

Harnessing Bacteriophages: Isolation, Purification, and Characterization of bacteriophages to combat Multidrug-Resistant Uropathogenic *E. coli*

Zoha Masood, Husnain Qamar, Muhammad Hamza Basit, Zahra Haider, Kausar Malik* and Samia Afzal*

Centre of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Bank Road, Thokar Niaz Baig, Lahore, Pakistan

ABSTRACT

Antimicrobial resistance is a significant global health threat, with an estimated 10 million annual deaths projected by the year 2050. A significant number of individuals die frequently due to infections caused by antimicrobial-resistant pathogens. To address this global issue, several alternative therapeutics are currently being explored. Amongst the proposed strategies, phage therapy is a promising approach that employs lytic bacteriophages to specifically target and kill bacterial cells. This present study focuses on the isolation of phages from the natural environment and the evaluation of their antibacterial potential against Multidrug-resistant uropathogenic *Escherichia coli* strains. The isolated phages exhibited noticeable lytic activity and stability across a varying range of environmental conditions. The phages remained stable within the temperature range of 4°C to 37°C, while a noticeable reduction in phage titre was observed at 65 °C and 95°C. Moreover, it showed stability within the pH range of 4-10, however at extreme acidic and alkaline conditions (pH 2 and pH 12), there was a tremendous decrease in phage titre. gradient PCR was performed for the identification of the phage family using the primers targeting the major capsid protein (MCP) of seven phage families. The results demonstrated that the phages belonged to the *Podoviridae* family. The host range analysis of the phages revealed narrow-host range activity, where phages were able to infect a narrow range of MDR bacterial strains. The findings indicated that the isolated phages are suitable candidates for phage therapy applications. Furthermore, the future prospects of this study include Transmission Electron Microscopy (TEM) and whole genome sequencing analysis to understand the complete molecular characterization of the isolated phages.

Article Information

Received 02 July 2025

Revised 05 September 2025

Accepted 14 September 2025

Available online 01 September 2026 (early access)

Authors' Contribution

ZM and HQ performed all experiments and wrote the manuscript. KM supervised the study that dealt with all troubleshooting areas. MHB and ZH helped in article write-up. SA performed systematic evaluation and proofread the article. All authors approved the manuscript.

Key words

Antimicrobial resistance, Uropathogenic *E. coli*, Bacteriophages, Phage therapy

INTRODUCTION

Antimicrobial resistance (AMR) is considered a major global health threat, as it has reduced the effectiveness of commonly prescribed antibiotics for bacterial infections. The World Health Organization (WHO) reported that approximately 10 million deaths will occur annually by the year 2050 if no alternative strategies are developed (Tang *et al.*, 2023). Additionally, the centers for disease control and prevention (CDC) states that the carbapenem-

resistant *Acinetobacter* family and carbapenem-resistant Enterobacterales order are listed in the 'urgent threat' category. Enterobacterales include ESBL vancomycin-resistant Enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), which cause serious, life-threatening infections (Bilal *et al.*, 2021). There has been a considerable increase in antimicrobial-resistant infections in South Asian countries, including Pakistan. According to a report in Pakistan, antibiotic-resistant *Escherichia coli* followed by MRSA, VRE, and *Klebsiella* spp., are responsible for several complicated infections (Bilal *et al.*, 2021).

Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are reported to be the most common infections suffered by people in Pakistan (Bilal *et al.*, 2021). *E. coli* is universally found everywhere and is the most common source of bacterial infections in both humans and animals (Yang *et al.*, 2017). UPEC causes septicemia, enteritis, and other clinical complications such as neonatal meningitis (Allocati *et al.*, 2013). The

* Corresponding author: samiaraza@live.com; kausarbasit786@yahoo.com
0030-9923/2026/0001-0001 \$ 9.00/0



Copyright 2026 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

emergence of multidrug-resistant *E. coli* strains, capable of withstanding several commonly used antibiotics, has further complicated treatment efforts (Galindo-Méndez, 2020). Various studies have reported that the antibiotic resistance traits in *E. coli* strains are often acquired through the process of horizontal gene transfer. This mechanism basically allows the transfer of genetic material, encoding antibiotic resistance and virulence factors, responsible for the pathogenicity of the microorganism (da Silva and Mendonça, 2012). A wide variety of antibiotics were conventionally used to treat infections caused by *E. coli* in humans and animals. However, according to a recent report, *E. coli* expresses extremely high levels of resistance against different classes of antibiotics like tetracycline, fluoroquinolones, aminoglycosides, and β -lactams (Li *et al.*, 2025). Antimicrobial resistance is dominant in pathogenic strains as compared to commensal bacterial strains (El-Baz *et al.*, 2022).

