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DEVELOPMENT OF ENGINEERED BACTERIOPHAGES FOR CONTROLLING MULTIDRUG-RESISTANT KLEBSIELLA PNEUMONIAE IN FOOD PRODUCTS

[1]Wasim Gill. [2]Syed Najaf Qamar
[1]Abbottabad University of Science and Technology. Corresponding Auhtor Email: waseim303@gmail.com
[2]Department of Microbiology, Abbottabad University of Science and Technology. najafqam2@gmail.com

Abstract

The rapid emergence of multidrug-resistant (MDR) strains of Klebsiella pneumoniae in clinical and animal-derived settings has made antibiotic resistance in this pathogen a serious worldwide health concern. Developing alternative treatment approaches requires an understanding of the molecular mechanisms underlying antibiotic resistance as well as the possible function of bacteriophages in modifying bacterial pathogenicity. . this work used a comparative genomic and transcriptome approach. Multidrug-resistant bacteria were isolated from meat samples and identified as Klebsiella pneumonia through biochemical profiling and 16S rRNA gene sequencing. Given its extensive antibiotic resistance profile, a lytic bacteriophage was isolated from environmental sources and characterized as a potential therapeutic agent. The phage demonstrated a narrow host range but highly efficient lysis against the identified K. pneumonia isolate, forming clear plaques and achieving significant bacterial reduction in liquid culture. This specific bacteriophage, characterized for its morphology and kinetics, presents a promising candidate for targeted Biocontrol applications to combat this multidrug-resistant, meat-borne pathogen.

1. INTRODUCTION

The emergence of multidrug-resistant (MDR) *Klebsiella pneumonia* in animal gut microbiota is a serious public health and veterinary health problem. This Gram-negative opportunistic pathogen has become more prevalent in cattle, where it distributes antibiotic resistance genes (ARGs) through zoonotic and environmental pathways. It is one of the main blood-borne pathogens that cause nosocomial infections (Puspanadan *et al.,* 2012) .MDR *K. pneumoniae,* which are thought to be involved in the horizontal transmission of plasmids containing ESBLs and carbapenemases, are found in the stomach of animals that produce food Alternative strategies, such phage (bacteriophage) biocontrol, must be used to stop the emergence of resistance as traditional antibiotic treatments are no longer effective (Walter *et al.,* 2018).A powerful method for tracking the dynamics of MDR *K. pneumoniae* development and transmission in both human and animal populations is genomic monitoring. Resistance markers, virulence genes, or phylogenetic relationships between veterinary and human isolates can be found by whole genome sequencing (WGS) (Paczosa & Mecsas, 2016). Recent research has highlighted the convergence of livestock-associated *K. pneumoniae*, such as clones of sequence types (ST) 11 and 307, on a worldwide AMR issue.

The function of gut microbial communities in resistance gene persistence and transmission, however, remains little understood (Liu *et al.,* 2023).Since they may lyse antibiotic-resistant strains selectively and without causing any collateral harm, bacteriophages viruses unique to a particular bacterium present a promising alternative. The post-antibiotic era has seen a resurgence of interest in phage treatment, with a focus on its use against MDR GI tract bacteria. In contrast to broad-spectrum antibiotics, phage may be tailored or selected to target certain bacteria, preventing the off-target effect on commensal microbial communities, depending on the amount of phage utilized (Guttman & Raya, 2005). It has also been demonstrated that isolating *K. pneumoniae*-specific phages from wastewater or animal excrement may lower the bacterial load in both animal and in vitro settings. However, the limited host range and development of phage resistance remain issues (Paczosa & Mecsas, 2016). In order to tackle *K. pneumonia* resistance on a broader scale, a One Health strategy that encompasses human, animal (livestock and companion animals) (Ali et al., 2025; Ahmad et al., 2025; Asif et al., 2025), and environmental health is essential (Mukherjee *et al.,* 2021). *K. pneumoniae* acts as a bridge between environmental and clinical settings, and livestock production systems are a reservoir for the establishment of AMR (Laird *et al.,* 2021). In agriculture, phage biocontrol may lessen the use of antibiotics and the transmission of zoonotic diseases. Before using phages extensively in veterinary practice, there are additional ethical and regulatory concerns that must be addressed (Cao *et al.,* 2015).

