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HOST RANGE DIVERSITY AND *ESCHERICHIA COLI* GROWTH REDUCTION EFFICACY OF NOVEL BACTERIOPHAGES

Zia Ullah[1], Mahnoor Abbasi[2], Ajmal Khan[3], Razia Misbah[4], Bilal Gohar[5], Muhammad Kamil Khan[6], Qarib Ullah[7], Samreen Khan[8], Sara Naeem[*9], Iftikhar Ullah[*10]

[1,2]*Abbottabad University of Science and Technology*
[3]*Department of Zoology, University of Swat, Main Campus, Charbagh, Khyber Pakhtunkhwa, Pakistan*
[4,6,10*]*Hazara University Mansehra,*
[5]*University of Peshawar, Center of Biotechnology and Microbiology,*
[7]*Department of Zoology, Shaheed Benazir Bhutto University Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan,*
[8]*Pakistan Institute of Engineering and Applied Science, Nilore, Islamabad*
[* 9]*Università Degli Studi Della Campania "Luigi Vanvitelli*

[1]ziahamdard13@gmail.com, [2]abbasimahnoor520@gmail.com,[3]ajmalkhan399@hotmail.com, [4]misbahmalik00@gmail.com,[5]bilalgohar489@gmail.com,[6]biologykame9@gmail.com, , [7]qaribullah.naderkhel1122@gmail.com, [8]samreen23@pieas.edu.pk,[9]sara.naeem@unicampania.it ,[10]iftikharullah485@gmail.com

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Corresponding Author: *
Iftikhar Ullah,
Sara Naeem

Abstract

The emergence of multidrug-resistant (MDR) Escherichia coli has created an urgent need for alternative antimicrobial treatments. Bacteriophages are bacterial viruses with high host specificity and potent lytic activity, making them promising candidates for biocontrol and therapeutic applications. This study aimed to isolate, characterize, and evaluate lytic bacteriophages against MDR E. coli clinical isolates, with an emphasis on host-range determination and potential to reduce bacterial growth. Sewage samples were collected and processed for phage isolation using the agar overlay technique. Two lytic bacteriophages, designated EcoPhage-1 and EcoPhage-2, were isolated against MDR E. coli. Host range analysis was performed using spot assays against 10 E. coli isolates and several non-target bacterial species. Bacterial growth reduction assays were conducted at different multiplicities of infection (MOIs) over 24 h, with spectrophotometric analysis. EcoPhage-1 lysed 80% of the tested E. coli isolates, while EcoPhage-2 lysed 60%. Neither phage showed lytic activity against non-E. coli species, indicating high specificity. Both phages remained stable at pH 4–9 and temperatures up to 50 °C. Growth reduction assays demonstrated significant suppression of bacterial growth, particularly at MOIs of 1 and 10, with complete inhibition observed for up to 18 h. The results suggest that these bacteriophages possess strong antibacterial potential and may serve as alternative therapeutic agents against MDR E. coli infections.

INTRODUCTION

E. coli is a Gram-negative facultative anaerobic bacterium commonly found in the intestinal tract of humans and animals. Although many strains are harmless commensals, pathogenic strains can cause severe intestinal and extraintestinal infections, including urinary tract infections, septicemia, neonatal meningitis, and gastroenteritis (Basavaraju & Gunashree, 2022). The increasing prevalence of multidrug-resistant (MDR) *E. coli* strains has significantly reduced the effectiveness of conventional antibiotics, creating a global public health challenge (Ullah et al., 2025).

Bacteriophages (phages) are viruses that specifically infect bacteria. They are considered the most abundant biological entities on Earth and have gained renewed attention as alternatives to antibiotics for their specificity, self-replication, and ability to lyse bacterial cells (Kasman & Porter, 2022). Unlike broad-spectrum antibiotics, phages target specific bacterial hosts without disturbing normal microbiota (Ullah et al., 2025).

Phage therapy, the use of bacteriophages to treat bacterial infections, has a history dating back to the early 20th century. Bacteriophages, or simply phages, are viruses that specifically infect bacteria. They were first discovered by Frederick Twort in 1915 and independently by Félix d'Hérelle in 1917, who observed their potential to eliminate bacterial cultures (Summers, 2012). Despite their early promise, the advent of antibiotics in the 1940s led to a decline in phage research and application in the Western world. However, the rise of antibiotic-resistant bacteria has rekindled interest in phage therapy as a viable alternative or complement to traditional antibiotics (Hesse & Adhya, 2019). The global health crisis posed by antibiotic resistance has prompted urgent calls for novel antimicrobial strategies (Brüssow, 2024). Multidrug-resistant (MDR) bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae represent significant challenges to healthcare systems worldwide. Phage therapy offers a targeted approach to combat these pathogens. Unlike broad-spectrum antibiotics, phages are highly specific to their bacterial hosts, thereby minimizing the impact on beneficial microbiota and reducing the risk of collateral damage (Fong et al., 2021).