In an effort to combat the current global crisis, alternative strategies are being extensively reviewed. Phage therapy has regained prominence in this regard for the effective treatment of pathogenic multidrug-resistant (MDR) bacterial strains due to its self-replicating nature, targeted action, and non-harmful characteristics (Chung *et al.*, 2023). The concept of phage therapy was first proposed by Felix d'Herelle and Frederick Twort back in the twentieth century, but this concept did not gain validity for extended clinical application because of the swift and efficacious introduction of antibiotics. However, the research interest has resurfaced in considering the implementation of phage therapy into practice (Saeed *et al.*, 2021). Bacteriophages offer several advantages over conventional antibiotic agents, particularly their high specificity to bacterial species or strains, without disrupting the normal microflora. Secondly, antibiotics have several harmful side effects against the bacterial host, while bacteriophages are safe to use and no adverse side effects have been reported so far (Mishra *et al.*, 2024).

Several phage-based products are commercialized; for instance, EcoShield™ is an FDA-approved phage formulation developed for control of the pathogenic strain of *E. coli* (Abuladze *et al.*, 2008). Furthermore, clinical studies and historical evidence have displayed the safety of phages in healthy volunteers, with no adverse side effects (Mishra *et al.*, 2024). Thus, the current study was carried out to isolate and evaluate the lytic activity of phages against MDR uropathogenic *E. coli*.

MATERIALS AND METHODS

Collection and processing of wastewater

Samples were collected from three different areas of Lahore: Rohi Nala, Harbanspura, and Wapda Town sewage. Samples were collected in large amounts,

approximately a liter from each site. Wastewater samples were poured into 50 mL Falcon tubes were kept overnight, to allow solid debris to settle down. The next day, these samples were centrifuged at 4000 rpm for 30 min and then syringe filtered using 0.45 μ m and 0.22 μ m membrane filter (Merck Millipore Millex cat #C7610).

Bacteriophage enrichment

Enrichment is an extremely important step for the multiplication and propagation of bacteriophages against their specific bacterial host. In a sterile Falcon tube, 10 mL of filtered sewage water and 1 mL of the MDR UPEC strain of early log phase were added together and incubated at 37 °C for 24 h. The next day, the sample was centrifuged at 4000 rpm for 30 min and then syringe filtered with a 0.45 μ m and 0.22 μ m pore size membrane filters (Merck Millipore Millex cat #C7610). The filtrate was stored at 4 °C until further use. This protocol was followed for all UPEC strains.

Isolation of bacteriophages

The “double agar overlay method” or the plaque test is the standard protocol employed for the isolation of bacteriophages in terms of plaque forming units per mL (PFU/mL) (Jurczak-Kurek *et al.*, 2016). A ten-fold serial dilution of phage lysate was made by mixing 900 μ L of SM buffer (100mM sodium chloride, 10mM magnesium sulphate, 50mM Tris-HCl of pH 5.0, and 0.01% (w/v) gelatin) and 100 μ L of lysate in a sterile Eppendorf. Alternate dilutions were selected, in which 1 mL of the early-log phage bacterial culture was added and incubated for 10 min at 37°C. This step ensures the adsorption of phages to the bacterial host. After 10 min, each tube was mixed with 0.7% soft agar and gently poured on solidified agar plates. The plates were then incubated overnight at 37°C. The next day, the plaques were counted and the bacteriophage titre was calculated.

Collection of phage lysate

A well-isolated, clear plaque was picked with the help of a sterile micropipette and transferred to a sterile Eppendorf containing 1 mL SM buffer. The suspension solution was centrifuged at 8000 rpm for 30 min. After centrifugation, the supernatant was collected in a separate tube and incubated with 1 mL of overnight bacterial culture for one hour, in order for phages to multiply and propagate. Post-incubation, the enriched solution was centrifuged again, then syringe filtered using a 0.22 μ m pore-sized filter. The lysate was stored at 4°C for further use. The process was repeated five times until a uniform and distinct morphology of UPEC phages was obtained.