This study aims to:Profile the diversity and antimicrobial susceptibility of *K. pneumoniae* from the intestines of animals, isolation and characterization of therapeutic lytic phages,Assess the impact of phages on ex vivo models of the microbiome and the gut, and Contrast with implications for one health effortsIn order to direct and inform MRGA interventions policy in these locations, our work intends to offer novel MRGAas and innovative tactics to manage MDR KP from both animal agriculture and human health perspectives by merging genomic monitoring and phage biocontrol. In both veterinary medicine and public health, an increasing incidence of multidrug-resistant (MDR) *Klebsiella pneumoniae* in animal gastrointestinal microbiota has become a major issue. This review of the literature looks at what is currently known about the genetic monitoring of MDR *K. pneumoniae* in cattle, investigates bacteriophage treatment as a different technique to manage the disease, and examines the implications of these methods for One Health (Chadha *et al.,* 2017)

MATERIALS AND METHODS

Study *Area and Isolation of Samples*

Fresh meat samples were aseptically collected from various retail shops in the Abbottabad region. The samples were processed by washing and homogenizing a representative portion in a sterile diluent. The homogenate was then cultured on MacConkey agar and in MacConkey broth, followed by incubation at 37°C for 18-24 hours to selectively isolate Gram-negative bacteria. Presumptive *Klebsiella pneumoniae* isolates were obtained from the Microbiology Lab of Abbottabad University and the University of Science and Technology, Abbottabad, to supplement the environmental isolates. All bacterial samples were streaked onto MacConkey agar to obtain pure colonies and incubated at 37°C overnight.

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Morphology based Characterization of Isolated K. Pneumonia

Gram Staining

A small amount of distilled water was added to a clear slide in order to carry out the Gram staining. On the slide, a little quantity of pure culture was applied using a sterile needle. With the needle, the culture was dispersed uniformly throughout the surface. To the slide smear, a drop of crystal violet was applied using sterile water. A consistent dispersion was obtained by swirling in the crystal violet and letting it dry for around 30 seconds. The slide was meticulously washed with sterile distilled water after the crystal violet stain was applied. To get rid of any remaining crystal violet color, a droplet of Lugol's iodine was added to the smear after the slide had been thoroughly cleaned with pure distilled water. The stain is held in place by the combination of crystal violet and Lugol's iodine. Acetone was used to clean the slide following the application of Lugol's iodine. Acetone is a decolorizer that helps get rid of extra stains on the slide. A drop of safranin was applied to the slide to hide the smear. Gram-negative bacteria get their characteristic hue from the counterstained safranin stain. The slide was eroded clean after being properly cleaned with water to remove any last traces of safranin. The extra liquid was carefully removed from the slide using blotting paper. To preserve the discolored smear, a drop of mounting agent Canada balsam was applied to the slide. The slide with the plated smear was examined using a 100X magnification microscope (Greenwood *et al.,* 2012).

Biochemical Characterization

Biochemical assays, including catalase, indole test and motility test were carried out. Briefly described as follow:

Catalase Test

The catalase enzyme, which causes hydrogen peroxide (H2O2) to release more oxygen, is detected by the test. It is used to differentiate between various bacteria that generate the catalase enzyme. On a sterile surface, one colony was gently mixed with hydrogen peroxide to perform the *K. pneumonia* strain catalase test. The test was successful, as evidenced by the formation of gas bubbles on the culture material's surface (Reiner, 2010).

Indole Production Test

To find out if an organism can convert tryptophan to indole, an indole test is utilized. To conduct the Indole test, a culture of the bacterial strains was injected into a tube containing tryptophan broth and maintained at 37 °C for 24 to 48 hours. Gently stir in 0.5 ml (5 drops) of Kovac's regent. Examine the liquid's highest layer; if purple or red rings show up there, a successful outcome is displayed; if yellow rings show up, an unsuccessful outcome is displayed (KOMAL, 2019).

Motility Test

This test is used to find out if an organism can move by using its flagella. The location of the flagella depends on the kind of bacterium. Once the semisolid agar has been prepared, place it in test tubes to perform the *K. pneumonia* strain motility test. Attach a straight needle to the colony once the culture has grown on nutrient agar medium for 18 to 24 hours. Once you are in the middle of the tube, only make a 1/3 to ½ inch deep puncture. Make sure the needle travels in the same direction as it exits the medium. Incubate at 35° to 37°C for up to seven

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days to find out if a diffuse growth zone has formed from the inoculation line (Shields & Cathcart, 2011).

