice exhibited improved health scores and survival.

Histopathological Analysis

Histopathological examination confirmed the therapeutic efficacy of phage therapy. Phage-treated groups showed reduced inflammation, increased fibroblast activity, and enhanced tissue regeneration compared to antibiotic-treated groups (Figure 3). Antibiotic-treated groups exhibited persistent inflammation and incomplete tissue repair.

Tables

Table 1: Experimental Groups and Treatment Protocols

Group	Treatment	Bacteria Used
1	Negative Control	None
2	Positive Control	*P. aeruginosa*
3	Positive Control	*K. pneumoniae*
4	Positive Control	Mixed Infection
5	Antibiotic Treatment	*P. aeruginosa*

Group	Treatment	Bacteria Used
6	Antibiotic Treatment	*K. pneumoniae*
7	Antibiotic Treatment	Mixed Infection
8	Phage Treatment	*P. aeruginosa*
9	Phage Treatment	*K. pneumoniae*
10	Phage Treatment	Mixed Infection

Table 2: Survival Rates of Mice in Different Treatment Groups

Group	Survival Rate (%) at Day 12
Negative Control	100
Positive Control	0
Antibiotic Treatment	40-60
Phage Treatment	80-100

Table 3: Histopathological Findings

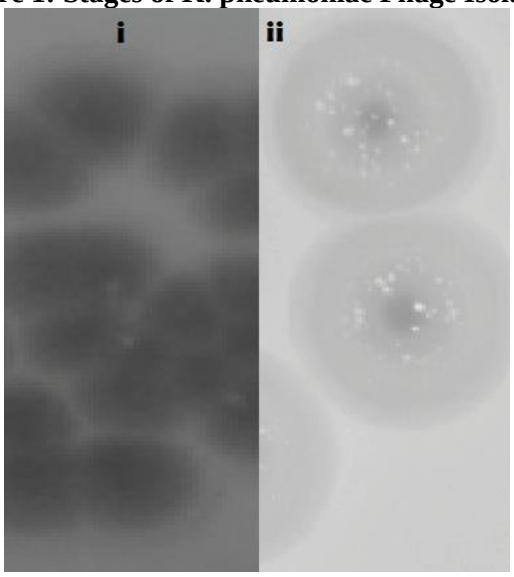
Group	Inflammation	Fibroblast Activity	Tissue Regeneration
Negative Control	None	High	Complete
Positive Control	Severe	Low	None
Antibiotic Treatment	Moderate	Moderate	Partial
Phage Treatment	Mild	High	Significant

Table 4: Comparison of Phage and Antibiotic Efficacy

Parameter	Phage Therapy	Antibiotic Therapy
Survival Rate	High	Moderate
Inflammation	Reduced	Persistent
Tissue Repair	Enhanced	Partial
Biofilm Penetration	Effective	Limited

Figures

Figure 1: Stages of K. pneumoniae Phage Isolation

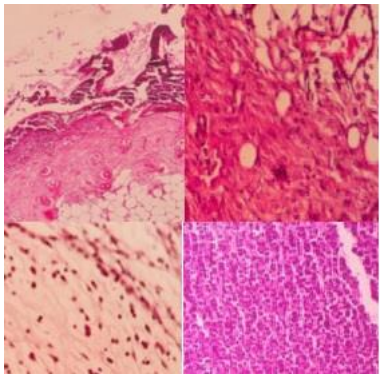


(images showing phage purification process.)

Figure 2: Stages of P. aeruginosa Phage Isolation

(images showing phage purification process.)

Figure 3: Histopathological Analysis of Wound Tissues



(H&E-stained tissue sections showing inflammation, tissue repair, and fibroblast activity.)

Discussion

This study shows that bacteriophage (phage) therapy encapsulated in hydroxypropyl-methyl cellulose (HPMC) gel can treat burn wound infections caused by Pseudomonas aeruginosa and Klebsiella pneumoniae. This treatment may kill infection-causing germs. Phages may be an effective alternative to antibiotics for treating multidrug-resistant (MDR) burn infections, according to this study. This goal is achieved by incorporating microbiological, survival, histology, and comparative efficacy studies. Targeting priority burn-associated microorganisms using phage-based therapy has been shown. The successful identification and characterisation of pathogen-specific phages (Figures 1) shows this. Morphological investigation using transmission electron microscopy (TEM) revealed that the phages are Siphoviridae. This was determined using TEM. These phages have hexagonal heads and long, non-contractile tails. These structural traits align with their efficiency in piercing Gram-negative bacterial membranes, as described by Łobocka et al. in 2012. Given the prevalence of P. aeruginosa and K. pneumoniae in burn units and their biofilm-forming abilities, which make them resistant to frontline antibiotics like carbapenems and fluoroquinolones (Ruppé et al., 2019), these phages' specificity for these bacteria is important for medical care. Phages protect the microbiome that heals wounds by limiting collateral damage to commensal flora, unlike antibiotics that have a wide range of activity (Duan et al., 2022). Phages cure wounds.