Host range

Twelve MDR strains of *Enterococcus faecalis*,

Escherichia coli, and MRSA were employed to test the host range of the isolated UPEC phages. Early-log phase bacterial strains were used to create a bacterial lawn on LB agar plates. Spot tests were carried out by adding 10 μ L of concentrated phage lysate to the bacterial lawn. The plates were incubated overnight at 37°C and then checked for clear zones the next day.

Effect of temperature

The thermal stability of the phages was assessed at various temperatures (-20°C, 4°C, 25°C, 30°C, 37°C, 50°C, 65°C, 95°C) using the standard double-agar overlay method. Phage lysates were serially diluted in SM buffer and incubated for 15 min with a control at 37 °C. The dilutions were mixed with the host bacterial strain and incubated for 10 min. After incubation, the dilutions were mixed with 0.7% soft agar and poured onto plates. The plates were allowed to solidify for 30 min and then placed in the incubator for 18-24 h. The next day, PFUs were counted for stability comparison.

Effect of pH

The stability of phages at different pH ranges was evaluated using the protocol designed by Jurczak-Kurek *et al.* (2016). In LB broth, 10-fold serial dilutions of the phage lysates were prepared and incubated at 37°C for 60 min at 2.0, 4.0, 7.5, 10.0, and 12.0 pH. A comparison control (dilution prepared in SM Buffer at pH 7.5) was also incubated at 37°C for one hour. Post-incubation, the standard double-agar overlay method was employed. Well-isolated, distinct plaques were counted to assess the stability of phages over a wide pH range.

Genomic DNA extraction and PCR-based family identification of phages

Purified, high-titer phage was obtained and processed for DNA extraction, using the double agar overlay method. Phage dilutions were incubated with host bacteria at 37°C for 10 min, mixed with 0.7% soft agar, and poured onto agar plates for 18-24 h. The plate containing well-isolated and distinct phages was used for DNA extraction. SM buffer was added to the plate, shaken for 4 h, and incubated overnight at 4°C. The buffer was eluted, centrifuged, and filtered through a 0.22 μ m membrane syringe filter (Merck Millipore Millex cat #C7610).

The standard phenol chloroform isoamyl alcohol (PCI) method was employed for the genomic DNA extraction of the UPEC phages. The phage lysate was initially treated with DNase (1U, thermo Cat # EN0525), RNase (7U, Sigma Aldrich, Cat # R6513), Proteinase K (20mg/mL, ThermoFisher cat # 25530049), 10% sodium dodecyl sulphate, and 0.5M EDTA, followed by incubation

at 55°C. After the addition of PCI (Invitrogen, Cat No:15-593-049), the mixture was centrifuged, and the aqueous layer was transferred into a sterile Eppendorf. The sample was then treated with sodium acetate and ethanol to allow DNA precipitation, followed by washing with ice-chilled 75% ethanol. The obtained, purified DNA was dissolved in nuclease-free water (Invitrogen Cat # 10977035). The concentration and quality of DNA were measured using a Nanodrop Spectrophotometer (Thermo Scientific Nanodrop, 2000). The sample was stored at -20°C until further use.

Primers for the bacteriophage major-capsid protein (MCP) were designed based on Born *et al.* (2019a). A gradient PCR was performed with temperatures ranging from 57°C to 62°C for the family identification of the isolated phages. PCR products were analyzed using a 2% agarose gel with a 50bp DNA ladder (Thermo-scientific #SM0371). The gel was run at 90V for 120 min and visualized with a UV transilluminator (BioRad). Statistical analysis was performed using GraphPad Prism 6.0, and phage titer was calculated using column graphs.

RESULTS

The current study focused on the isolation of lytic phages from natural environment against the MDR UPEC strains. *Escherichia coli* strains were isolated from contaminated hospital wastewater. *E. coli* showed light pink colonies with a moist appearance on MacConkey Agar. The Kirby-Bauer disc diffusion method was used for the antibiotic susceptibility testing. The method was performed according to the guidelines provided by the clinical and laboratory standards institute (CLSI). Table 1 indicates the antibiotic profiling results of two UPEC strains. The strains indicated resistance to more than three classes of antibiotics and hence the bacterial strains were classified as MDR strains.



Fig. 1. Isolated light pink colonies of MDR *E. coli*, with moist and non-mucoid appearance, having a depression in the middle of the colonies.

Table I. Antibiotic susceptibility report of bacterial strains (UPEC 1 and UPEC 2).