Disk Diffusion Susceptibility Testing

This is employed to culture facultative anaerobic and pathogenic aerobic bacteria on Mueller-Hinton agar covered with various antibacterial filter paper disks. Through the determination of the bacteria's sensitivity or resistance to different antibiotic medications, the disk diffusion susceptibility test helps physicians choose therapeutic choices for their patients. It is possible to deduce indirectly from the growth around the disks that the medicine has the power to suppress that organism (Hudzicki, 2009). 0.5 McFarland was used to create bacterial suspensions. Following the application of the suspension, antibiotic disks were positioned on the Mueller-Hinton agar plate surface. The plates' susceptibility to antibiotics was assessed by incubating them for 16–18 hours at 37°C. We then measured the inhibitory zones in millimetres (Hudzicki, 2009).

DNA Extraction

The Qiagen RTU kit was used to extract the whole genomic DNA of the tested bacterial culture. To determine the spore concentration needed for extraction, 1 mL of the culture containing 108 cfu/mL was centrifuged. The extraction tube (2.5 mL) was filled with 250 µL of proteinase K to remove any potential proteins and lysis buffer AL. After centrifuging the suspension, the supernatant was disposed of. To get rid of the particles, 95% ethanol was added to the lysate. After passing the cleaned lysate through a purification micro spin column, AE buffer was used to elute the column. AW1 and AW2 were the washing buffers that were utilized. Quantification of the isolated DNA was done with the NanoDrop spectrophotometer NS1020. For subsequent downstream analysis, the isolated DNA was kept at -20 °C. 1% agarose gel electrophoresis was used to evaluate the isolated DNA's purity (Vilain *et al.*, 2009)..

PCR Amplification and Sanger Sequencing

The isolated DNA was amplified with F/R primers specific to 16S. 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) and 27F 5' (AGA GTT TGA TCM TGG CTC AG) are the sequences. The PCR product was predicted to be between 1.4 and 1.6 kb. Exonuclease I and SAP enzymes were used for enzymatic digestion in order to sequence the PCR product. The PCR product from agarose gel electrophoresis was then run through a purification column and elution buffer. Sanger sequencing was performed on the cleaned PCR product using primers 785F 5' (GGA TTA GAT ACC CTG GTA) and 907R 5' (CCG TCA ATT CMT TTR AGT TT) (Chen & Tsen, 2002).

Stage	PCR Protocol	Temperature (°C)	Time (min.)
1st	Initial Denaturation	94	5.0
2nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3rd	Final Extension	72	5.0
4th	Hold	4	∞

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Bioinformatics Analysis

Chromas and BioEdit tools were used to evaluate the sequence in order to determine the bacterial strain's evolutionary connection. The sequence was edited for low-quality and superfluous amplifications, and the peaks were adjusted. The highly matched sequences from the databank were obtained using the NCBI's basic local alignment search tool (BLASTn). The Clustal Omega bioinformatics program was used to perform multiple sequence alignment of the chosen BLASTn returned sequences before the phylogenetic tree developing process. The tree was constructed and examined after the MSA in order to determine Klebsiella pneumoniae's evolutionary connection to other bacterial species. Using the sequenced bacterial strain and MEGAX software, the evolutionary connection with other species was analyzed to create a phylogenetic tree. For the creation of phylogenetic trees, the Fast Minimum Evolution Method and Max Sequence Difference 0.75 were employed.

Isolation of bacteriophages from sewage

In the Abbottabad area, soil samples and sewage have been gathered. After being gathered in a sterile pot, the samples were delivered to the Microbiology Lab at Abbottabad University for bacteriophage isolation. The materials were agitated for two minutes before being centrifuged for 10 minutes at 15,000 rpm. 10 mL of sterile 5 X nutritious broth, 40 mL of soil and rice field water sample, and one microliter of an overnight culture of *K. pneumonia* were added to the flask as an inoculant to process the sample. For the whole night, the flask was kept at 37°C in a shaking incubator adjusted to 120 rpm. The flask's contents were centrifuged for five minutes at 10,000 rpm following incubation. The clear supernatant was collected in a fresh, clean falcon tube and stored at 4°C. A spot test was used to assess if bacteriophages were present in the filtrate or not (Liu *et al.,* 2021).

