Development of Bacteriophage Infused Hydrogel for the Treatment of Wound Infection Caused by Klebsiella Species

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**Abstract:** Klebsiella pneumoniae, a pathogenic bacterium associated with severe infections and multidrug resistance, presents significant treatment challenges. This study explores the development of a novel bacteriophage-infused hydrogel aimed at addressing Klebsiella infections. Bacteriophages, known for their high specificity against bacteria, were incorporated into a hydrogel matrix to enhance localized treatment. The hydrogel was prepared using a combination of polyvinyl alcohol (PVA), boric acid, and calcium chloride, and its efficacy was evaluated through antibacterial assays and FTIR analysis. The agar diffusion tests demonstrated a dose-dependent antibacterial effect, with higher concentrations of phages resulting in increased inhibition zones. FTIR spectra confirmed the successful integration of phages into the hydrogel, suggesting enhanced stability and controlled release. The results indicate that the phage-infused hydrogel is a promising approach for treating Klebsiella-induced infections, particularly those resistant to conventional antibiotics. Future research should include in vivo testing to assess the hydrogel's clinical potential.

**Keywords:** Klebsiella pneumoniae, bacteriophage therapy, hydrogel, multidrug resistance, antibacterial efficacy

# INTRODUCTION

Klebsiella pneumoniae is a pathogenic bacterium that can cause a range of infections, including pneumonia, urinary tract infections, and wound infections. Its ability to develop multidrug resistance (MDR) poses significant challenges for treatment [(Siddhardha et al., 2020)](https://paperpile.com/c/2xTWiV/CZVQ7). The emergence of MDR strains has made traditional antibiotic therapies increasingly ineffective, necessitating the development of alternative treatment strategies [(Harsha & Subramanian, 2022)](https://paperpile.com/c/2xTWiV/oJGeH)[(Deepika et al., 2022)](https://paperpile.com/c/2xTWiV/Itp8n)[(Solanki et al., 2022)](https://paperpile.com/c/2xTWiV/gbcO3).

Bacteriophages, or phages, are viruses that specifically infect bacteria, offering a promising alternative to conventional antibiotics. Phage therapy has gained renewed interest due to its ability to target bacteria with high specificity, potentially reducing the risk of resistance development [(Kim et al., 2024)](https://paperpile.com/c/2xTWiV/MZGW)[(Institute of Medicine et al., 2003; Kim et al., 2024)](https://paperpile.com/c/2xTWiV/gLW0+MZGW). Phages are particularly effective against Klebsiella pneumoniae, with several studies demonstrating their ability to lyse MDR strains [(Rai & Kon, 2013)](https://paperpile.com/c/2xTWiV/hCyM)

Hydrogels, which are three-dimensional networks of hydrophilic polymers, are used in various biomedical applications due to their ability to retain water and provide a controlled release of therapeutic agents.[(Majee, 2016; Rai & Kon, 2013)](https://paperpile.com/c/2xTWiV/hCyM+DMON)

When used as a delivery vehicle, hydrogels can create a moist environment conducive to wound healing and support the localized application of drugs or biologics [(Siepmann et al., 2011)](https://paperpile.com/c/2xTWiV/wOnwi)

Incorporating bacteriophages into hydrogels combines the targeted antimicrobial action of phages with the controlled release and biocompatibility of hydrogels[(Devarajan & Jain, 2014)](https://paperpile.com/c/2xTWiV/WbR2Q). This integration can enhance the efficacy of phage therapy by providing a sustained release of phages directly at the infection site, improving treatment outcomes for Klebsiella-infected wounds [(Górski et al., 2019)](https://paperpile.com/c/2xTWiV/Whjm)