S. No	Antibiotics	UPEC 1	UPEC 2
1	Oxazolidinone		
	Linezolid	R	R
	Tedizolid	R	R
2	Macrolide		
	Erythromycin	R	R
3	Lincosamide		
	Clindamycin	R	R
4	Fluoroquinolone		
	Ciprofloxacin	R	R
5	Tetracycline		
	Doxycycline	R	R
6	Sulfonamide- Trimethoprim		
	Trimethoprim-Sulfamethoxazole	R	R
7	Cephalosporin		
	Cefoxitin	R	R
	Ceftriaxone	R	R
	Cefixime	S	R
	Cefotetan	S	S
8	Aminopenicillin		
	Amoxicillin Clavulanic acid	R	R
9	Aminoglycoside		
	Amikacin	R	R
	Tobramycin	R	S
10.	Phenicol		
	chloramphenicol	R	R
11.	Carbapenem		
	Imipenem	R	S
12.	Glycylcycline		
	Tigecycline	R	R
13.	Quinolone		
	Levofloxacin	R	R
14.	Miscellaneous		
	Fusidic acid	-	-
	Fosfomycin	R	R
	Methylsulfonylmethane	S	R

Following the standard procedure of enrichment, biological wastewater samples were incubated with the host bacterial strains. Phages were isolated and purified using the standard double-layer overlay agar method. To obtain purified and uniform-sized phages against MDR UPEC, plaques were picked up by a sterile micropipette tip and dispensed into SM buffer; this process was repeated

several times. The morphology of each bacteriophage is shown in [Figures 2 A, B, C](#). Two bacteriophages were isolated against the UPEC strain 1 from Rohi Nala and Harbanspura sewage and were named as *Escherichia* phage E1 and *Escherichia* phage E2, respectively. One bacteriophage was isolated against the UPEC strain 2 from Rohi Nala sewage and was named as *Escherichia* phage E3. The host range of bacteriophages was assessed using the spot test, and clearance of the bacterial lawn demonstrated the lytic activity against the MDR *E. coli* and has been presented in [Table II](#).

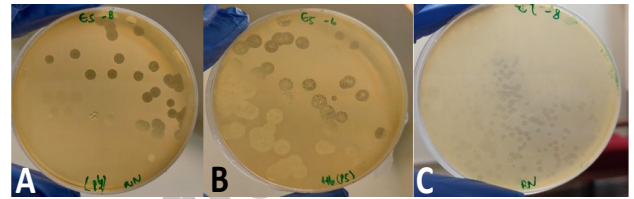


Fig. 2. (A) shows clear plaques isolated from Rohi Nala against the ES5 strain. (B) shows almost the same morphology plaques as in A, clear and larger in size against ES but isolated from Harbanspura. (C) shows small plaques with different morphology as compared to other against UPEC E4. All phages show lytic activity against their host.

Table II. Host range of *Escherichia* phage E1, *Escherichia* phage E2, and *Escherichia* phage E3.

S. No	Bacterial host	E1	E2	E3
1	MDR UPEC 1	+	-	+
2	MDR UPEC 2	-	-	-
3	MDR UPEC 3	-	+	-
4	MDR UPEC 6	-	-	+
5	MRSA 1	-	-	-
6	MRSA 2	-	-	-
7	MRSA 3	-	-	-
8	<i>Enterococcus faecalis</i> 1	-	-	-
9	<i>Enterococcus faecalis</i> 2	-	-	-
10	<i>Enterococcus faecalis</i> 3	-	-	-
11	<i>Enterococcus faecalis</i> 4	-	-	-
12	<i>Enterococcus faecalis</i> 5	-	-	-

+, sign indicates positive phage activity; -, indicates no phage activity.

The thermal stability of the phages was assessed at different temperatures, and the PFU was calculated using the “Double agar overlay method”. The phages showed infectivity at -20°C, 30°C, and 37°C, while phage activity started declining at 50°C, and there was an abrupt loss in phage activity at extreme temperature ranges like 65°C and 95°C ([Fig. 3A](#)).