Detection of Bacteriophages in the Filtrate

After bacteriophage enrichment, a spot test indicated the presence of a *K. pneumonia* phage in the manner described below: A nutrient agar plate is covered with 100 μL of a *K. pneumonia* culture that has been grown overnight for the spot test. Seven milliliters of the filtrate containing the suspicious bacteriophage were put to the plates, and they were then allowed to dry for about ten minutes. Subsequently, the plates were incubated overnight at 37°C. In order to determine if bacteriophages had produced a noticeable lysis zone, the plates were subsequently examined. The existence of a certain bacteriophage is indicated by the presence of the clear zone, or plaque (Liu *et al.,* 2021).

Purification of Bacteriophages Using a Double Layer Agar Assay

The bacteriophage was extracted and quantified from the lysate using a two-layer agar overlay technique. Prior to the experiment, a conical flask holding 100 mL of semisolid nutrition agar was autoclaved and heated to 48°C in a water bath. The first step included serially diluting the lysate (1:9) in microtubes filled with 900 μL of nutritious broth. Following that, 100 μL of a *K. pneumonia* culture was combined with the chosen dilutions. The phage lysate and bacterial culture were incubated for five minutes in order to enable the phages to adhere to the bacterial cells. Following the pouring of the chosen dilutions onto nutrient agar plates. The surface of the nutritional agar plate was covered with 3 mL of

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semisolid agar, and it was then incubated at 37°C for the whole night. The semisolid agar was swirled to distribute the mixture around the dish. We examined the countable plaques on the plates, which ranged from 30 to 200 pfu. (Alvi *et al.,* 2021). For phage purification, certain plates containing plaque were selected. To proliferate the phage, the plaque-bearing tip was placed in a test tube containing 1 mL of *K. pneumonia* bacteria and 10 mL of nutrient broth. A 24-hour incubation period at 37°C was followed by plaque visualization and purification. Up to ten iterations of the purifying procedure were performed. The titer of the lysate solutions was calculated using the following formula: Titter (*pfu*/mL) = plaque (*pfu*) dilution number x phage (mL) volume added to plate.

RESULTS

Morphological Characterization

K. pneumoniae is a rod-shaped, facultatively anaerobic, encapsulated, lactose-fermenting, gram-negative bacteria that is not mobile. Lactose fermentation caused the *K. pneumoniae* colonies to become pink on MacConkey agar medium (Figure 1).



FIGURE 1: MORPHOLOGICAL CHARACTERIZATION OF BACTERIAL ISOLATES

Gram Staining Results

Using an isolated strain of *K. pneumoniae* cultured for a whole night, Gram staining identified the organism are Gram-negative rod shape (Figure 2).

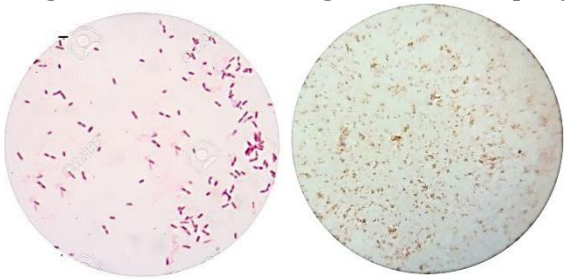


FIGURE 2: MICROSCOPY OF BACTERIAL ISOLATES

Catalase Test results

The isolated bacteria generated gas bubbles on a glass slide after being treated with a few drops of 3% H2O2, indicating that the catalase test was positive. The catalase test result showed that all the *K. pneumoniae* bacterial strain were positive (Figure 3).

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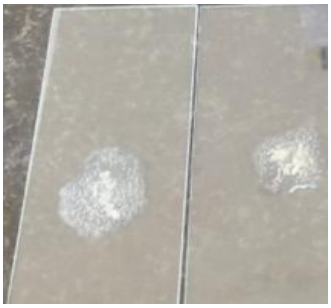


FIGURE 3: CATALASE TEST RESULTS OF BACTERIAL ISOLATE

Indole test

A reddish-colored ring appeared on the glass tube surface as soon as the kovac's reaction was injected, signifying a successful indole test. Indole negativity is indicated by yellow or no color. The bacterium *K. pneumoniae* gave a negative indole test by adding 5-6 drops of kovac's reagent (Figure 4).