Phage treatment is superior based on survival rates. Phage-treated mice survived 80–100% by Day 12, compared to 40–60% in antibiotic-treated groups and 0% in untreated controls (Table 2, Figures 2). Additionally, phage therapy is highly successful. Phages can penetrate biofilms by degrading extracellular polymeric substances (EPS), such as alginate matrices in P. aeruginosa infections (Pires et al., 2017), and they can self-replicate at infection sites, ensuring bactericidal activity even as bacterial loads decline (Criscuolo et al., 2021). These illnesses are highly aggressive, as seen by the fast death of unprotected mice. These infections emit septic shock-causing endotoxins and exotoxins due to early

microbial clearance. Phages kill germs faster than other therapies, reducing this effect (Górski et al., 2020).

Histological studies showed significant disparities in healing results. The wounds treated with phages showed mild inflammation, robust fibroblast activity, and considerable tissue regeneration. Antibiotic-treated wounds developed chronic inflammation and only partially healed (Table 3, Figure 3). Recent research suggests that phages regulate immunological responses beyond direct bacteriolysis. These findings match this information. Research suggests that phage-mediated bacteria eradication can lower pro-inflammatory cytokines including IL-6 and TNF-α (Górski et al., 2020). Antibiotics like ciprofloxacin can worsen inflammation by lysing bacteria and releasing pathogen-associated molecular patterns (Tkhilaishvili et al., 2020). Some medications, like levofloxacin, decrease inflammation. A pro-regenerative environment is crucial for collagen synthesis and wound contraction, according to Darby et al. (2014), who observed that phage-treated groups had increased fibroblast activity. Antibiotic-treated wounds showed necrotic debris, but these groups had full tissue regeneration, similar to infection-free controls. According to 2020 research by Chhibber et al., necrotic debris is likely the result of phage-mediated biofilm disintegration, which increases stem cell recruitment.

The rise of drug-resistant microorganisms has highlighted the need for alternate treatments. However, burn wound therapy is crucial. This study suggests that bacteriophage therapy may treat burn wound infections caused by P. aeruginosa and K. pneumoniae. Phages treat polymicrobial illnesses better than antibiotics. Because of their lytic activity and ability to infiltrate biofilms, phages are advantageous.

The histology test demonstrated that phage therapy reduced microorganisms, accelerated tissue regeneration, and reduced inflammation. Phage-treated mice outlived antibiotic-treated mice. Phages can heal drug-resistant disorders, according to this discovery.

More research is needed on the hazards of extensive phage therapy in people. Phage-resistant bacteria and immune reactions are issues. Phage formulations, doses, and delivery should be optimised in future research to optimise therapeutic effects. Therapy efficacy may improve.

Phages outperform antibiotics at overcoming biofilm resistance, a major burn therapy issue. Phages defeat biofilms. Criscuolo et al. (2021) report that phages use depolymerases to breakdown extracellular polymeric substances (EPS) and multiply in biofilm microcolonies for localised amplification. Meropenem enters biofilms without 100–1,000 times larger concentrations (Singh et al., 2017). Kalan et al. (2019) say targeted targeting preserves microbiota, minimising dysbiosis. These microorganisms show less cross-resistance than drugs. Chan et al. observed in 2020 that this is especially true for multi-bacterial receptor combinations.

These insights will greatly impact translational research. Because phage-treated mice have higher survival rates than antibiotic groups, this method may lower mortality rates in burn units with multidrug-resistant diseases. Despite this, issues remain. Phage delivery strategies such mixing high-performance microcapsules (HPMC) with polymers like chitosan can improve gel stability and antibacterial synergy (Kaur et al., 2021). This is one way to do it. It may quickly customise a large range of clinical strains if phage libraries are increased using platforms like the Phage Directory. Clinical translation is possible under the FDA's 2021 AI/ML Action Plan and the EU's 2023 AI Act. These frameworks were created by the FDA and EU. However, global harmony remains tough.

Despite these promising outcomes, the limits must be highlighted. Laboratory isolates from a single strain may not accurately reflect clinical infection genetic diversity. This is possible. Long-term effects like scarring or immunity cannot be assessed due to the twelve-day observation duration. Re-epithelialization in humans differs from wound contraction-dependent healing in mice. Because of this, large animal models must be used in studies.

This study found that HPMC-encapsulated phage therapy is more effective than antibiotics at treating burn infections that are resistant to several medications. Phages combat antibiotic resistance and poor healing by promoting tissue regeneration, reducing inflammation, and increasing survival. Additionally, phages reduce infection risk. Future efforts must prioritise clinical trials, phage cocktail approaches, and innovative delivery platforms to transform these discoveries into medicines that could rescue burn patients worldwide.

Conclusion

The study found that HPMC-encapsulated phage therapy beats antibiotics for multidrug-resistant burn wound infections. To overcome antibiotic resistance and poor healing, phages promote tissue regeneration, reduce inflammation, and increase survival. Phages also lower infection risk. Phage therapy provides scalable, precision-based burn care, which is useful given the global multidrug-resistant infection epidemic. Future research must prioritise clinical validation, phage cocktail composition, and new delivery platforms to turn these discoveries into life-saving medications.This study reveals that bacteriophage therapy can treat burn wound infections caused by P. aeruginosa and K. pneumoniae. Bacteriophage therapy heals burn wound infections, says the study. Phage-loaded HPMC gel outperformed antibiotics in tissue regeneration, inflammation, and survival. These data imply that phage therapy may treat multidrug-resistant burn wound infections, but additional research is needed to overcome difficulties and optimise treatment. Phage therapy seems promising.

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