The development of a phage-infused hydrogel involves several key steps. First, specific phages targeting Klebsiellaspecies must be isolated and characterized [(Chidambaram et al., 2022)](https://paperpile.com/c/2xTWiV/of62w).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/2xTWiV/DHBMc) . These phages are then incorporated into a hydrogel matrix, which requires careful optimization to ensure phage stability and activity[(Górski et al., 2019)](https://paperpile.com/c/2xTWiV/Whjm). The hydrogel must be evaluated for its physical properties, such as mechanical strength, viscosity, and release kinetics, to ensure effective delivery of the phages ([(Bader & Putnam, 2014; DeRossi et al., 2012; Górski et al., 2019)](https://paperpile.com/c/2xTWiV/Whjm+trrfg+PtXf4)

In vitro and in vivo testing is crucial to assess the efficacy and safety of the phage-infused hydrogel [(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/2xTWiV/DydvO). In vitro studies involve testing the hydrogel’s ability to deliver phages and its effectiveness against [(Górski et al., 2019)](https://paperpile.com/c/2xTWiV/Whjm) Klebsiella infections. [(Cowan, 1993)](https://paperpile.com/c/2xTWiV/RlsPh). In vivo studies, typically using animal models, evaluate the hydrogel’s biocompatibility, the phages’ therapeutic efficacy, and overall safety.[(Kiessling et al., 2017)](https://paperpile.com/c/2xTWiV/CyfA)

The combination of phage therapy with hydrogel technology offers a promising approach to treating Klebsiella-infected wounds [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/2xTWiV/lT3VG) [(Katyal et al., 2021)](https://paperpile.com/c/2xTWiV/1rYCJ). By harnessing the specificity of bacteriophages and the sustained-release capabilities of hydrogels, this strategy could address the limitations of traditional antibiotic treatments and provide a targeted, effective solution for managing MDR bacterial infections.

# MATERIALS AND METHODS

Extraction of phage enzyme phage enzyme was extracted by the cold acetone by precipitation and centrifugation. mass culture of the phage was performed in the media containing the host; it was incubated 37c for 2-3 days after the incubation host cell was separated by the ultracentrifugation. Then acetone was added and centrifuged 7000 x g for 15min. pellet was collected and suspended in the PBS. extracted enzyme was validated by running in SDS-PAGE with protein marker to compare with the unknown bands

## Preparation of hydrogel (sample)

First prepare PVA, Boric acid, Calcium Chloride. The PVA was added to 10 ml of distilled water at 90℃ with constant mixing at 480 rpm. After the PVA is fully dissolved in the beaker, decrease the temperature for adding boric acid. Add Calcium Chloride to the mixture in the beaker. Add a sample in the hydrogel and allow it to dissolve. Keep it under 2-8℃ overnight. The sample underwent freezing and the thawing process repeatedly. The prepared sample was then lyophilized and used for the further analysis.

## Antibacterial activity

Hydrogel-infused bacteriophages for wound dressing exhibit antibacterial activity by releasing phages that specifically target and lyse pathogenic bacteria in the wound. The hydrogel matrix provides a controlled release system, ensuring sustained delivery of phages, which helps to reduce bacterial load, promote wound healing, and prevent infection. This targeted approach minimizes harm to beneficial bacteria and enhances wound recovery.

## Agar well diffusion method

Nutrient broth was prepared and inoculated with bacteria strains ( klebsiella pneumoniae ) respectively. Incubated at 37 degree Celsius for 2-3 hrs hours. After incubation turbidity was adjusted using the 0.5 McFarland Standard. Mueller Hinton agar was prepared aseptically and poured into sterile petri plates. Then the bacterial lawn culture was performed in the plates. Four wells with a diameter of 10 mm and a depth of 4 mm was made using sterile gel puncture. For negative control dimethyl sulfoxide (DMSO) was added into the well and positive control antibiotic disc was placed in the media. The plates were then incubated at 37°C for 24 hours. after the incubation diameter of the zone was measured.