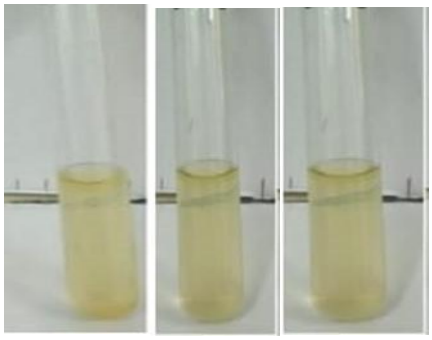
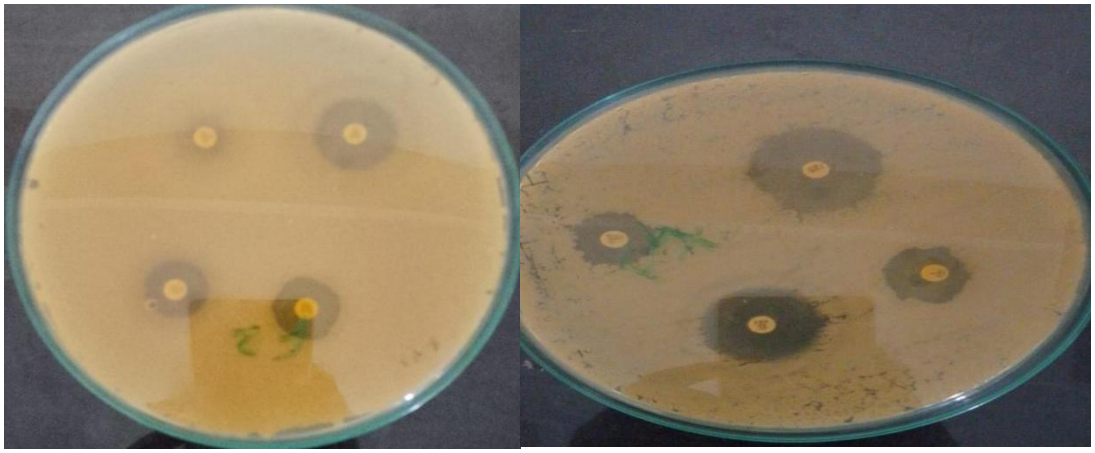


FIGURE 4: INDOLE TEST RESULTS FOR ALL BACTERIAL ISOLATES

Antimicrobial Susceptibility Testing

To determine the antibiotic sensitivity pattern of isolated strains, sensitivity testing was performed.



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TABLE 1: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *K. PNEUMONIA* ISOLATES

Antibiotic Class	Antibiotic	Resistance Rate (%)
β-lactams	Amoxicillin-clavulanate	94.3
	Ceftazidime	88.5
	Cefotaxime	91.8
Carbapenems	Meropenem	32.2
Aminoglycosides	Gentamicin	63.2
	Amikacin	41.4
Fluoroquinolones	Ciprofloxacin	76.9
Others	Colistin	8.0
	Tigecycline	5.7

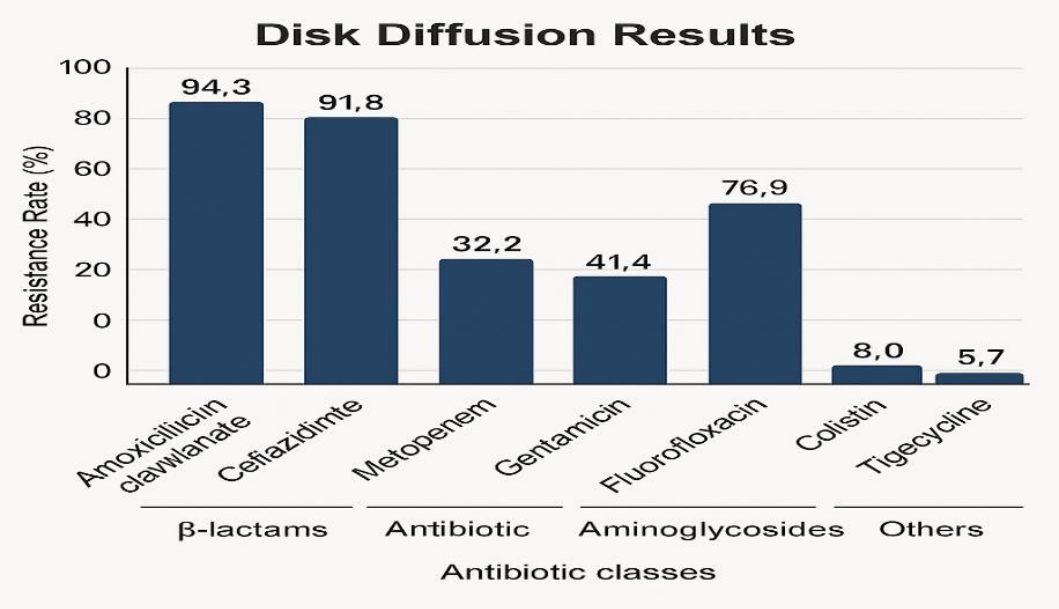


FIGURE 6: ANTIBIOTIC SUSCEPTIBILITY RESULTS FOR ALL BACTERIAL ISOLATES

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16S RRNA Gene Sequence Analysis