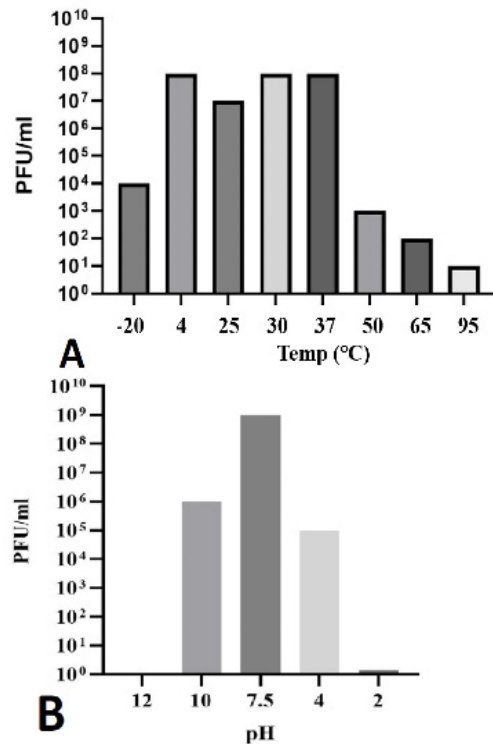


Fig. 3. Effect of different temperature (A) and pH 2 to 12 (B) on activity pf phages. The figure shows the average change in PFU/ml of three isolated phages at varying temperature and pH.

The stability of phages was also assessed at acidic and alkaline pH ranges, where phage dilutions were prepared in LB broth containing 1M HCl and 1M NaOH. The resulting PFU was calculated and compared with the control using the plaque assay or the standard “Double agar overlay”

method. The results indicated that the phages were stable at a pH range of 4, 7.5 and 10. Whereas, the phages lost their activity at extreme pH levels 2 and 12 (Fig. 3B).

Bacteriophage Major Capsid Proteins (MCP) Primers (Table III) were optimized at the 1:10 dilution. *Escherichia* phage E1 showed a band at 60°C and belonged to the SP6 family. Whereas *Escherichia* phage E3 showed a visible band at 59°C that belonged to the T7 family, while *Escherichia* phage E2 showed no band on 2% agarose gel with any of the mentioned sets of primers, indicating high genetic diversity of phage E2. Visible bands can be seen in Figure 4A, B.

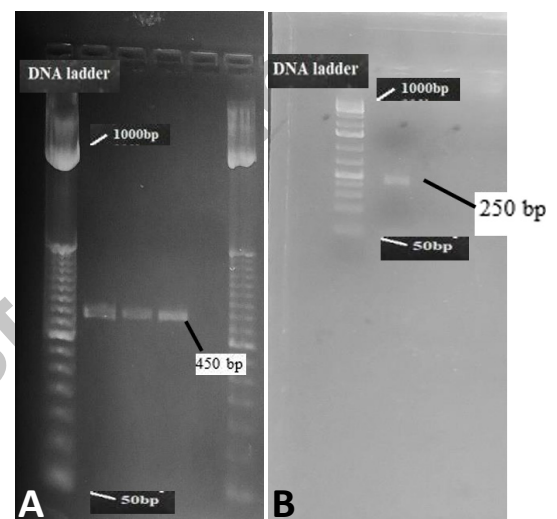


Fig. 4. The isolated phage was discovered to be a member of the T7-like phage MCP-based family and the SP6-like phage MCP-based family. (A) Showed bands of 450 bp bands of ES RN phage using a 50 bp ladder. (B) Showed bands of size 250 bp bands of E4RN phage.

Table III. Bacteriophage major capsid proteins (MCP) primers.

S. No	Name of primer	Sequence of Primers	No. of bases of primers
1	T4-fw	CCCTGCTGTTCCAGATCGANA ARG ARG C	28
2	T4-rev	CTGCCTGGCGTACTGGTCDATRWANAC	27
3	T7-fw	GACAAGCGGAAGGACATCAANCAYACNGARA	31
4	T7-rev	CGCGTAGTTGGCGGCRTTNGGCATNA	26
5	N4- fw	GGATGATCGTAATATTAATGATCAGGGNATHRAYGC	36
6	N4-rev	GACATAAAGCCCATTTCGCCRWANGGRTC	29
7	GJ1-fw	GGCTGCGCGTATGATTAGGAYATHGAYGA	29
8	GJ1-rev	CCAATGCATCACCGCGTAAACNGTNGC	27
9	SP6-fw	CACCGTGATTGCGCGTAAACNGTNGC	27
10	SP6-rev	TTCCCAACGATCCGGAATNGCNCCYTC	27
11	FO1-fw	CGCCATTGAAGAACTGCGTRWRCAATGGA	30
12	FO1- rev	GGCATCATATAGGAATGCGCYTCRAARTC	29
13	Vi1-fw	GCCGATTAATATTGCGATGGAYTTYTT	27
14	Vi1-rev	CCAGCATAAAGGTCATAAATTCCAYTTYTC	31