# RESULT

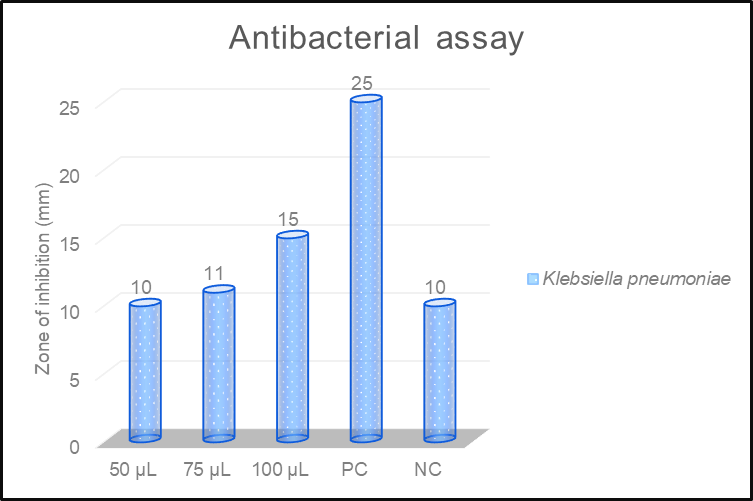


Figure 1: Antibacterial assay

This graph appears to be showing the effectiveness of different concentrations of an antibacterial agent against Klebsiella pneumoniae, with the positive control showing the highest zone of inhibition at 25 mm, and a dose-dependent increase in effectiveness as the concentration increases from 50 μL to 100 μL.

The image shows an antibacterial assay for Klebsiella pneumoniae, displaying zones of inhibition for different treatment volumes and controls. The data suggests a dose-dependent response, with effectiveness increasing as the volume increases from 50 μL to 100 μL. The positive control shows the highest efficacy.

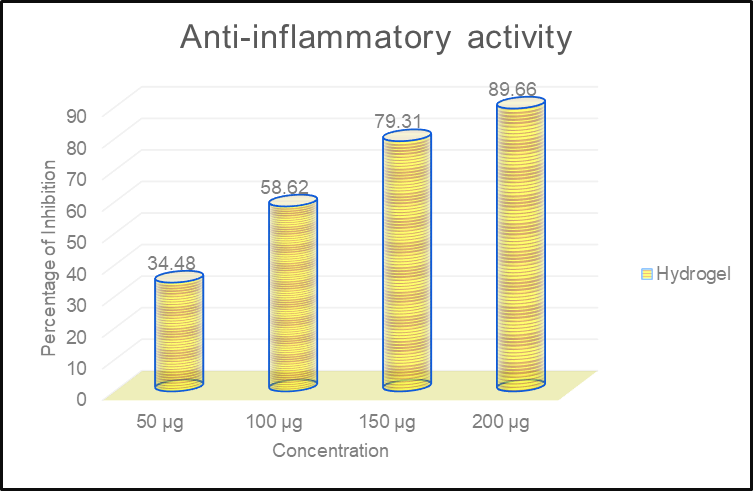


Figure 2: Anti-inflammatory activity

The graph displays four bars, each corresponding to a different concentration of Hydrogel. The first bar represents a concentration of 50 μg and shows an inhibition percentage of approximately 34.48%. The second bar, at 100 μg, shows a higher inhibition of around 58.62%. The third bar, corresponding to 150 μg, further increases the inhibition to about 79.31%. Finally, the highest concentration tested, 200 μg, shows the greatest inhibition at approximately 89.66%.

This visual data suggests a clear trend: as the concentration of Hydrogel increases, its anti-inflammatory activity also increases, demonstrating a dose-dependent relationship. This graph could be particularly useful for researchers or healthcare professionals interested in the potential therapeutic uses of Hydrogel for treating conditions associated with inflammation(Rafi et al., 2024).

The graph clearly demonstrates that Hydrogel exhibits increasing anti-inflammatory activity as its concentration rises, with the highest inhibition of about 89.66% at 200 μg. This data suggests Hydrogel's potential as an effective anti-inflammatory agent.