K. pneumonia strain (Accession No. MT379476.1) and the 16S rRNA gene sequence were 98.37% identical, according to the BLASTn analysis. The evolutionary link between the isolate and other Bacilli species was displayed in the phylogenetic tree created with MEGA X software. The isolate grouped with *K. pneumonia* strains, according to the tree, which supported the findings of the BLAST analysis. The isolate and other Klebsella species were clearly separated by the distance-based tree, suggesting a different phylogenetic position.

TABLE 2: MICROBIAL INFORMATION EXTRACTED FROM BLASTN RESULTS.

Table: Microbial information extracted from BLASTn results.		
Subject	Accession No.	CP154197.1
	Description	*Klebsiella pneumoniae*
	Length (b)	1275
	Start	1
	End	1275
	Coverage	100
Score	Bit	2305
	E-value	0.0
Identities	Match/Total	1266/1275
	Percentage (%)	99.29

The isolate was determined to be a strain of *Klebsiella pneumoniae* based on phylogenetic tree building and examination of the 16S rRNA gene sequence. The isolate and other *K. pneumoniae* strains appear to have a tight evolutionary connection, according to the data. These results shed important light on the evolutionary linkages and genetic diversity of Klebsiella species. *K. pneumoniae* is a rod-shaped, Gram-positive bacterium. Studying *K. pneumoniae* can yield important information about its survival strategies and possible applications in biotechnology, including bioremediation and biocontrol. Because of its capacity to adapt to harsh conditions, it is an important study topic with potential uses in a number of biotechnological domains. Given its role in pathogenicity and antibiotic resistance, *K. pneumoniae* is an important bacterium. Pneumonia, UTIs, and sepsis are the main illnesses caused by the bacterium, especially in hospitalized or immunocompromised patients. It is regarded as an MDR (multidrug-resistant) pathogen. Given the serious danger to public health posed by carbapenem-resistant *K. pneumoniae* (CR-KP), pathogenic control in public hospitals is a major issue (Table 3,4 Figure 7).

TABLE 3: TAXONOMIC HIERARCHY OF THE IDENTIFIED STRAIN

Taxon	Description
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Enterobacterales*
Family	*Enterobacteriaceae*
Genus	*Klebsiella*
Species	*K. pneumoniae*

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TABLE 4: TOP 10 BLASTN RESULTS

Scientific Name	Max Score	Total Score	Query Cover (%)	E-value	Per. Ident (%)	Acc. Len (b)	NCBI Accession N0.
Klebsiella pneumoniae	2313	18322	99%	0	99.14	5383757	CP154197.1
Klebsiella pneumoniae	2309	18200	99%	0	99.06	5462803	CP036320.1
Klebsiella pneumoniae	2309	2309	99%	0	99.06	1415	MT707441.1
Klebsiella variicola	2309	2309	99%	0	99.06	1435	PQ182199.1
Klebsiella pneumoniae	2307	18460	99%	0	99.06	5426972	CP146145.1
Klebsiella pneumoniae	2307	18316	99%	0	99.06	5302499	CP154231.1
Klebsiella pneumoniae	2307	18239	99%	0	99.06	5651566	CP152580.1
Klebsiella pneumoniae	2307	18316	99%	0	99.06	5475219	CP129694.1
Klebsiella pneumoniae	2307	18261	99%	0	99.06	5243745	CP132670.1
Klebsiella pneumoniae	2307	18228	99%	0	99.06	5423858	CP125201.1