DISCUSSION

Antibiotic resistance is a global issue and a potential source of threat to the human population. Millions of people are dying every year because of infections caused by resistant pathogens (Bassetti *et al.*, 2017; Walsh *et al.*, 2023). The rise in antimicrobial resistance is due to the overuse of antimicrobial products, especially antibiotics. Overuse, misuse, self-medication, lack of awareness, and lack of proper treatment are the main causes of antibiotic resistance (Hayat *et al.*, 2022). There is a tremendous increase in antibiotic resistance in Pakistan. Reportedly, Pakistan experienced a 65% increase in the use of antibiotics, from an estimated 800 million to 1.3 billion dollars between 2000 and 2015 (Qu *et al.*, 2019).

To effectively tackle the issue of antibiotic resistance, phage therapy is being considered as a viable solution. Bacteriophages can be successfully employed as a sustainable alternative to antibiotics, as they are highly specific to their host target and have minimal overall damage to beneficial microbiota. They also decrease the chances of resistance developments unlike conventional antibiotics (Subramanian, 2024). Virulent bacteriophages possess special mechanisms for infecting and specifically targeting bacterial cells (Taati-Moghadam *et al.*, 2020). For instance, the antibacterial potential of lytic phage, isolated against MDR UPEC strain was reported in a study by Nawaz *et al.* (2024). Lack of virulent and lysogenic genes was observed by the complete molecular characterization analysis. The phage also demonstrated stability in human blood and was effective in reducing bacterial load by 99% on contaminated surfaces and blood samples.

Biological wastewater is reported to be a rich source of organic and inorganic material; hence, sewage wastewater was collected from different areas in Lahore for the isolation of lytic phages against MDR *E. coli*. Three phages were isolated against two UPEC strains. *Escherichia* phage E1 and *Escherichia* phage E2 were isolated from Rohi Nala and Harbanspura sewage against UPEC strain 1, whereas *Escherichia* phage E3 was isolated from Rohi Nala against UPEC strain 2. The “Double Agar layer overlay” method was used for the isolation and purification of phages. The method was repeated consecutively until the morphology and size of the phages were consistent. Phages isolated against the UPEC strain maintained a clear zone showing lytic activity against its bacterial host. Moreover, semitransparent zones and haloes were observed around clear plaques. This is essentially due to the presence of phage enzymes such as hydrolytic depolymerases, which are used to destroy bacterial cells or degrade extracellular polymeric substances (Jurczak-Kurek *et al.*, 2016).

The stability of the isolated phages was assessed

at a wide range of temperatures and pH. The phages were stable and showed no reduction in the phage titre at temperatures 4°C, 25°C, 30°C, and 37°C. However, at extreme temperatures of 65°C and 95°C, the phage titre was significantly reduced. Similar thermal stability of lytic phages has been observed in previous studies by (Carey-Smith *et al.*, 2006; Jamalludeen *et al.*, 2007). Moreover, the phages indicated stability at varying pH values. No visible reduction in the phage titre was observed at pH values 4, 7.5, and 10. However, at extreme acidic (pH:2) and alkaline (pH:12) conditions, a tremendous decline in the phage titre was noticeable. The results align with a previous study reported by Mohamed *et al.* (2025) where the isolated phage became inactive at extreme pH conditions.

For the evaluation of the host range of phages, 12 MDR bacterial strains of *Enterococcus faecalis*, *Escherichia coli*, and MRSA were used. *Escherichia* phage E1 and phage E2 showed activity against one MDR UPEC strain, while *Escherichia* phage E3 was active against two MDR UPEC strains. This indicated that both the phages were highly specific and were hence classified as narrow-host range phages. Major capsid protein (MCP) primers were designed and used for the partial molecular characterization and family identification of phage families. Numerous phage genomes have been sequenced, revealing phylogenetic relationships among various phage families (Born *et al.*, 2019).

Primers of seven bacteriophage families as designed by Born *et al.* (2019) were used for characterization by gradient PCR assays with a temperature range of 57°C to 62°C. Using PCR assays, it was revealed that the phage E1 annealed to the SP6 primer pair at 60 °C and phage E3 annealed to the T7 primer pair at 59 °C. Both the phages belonged to the Podoviridae family. Another phage, *Escherichia* phage E2 isolated from Harbanspura sewage, showed no specific amplification with the primer pairs, thereby suggesting high genetic diversity of phage E2. It indicates that *Escherichia* phage E2 may lack specific sequence homology with the conserved regions targeted by the primers.