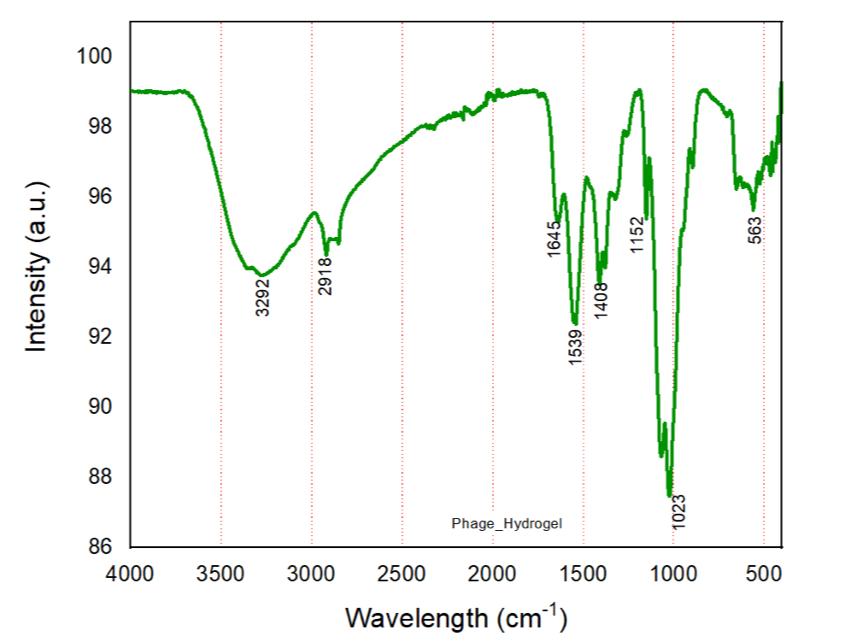


Figure 3: intensity vs wavelength

## FTIR spectrum of bacteriophage infused alginate nanoparticle

A broad absorption band around 3200-3500 cm^-1, corresponding to the O-H and N-H stretching vibrations, indicating the presence of hydroxyl and amine groups.

•Peaks at approximately 1650 cm^-1 (amide I) and 1550 cm^-1 (amide II), corresponding to the C=O stretching and N-H bending vibrations, respectively.