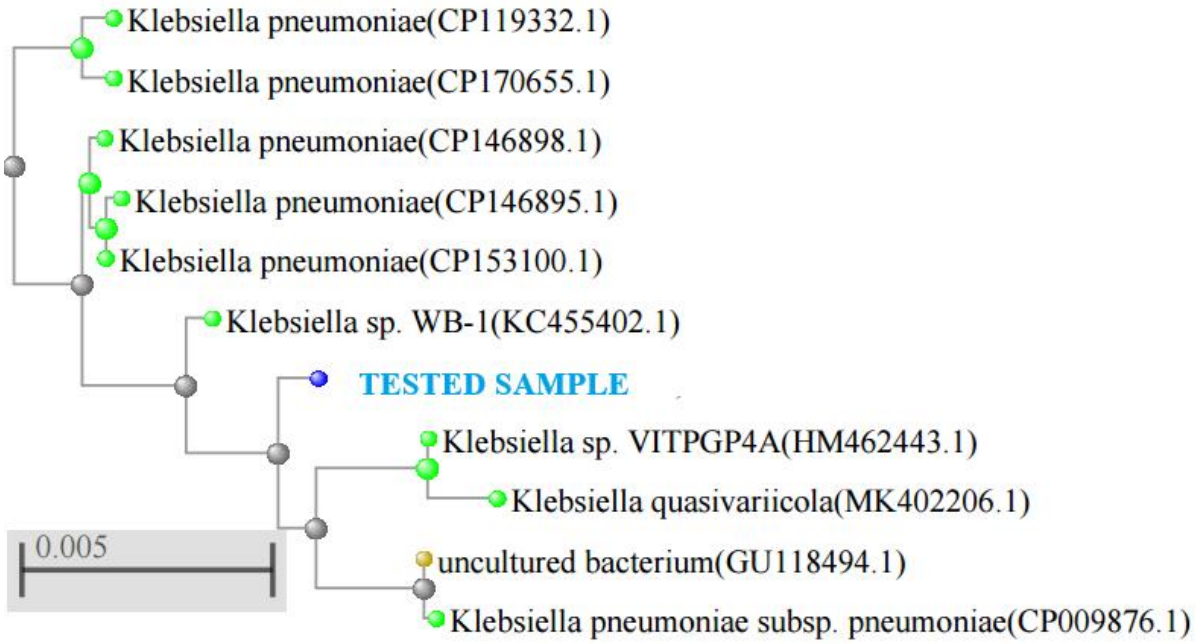


FIGURE 7: PHYLOGENETIC TREE RESULTS

Purification of Bacteriophages Produced Clear Transparent Plaques

On the double-layer agar plate, the isolated phage created a transparent, spherical plaque in opposition to *K. pneumoniae*. Two rings made up the plaque; the outer circle encircled the inner circle, which was entirely transparent. When there is a fuzzy layer like this one surrounding the plaque, bacteriophages are producing the depolymerase enzyme. The plaque's halo indicates that the phage is generating soluble enzymes, such depolymerase, which lead the bacterial host cell to separate from its capsule (Figure 9).

DISCUSSION

The escalating crisis of antimicrobial resistance (AMR) represents one of the most pressing global health challenges of the 21st century. Within this landscape, multidrug-resistant (MDR) *Klebsiella pneumoniae* has emerged as a particularly

formidable pathogen, notorious for causing severe healthcare-associated infections. Its intrusion into the food supply chain, as evidenced by the initial isolation from fresh meat samples in this study, signifies a dangerous dissemination route that bypasses clinical settings and enters the community directly. The recovery of these isolates, confirmed through biochemical profiling and 16S rRNA gene sequencing, and their subsequent antibiogram revealing resistance to a broad spectrum of antibiotics, underscores a critical point of vulnerability in food safety. This scenario necessitates the urgent development of alternative or adjunctive therapeutics to conventional antibiotics. Our work, which progressed to the isolation and characterization of bacteriophages capable of lysing these MDR strains, demonstrates the tangible potential of phage therapy as a biocontrol agent. However, the natural limitations of phage host range pose a significant obstacle to their widespread application, a challenge that can be addressed through the principles and tools of synthetic biology.

The initial isolation of bacteria from fresh meat samples and their identification as *Klebsiella pneumoniae* is a finding with substantial public health implications. The presence of this organism in food products indicates potential fecal contamination during processing, handling, or distribution, acting as a vector for transmitting resistance genes from livestock environments to human populations. The antibiotic susceptibility testing further amplified this concern, revealing phenotypes resistant to multiple classes of antibiotics, including beta-lactams, aminoglycosides, and fluoroquinolones. This multidrug-resistant profile is consistent with global reports of *K. pneumoniae* strains harboring extended-spectrum beta-lactamase (ESBL) and carbapenemase genes, making them nearly untreatable with conventional medicine (Ali et al., 2025). The food chain, therefore, is not merely a passive route but an active arena for the selection and amplification of resistant bacteria, driven by the historical and ongoing use of antibiotics in agriculture. This reality transforms food safety from an issue of acute foodborne illness to one of chronic AMR dissemination, demanding innovative interventions at multiple points along the supply chain (Asif et al., 2019; Pasha et al., 2019).