In conclusion, phage therapy has recently gained importance because of its ability to specifically target antimicrobial-resistant bacteria. Unlike broad-spectrum antibiotics, phages have minimal side effects on the surrounding microbiota, thus holding significant potential for antibiotic-resistant infections (Yang *et al.*, 2012). The future aspects of the study include the complete molecular characterization of phage MM3 using standard techniques such as TEM analysis and whole genome sequencing. Despite some challenges, phage therapy has the potential to emerge as an alternative treatment to address antimicrobial resistance.

DECLARATIONS

Acknowledgement

The authors would like to express their sincere gratitude to Centre of Excellence in Molecular Biology, University of The Punjab for continuous support, resources, and research facilities that made this study possible.

Funding

This study was supported by institutional budget. No external funding was obtained for this study.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Abuladze, T., Li, M., Menetrez, M.Y., Dean, T., Senecal, A. and Sulakvelidze, A., 2008. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*, **74**: 6230–6238. <https://doi.org/10.1128/AEM.01465-08>
- Allocati, N., Masulli, M., Alexeyev, M.F. and Di Ilio, C., 2013. *Escherichia coli* in Europe: An overview. *Int. J. Environ. Res. Publ. Hlth.*, **10**: 6235–6254. <https://doi.org/10.3390/ijerph10126235>
- Bassetti, M., Poulakou, G., Ruppe, E., Bouza, E., Van Hal, S.J. and Brink, A., 2017. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: A visionary approach. *Intens. Care Med.*, **43**: 1464–1475. <https://doi.org/10.1007/s00134-017-4878-x>
- Bilal, H., Khan, M.N., Rehman, T., Hameed, M.F. and Yang, X., 2021. Antibiotic resistance in Pakistan: A systematic review of past decade. *BMC Infect. Dis.*, **21**: 244. <https://doi.org/10.1186/s12879-021-05906-1>
- Born, Y., Knecht, L.E., Eigenmann, M., Bolliger, M., Klumpp, J. and Fieseler, L., 2019. A major-capsid-protein-based multiplex PCR assay for rapid identification of selected virulent bacteriophage types. *Arch. Virol.*, **164**: 819–830. <https://doi.org/10.1007/s00705-019-04148-6>
- Carey-Smith, G.V., Billington, C., Cornelius, A.J., Hudson, J.A. and Heinemann, J.A., 2006. Isolation and characterization of bacteriophages infecting *Salmonella* spp. *FEMS Microbiol. Lett.*, **258**: 182–186. <https://doi.org/10.1111/j.1574-6968.2006.00217.x>
- Chung, K.M., Liao, X.L. and Tang, S.S., 2023. Bacteriophages and their host range in multidrug-resistant bacterial disease treatment. *Pharmaceuticals*, **16**: 1467. <https://doi.org/10.3390/ph16101467>
- da Silva, G.J. and Mendonça, N., 2012. Association between antimicrobial resistance and virulence in *Escherichia coli*. *Virulence*, **3**: 18–28. <https://doi.org/10.4161/viru.3.1.18382>
- El-Baz, R., Said, H.S., Abdelmegeed, E.S. and Barwa, R., 2022. Characterization of virulence determinants and phylogenetic background of multiple and extensively drug resistant *Escherichia coli* isolated from different clinical sources in Egypt. *Appl. Microbiol. Biotechnol.*, **106**: 1279–1298. <https://doi.org/10.1007/s00253-021-11740-x>
- Galindo-Méndez, M., 2020. Antimicrobial resistance in *Escherichia coli*. In: *E. coli infections importance of early diagnosis and efficient treatment*. Intech Open. <https://doi.org/10.5772/intechopen.93115>
- Jamalludeen, N., Johnson, R.P., Friendship, R., Kropinski, A.M., Lingohr, E.J. and Gyles, C.L., 2007. Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic *Escherichia coli*. *Vet. Microbiol.*, **124**: 47–57. <https://doi.org/10.1016/j.vetmic.2007.03.028>
- Jurczak-Kurek, A., Gąsior, T., Nejman-Faleńczyk, B., Bloch, S., Dydecka, A., Topka, G., Necel, A., Jakubowska-Deredas, M., Narajczyk, M., Richert, M., Mieszkowska, A., Wróbel, B., Węgrzyn, G. and Węgrzyn, A., 2016. Biodiversity of bacteriophages: Morphological and biological properties of a large group of phages isolated from urban sewage. *Sci. Rep.*, **6**: 34338. <https://doi.org/10.1038/srep34338>
- Hayat, K., Fatima, N., Umer, M.F., Khan, F.U., Khan, F.U., Najeeb, ZuR., Ghaffari, M.A., Raza, S.Q., Liu, W., Chen, C. and Fang, Y., 2022. Understanding of future prescribers about antimicrobial resistance and their preparedness towards antimicrobial stewardship activities in Pakistan: Findings and implications. *Front. Pharmacol.*, **13**: 771083. <https://doi.org/10.3389/fphar.2022.771083>
- Li, X., Xu, S., Tan, L., Yan, X., Wang, X., Li, Z., Chen, L. and Zhang, W., 2025. Characterization of a novel phage vB_EcoP_P64441 and its potential role in controlling uropathogenic *Escherichia coli* (UPEC) and biofilms formation. *Virology*, **609**: 110570. <https://doi.org/10.1016/j.virol.2025.110570>