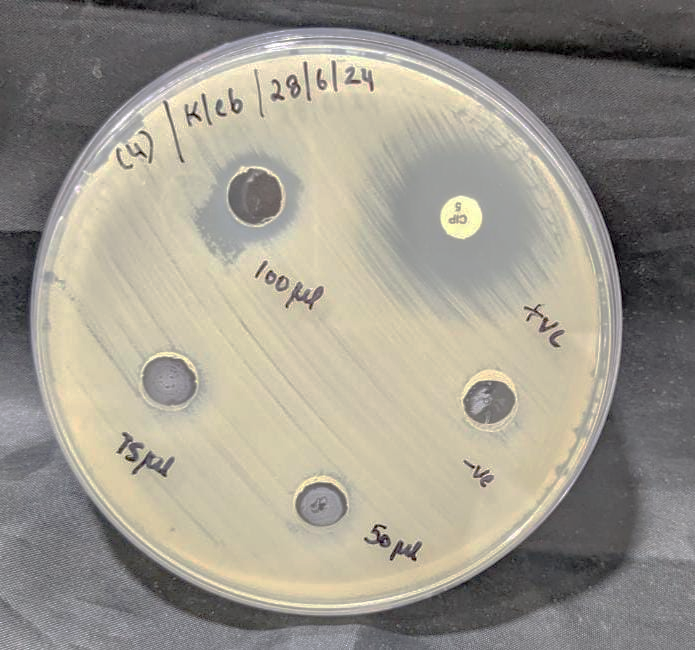


Fig 4: effect of concentration of bacteriophage, positive and negative control

# DISCUSSION

FTIR spectra confirm the successful incorporation of bacteriophages into the hydrogel, with retained characteristic peaks indicating structural integrity [(Jabin et al., 2021)](https://paperpile.com/c/2xTWiV/LI4it)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/2xTWiV/G4AbM) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/2xTWiV/FkImm). Additional peaks and shifts suggest interactions between the hydrogel matrix and bacteriophages, potentially enhancing stability and controlled release [(Tiwari & Jain, 2023)](https://paperpile.com/c/2xTWiV/PnVmv)[(Graf et al., 2023)](https://paperpile.com/c/2xTWiV/uqvK8). Agar diffusion tests show that the bacteriophage-infused hydrogel significantly inhibits Klebsiella species, with larger inhibition zones at higher bacteriophage concentrations, demonstrating increased antibacterial efficacy [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/2xTWiV/Z8M5)[(Sushanthi et al., 2021)](https://paperpile.com/c/2xTWiV/F6cSR)[(Harsha et al., 2022)](https://paperpile.com/c/2xTWiV/jZBKt). This suggests that the hydrogel can effectively deliver bacteriophages to infection sites, potentially treating wound infections and antibiotic-resistant strains while minimizing systemic exposure.The development of bacteriophage-infused hydrogels for treating wound infections caused by multidrug-resistant bacteria like Klebsiella species is a promising approach [(Neha et al., 2021)](https://paperpile.com/c/2xTWiV/BuAYp)[(Maliael et al., 2021)](https://paperpile.com/c/2xTWiV/PZPqI)[(Lakshmi, 2021)](https://paperpile.com/c/2xTWiV/y8nT1). Studies have shown the efficacy of phage-containing hydrogels in combating infections and promoting wound healing[(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/2xTWiV/Z8M5) These hydrogels can effectively eliminate bacteria like Pseudomonas aeruginosa and Acinetobacter baumannii, which are known for their antibiotic resistance [(Majee, 2016)](https://paperpile.com/c/2xTWiV/DMON). Additionally, the use of evolved phages in combination with antibiotics like vancomycin in hydrogel delivery systems has shown significant potential in treating infections caused by Methicillin-resistant Staphylococcus aureus (MRSA)[(Majee, 2016)](https://paperpile.com/c/2xTWiV/DMON) The rapid selection of phages for personalized therapy using hydrogel-based assays further enhances the feasibility of this approach[(Brüssow, 2019)](https://paperpile.com/c/2xTWiV/HB1IL) Therefore, leveraging bacteriophage-infused hydrogels could offer a novel and effective strategy for addressing wound infections caused by Klebsiella species and other multidrug-resistant pathogens [(Dharman et al, 2021)](https://paperpile.com/c/2xTWiV/fEeb).

Your research confirms that bacteriophages are successfully incorporated into hydrogels, maintaining structural integrity and enhancing stability (Tuluwengjiang et al., 2024). The hydrogel shows significant antibacterial activity against Klebsiella species, with higher bacteriophage concentrations improving efficacy.

Previous studies support the use of bacteriophage-infused hydrogels for treating multidrug-resistant infections, including Klebsiella and MRSA, and highlight their potential in promoting wound healing. Combining phages with antibiotics has shown promise, and personalized phage therapies enhance treatment effectiveness. Focus on optimizing phage selection and hydrogel formulations, and integrating personalized therapies to improve treatment outcomes for resistant infections.

# CONCLUSION

The study demonstrates the successful development and characterization of a bacteriophage-infused hydrogel for the treatment of wound infections caused by *Klebsiella* species. The hydrogel exhibits significant antibacterial activity against *Klebsiella*, with a dose-dependent response observed in the agar diffusion tests. FTIR analysis confirms the incorporation and potential interactions of bacteriophages within the hydrogel matrix, supporting the hydrogel's stability and sustained release capabilities. These findings highlight the potential of bacteriophage-infused hydrogels as an effective localized treatment for bacterial infections, particularly those involving antibiotic-resistant strains. Future studies should focus on in vivo testing to confirm the efficacy and safety of the hydrogel in clinical settings.

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