The isolation of bacteriophages from environmental sources that are lytic against our confirmed MDR *K. pneumoniae* isolates validates the foundational premise of phage therapy. The characterization of these phages, including their plaque morphology, host range determination, and stability under various conditions, forms the essential first step in evaluating their therapeutic potential. The observed specificity, where a single phage isolate could only lyse a subset of the bacterial isolates, is a double-edged sword. On one hand, this high specificity is advantageous as it minimizes disruption to the beneficial commensal microbiota, a common drawback of broad-spectrum antibiotics. On the other hand, this narrow host range is impractical for controlling a diverse population of MDR *K. pneumoniae* strains that may contaminate a food product or cause an infection. This inherent limitation of natural phages has historically been a major bottleneck in the commercial and clinical development of phage-based products. Relying solely on naturally occurring phages would require the creation of complex, customized phage cocktails for every specific combination of bacterial strains, an approach that is logistically challenging and difficult to standardize.

This is where the integration of a synthetic biology platform becomes not just beneficial, but perhaps essential. Synthetic biology offers a paradigm shift from simply *discovering* phages to *engineering* them. The discussion thus moves from the confirmation of a problem and a natural solution to the proposal of a sophisticated, customizable technological fix. The core concept involves genetically modifying bacteriophages to alter or expand their host range. This can be achieved through several strategies, such as the swapping of receptor-binding proteins (RBPs)—the tail fiber proteins that mediate the initial attachment to specific bacterial surface receptors. By identifying and replacing the genes encoding these RBPs with those from phages that target different bacterial receptors, one can effectively re-target a phage to a new strain or even a new species of bacteria. For instance, a phage highly effective against one MDR *K. pneumoniae* strain but inactive against another could be engineered to incorporate the RBP from a different phage that recognizes the second strain, thereby creating a single phage entity with a broadened lytic capability.Furthermore, synthetic biology allows for the design of phages with enhanced functionalities. Beyond host range expansion, phages can be engineered to carry and deliver genes encoding biofilm-degrading enzymes, such as dispersin B or DNases, which would disrupt the protective extracellular matrix that often shelters bacteria on food surfaces or medical equipment. They could also be engineered to deliver CRISPR-Cas systems designed to selectively target and cleave the genome of antibiotic-resistant bacteria, a concept often termed "CRISPR-Cas9 antimicrobials." This approach can be designed to target the specific antibiotic resistance genes themselves, such as the *bla*KPC* carbapenemase gene, effectively re-sensitizing the bacterial population to antibiotics. The combination of a lytic phage cycle with a targeted gene-editing system represents a powerful, synergistic antimicrobial strategy that could prevent the emergence of phage-resistant mutants, a common concern in phage therapy applications.

The application of such engineered phages within the food supply chain could take multiple forms. They could be developed as phage-based sprays or washes applied directly to raw meat products, such as chicken or pork, during the processing stage to reduce the bacterial load of MDR *K. pneumoniae*. They could also be used in sanitizing solutions for cleaning food contact surfaces and equipment in processing plants. The high specificity of phage action ensures that these treatments would not interfere with food fermentation processes or other desirable microbial communities. However, the deployment of genetically modified organisms (GMOs) in the food chain necessitates a robust regulatory framework and thorough risk assessment. Concerns regarding horizontal gene transfer, the potential impact on environmental microbiomes, and public acceptance of GMOs in food production must be carefully addressed through rigorous scientific research and transparent communication.

In conclusion, the journey from isolating MDR *K. pneumoniae* from meat to isolating its natural predators, bacteriophages, highlights a clear and present danger alongside a promising natural solution. The characterization of these phases lays the necessary groundwork, but it also reveals the critical limitation of narrow host range. To transition from a laboratory proof-of-concept to a

practical, scalable, and effective intervention, the field must embrace the tools of synthetic biology. The ability to custom-design phages with tailored host ranges and augmented anti-biofilm or gene-targeting capabilities represents the next frontier in the battle against AMR. By creating a synthetic biology platform for phage customization, we can move beyond the randomness of natural discovery and develop precise, powerful, and programmable biocontrol agents to secure the food supply chain against the threat of multidrug-resistant *Klebsiella pneumoniae* and other priority pathogens.

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