Host range is a critical characteristic determining the therapeutic applicability of bacteriophages (Subramanian, 2024). Phages with broader host ranges can target multiple bacterial strains, whereas narrow host-range phages offer highly specific antibacterial action (Hyman, 2019). Additionally, the ability of phages to reduce bacterial growth over time is essential for evaluating their therapeutic potential (Khan & Waqar).

The present study aimed to isolate lytic bacteriophages against MDR *E. coli*, determine their host range, evaluate their potential to reduce bacterial growth, and assess their thermal and pH stability.

Materials and Methods

Bacterial Isolates

Ten clinical isolates of MDR *E. coli* were obtained from urine swab samples collected from hospitalized patients. Additional bacterial species, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*, were used for host-specificity testing (Jalil & Al Atbee, 2022).

Identification of isolates was performed using standard microbiological procedures, including Gram staining, biochemical tests, and API 20E identification kits. Antibiotic susceptibility was determined by the Kirby–Bauer disk diffusion method according to CLSI guidelines (Fatima et al., 2020).

Collection of Environmental Samples

Sewage water samples were collected from municipal drainage systems and hospital wastewater outlets using sterile containers. Samples were transported to the laboratory at 4°C and processed within 6 h (Al Salah et al., 2020).

Isolation of Bacteriophages

Phages were isolated using the enrichment method. Briefly, a 10 mL sewage sample was centrifuged at 10,000 rpm for 10 min and filtered through a 0.22 µm membrane filter. Five milliliters of filtrate were mixed with 5 mL double-strength LB broth and 1 mL overnight culture of MDR *E. coli*. The mixture was incubated at 37°C for 24 h with shaking (Kenney & Gómez-Duarte, 2024). Following incubation, the culture was centrifuged and filtered again. Presence of phages was confirmed by the double-layer agar overlay method. Clear plaques were picked and purified through three successive plaque isolations (Abebe et al., 2025).

Phage Propagation and Titer Determination

Purified phages were propagated using exponentially growing host bacteria. Phage titers were determined by plaque assay and expressed as plaque-forming units per milliliter (PFU/mL) (Kosznik-Kwaśnicka et al., 2023).

Host Range Determination

Host range analysis was conducted using spot assays. Bacterial lawns were prepared by mixing 100 µL of bacterial culture with molten soft agar and overlaying the mixture onto LB agar plates. Ten microliters of phage suspension were spotted onto the bacterial lawn and incubated overnight at 37°C (Paranos et al., 2024).

Clear plaques were interpreted as positive lysis, turbid zones as partial lysis, and the absence of plaques as resistance.

Bacterial Growth Reduction Assay

Growth inhibition assays were performed in LB broth using different multiplicities of infection (MOI 0.1, 1, and 10). Overnight cultures of *E. coli* were adjusted to OD600 = 0.1. Phages were added accordingly and incubated at 37°C.

Optical density measurements at 600 nm were recorded every 2 h for 24 h using a spectrophotometer. Control tubes without phage were included (Seo et al., 2016).

Thermal and pH Stability

Phage suspensions were incubated at temperatures ranging from 4°C to 80°C for 1 h. For pH stability, phages were incubated in SM buffer adjusted to pH 2–11 for 24 h. Residual titers were determined by plaque assay (Pradeep et al., 2022).

Results

Isolation and Morphology of Bacteriophages

Two distinct lytic bacteriophages, EcoPhage-1 and EcoPhage-2, were isolated from sewage samples and were active against Eco1. Plaques produced by EcoPhage-1 were large and clear (3–5 mm), whereas EcoPhage-2 produced smaller, clear plaques (1–2 mm).