- Mishra, V., Bankar, N., Tiwade, Y. and Ugemuge, S. 2024. How phage therapy works, its advantages and disadvantages: Mini review. *J. Pure appl. Microbiol.*, **18**: 177–184. <https://doi.org/10.22207/JPAM.18.1.49>
- Mohamed, A.A., El-Zayat, E.M. and El-Shibiny, A., 2025. Efficacy of phage vB_Ps_ZCPS13 in controlling Pan-drug-resistant *Pseudomonas aeruginosa* from urinary tract infections (UTIs) and eradicating biofilms from urinary catheters. *Virol. J.*, **22**: 236. <https://doi.org/10.1186/s12985-025-02848-x>
- Nawaz, A., Zafar, S., Alessa, A.H., Khalid, N.A., Shahzadi, M., Majid, A., Badshah, M., Shah, A.A. and Khan, S., 2024. Characterization of ES10 lytic bacteriophage isolated from hospital waste against multidrug-resistant uropathogenic *E. coli*. *Front. Microbiol.*, **15**. <https://doi.org/10.3389/fmicb.2024.1320974>
- Qu, J., Huang, Y. and Lv, X., 2019. Crisis of antimicrobial resistance in China: Now and the future. *Front. Microbiol.*, **10**. <https://doi.org/10.3389/fmicb.2019.02240>
- Saeed, D.K., Farooqi, J., Shakoor, S. and Hasan, R., 2021. Antimicrobial resistance among glass priority pathogens from Pakistan: 2006–2018. *BMC Infect. Dis.*, **21**: 1231. <https://doi.org/10.1186/s12879-021-06795-0>
- Subramanian, A., 2024. Emerging roles of bacteriophage-based therapeutics in combating antibiotic resistance. *Front. Microbiol.*, **15**. <https://doi.org/10.3389/fmicb.2024.1384164>
- Taati Moghadam, M., Khoshbayan, A., Chegini, Z., Farahani, I. and Shariati, A., 2020. Bacteriophages, a new therapeutic solution for inhibiting multidrug-resistant bacteria causing wound infection: Lesson from animal models and clinical trials. *Drug Design, Dev. Ther.*, **14**: 1867–1883. <https://doi.org/10.2147/DDDT.S251171>
- Tang, K.W.K., Millar, B.C. and Moore, J.E., 2023. Antimicrobial resistance (AMR). *Br. J. biomed. Sci.*, **80**. <https://doi.org/10.3389/bjbs.2023.11387>
- Walsh, T.R., Gales, A.C., Laxminarayan, R. and Dodd, P.C., 2023. Antimicrobial resistance: Addressing a global threat to humanity. *PLoS Med.*, **20**: e1004264. <https://doi.org/10.1371/journal.pmed.1004264>
- Yang, L., Liu, Y., Wu, H., Song, Z., Høiby, N., Molin, S. and Givskov, M., 2012. Combating biofilms. *FEMS Immunol. Med. Microbiol.*, **65**: 146–157. <https://doi.org/10.1111/j.1574-695X.2011.00858.x>
- Yang, X., Yang, J., Wang, L., Ran, B., Jia, Y., Zhang, L., Yang, G., Shao, H. and Jiang, X., 2017. Pharmaceutical intermediate-modified gold nanoparticles: Against multidrug-resistant bacteria and wound-healing application via an electrospun scaffold. *ACS Nano*, **11**: 5737–5745. <https://doi.org/10.1021/acsnano.7b01240>