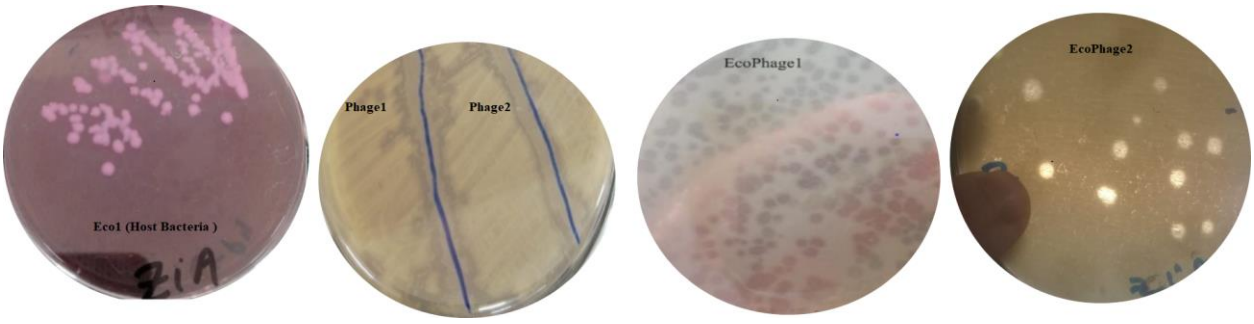

Figure 1: Isolated Bacteriophages against Host Bacteria Eco1

Host Range Analysis

EcoPhage-1 lysed 8 out of 10 MDR *E. coli* isolates (80%), while EcoPhage-2 lysed 6 isolates (60%). Neither phage infected *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, or *S. aureus*, indicating high specificity toward *E. coli*.

Table 1. Host Range of Isolated Phages

Bacterial isolate	EcoPhage-1	EcoPhage-2
E. coli EC1	+	+
E. coli EC2	+	+
E. coli EC3	+	-
E. coli EC4	+	+
E. coli EC5	+	-
E. coli EC6	+	+
E. coli EC7	+	+
E. coli EC8	-	-
E. coli EC9	+	-
E. coli EC10	+	+
K. pneumoniae	-	-
P. aeruginosa	-	-
S. aureus	-	-

(+: lysis observed; -: no lysis)

Bacterial Growth Reduction

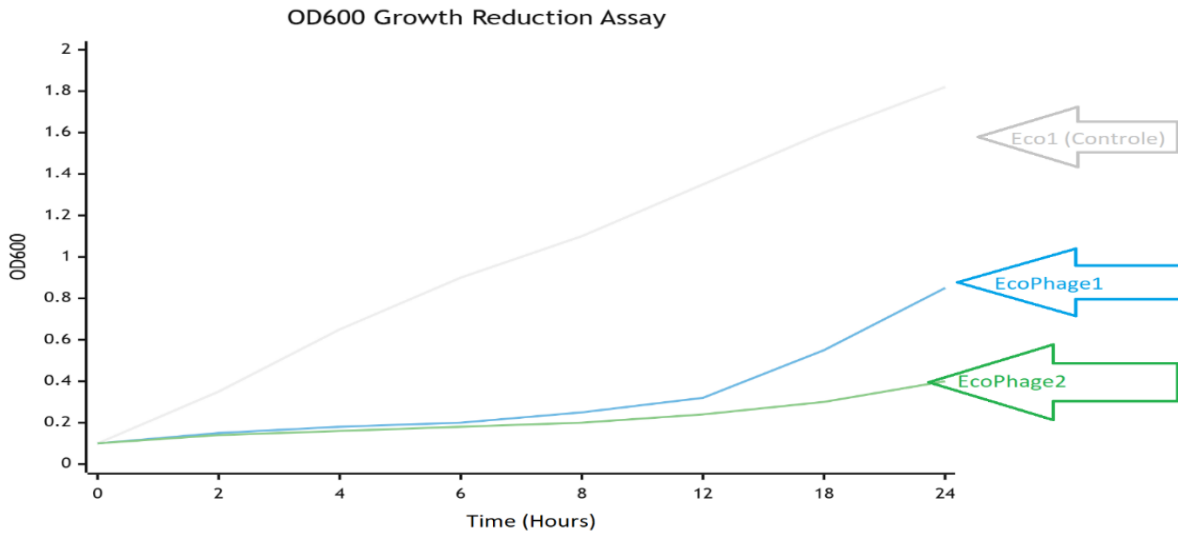

Figure 2: Bacterial growth reduction essay by EcoPhage1 and EcoPhage2

Both phages significantly inhibited bacterial growth compared with controls. At MOI 1 and 10, EcoPhage-1 maintained suppression for 18 h, while EcoPhage-2 showed inhibition up to 24 h.

Thermal and pH Stability : Both phages remained stable between 4°C and 50°C. A significant reduction in viability occurred above 60°C, and complete inactivation was observed at 80°C.

The phages retained activity within pH 4–9 but showed reduced infectivity at extreme acidic and alkaline conditions.

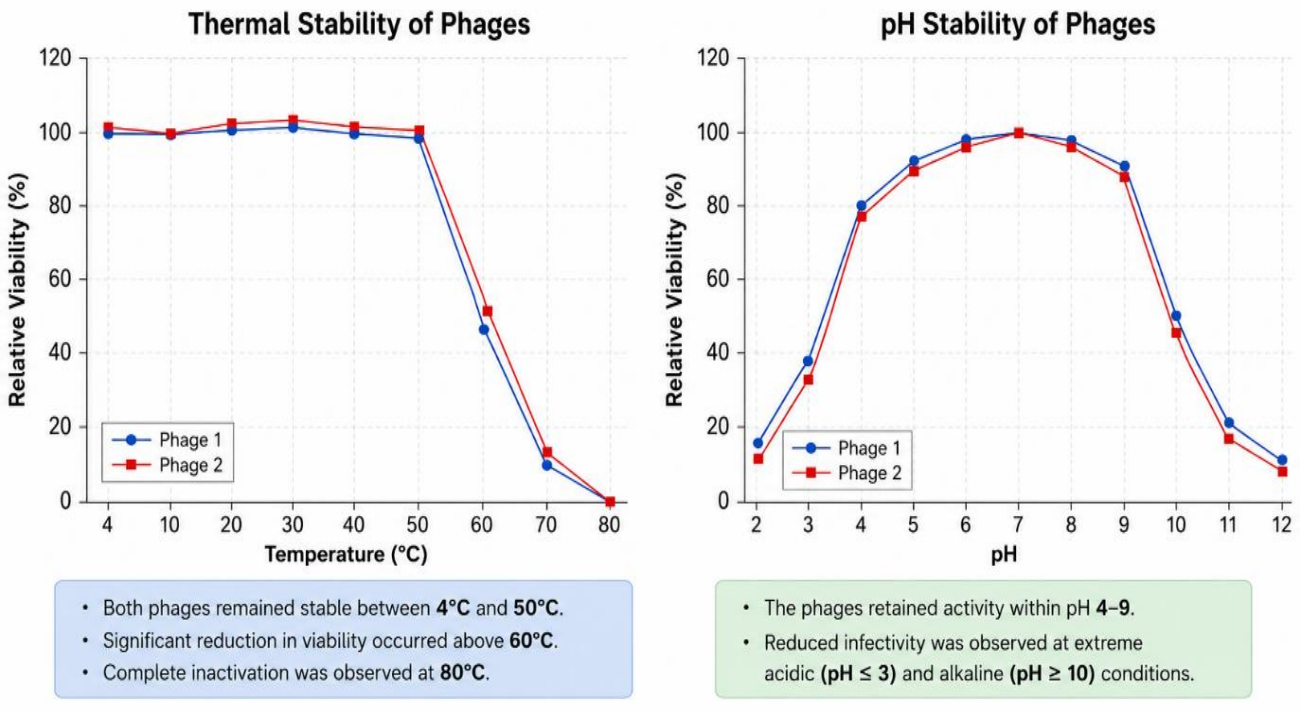

Figure 3: pH and thermal stability of EcoPhage1 and EcoPhage2

Discussion

The current study demonstrated successful isolation and characterization of lytic bacteriophages active against MDR *E. coli*. The isolated phages exhibited clear plaques, indicating strong lytic activity consistent with previous studies.

Host range analysis revealed that EcoPhage-1 possessed broader lytic activity than EcoPhage-2. Similar findings have been reported (Göller et al., 2021)where certain phages infect a wide spectrum of *E. coli* strains while remaining species-specific. Such specificity minimizes disruption of beneficial microbiota and supports their therapeutic potential.

Growth reduction assays indicated substantial inhibition of bacterial growth at higher MOIs. EcoPhage-2 demonstrated prolonged bacterial suppression up to 24 h, suggesting superior lytic efficiency. Comparable observations have been documented in previous studies (Manohar et al., 2018) evaluating phage efficacy against MDR bacteria.

Thermal and pH stability are important for phage formulation and storage. The isolated phages maintained viability under moderate environmental conditions, similar to other reported lytic *E. coli* phages.

Both phages Ecophage1 and Ecophage2 remained stable between 4°C and 50°C. A significant reduction in viability occurred above 60°C, and complete inactivation was observed at 80°C. The phages retained activity within pH 4–9 but showed reduced infectivity at extreme acidic and alkaline conditions. A similar study was conducted by (Yuan et al., 2021) through stability tests DY1 was very stable at temperatures ranging from 20 to 50 °C and pH levels from 5 to 11. The study supports the growing evidence that bacteriophages can serve as promising alternatives to antibiotics for MDR bacterial infections. However, additional genomic characterization, animal studies, and clinical trials are required before therapeutic application.

Conclusion

The isolated bacteriophages EcoPhage-1 and EcoPhage-2 demonstrated effective lytic activity against MDR *E. coli* isolates, with strong growth-reducing capability and high host specificity. Both

phages remained stable under a range of temperature and pH conditions. These findings highlight their potential application in phage therapy and bacterial biocontrol strategies against MDR *E. coli* infections.

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