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Isolation and Characterization of Bacteriophage Targeting *E. Coli*: A Study on Host Interaction

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ABSTRACT

Bacteriophages, viruses that specifically infect bacteria, have emerged as promising alternatives to antibiotics in combating multidrug-resistant bacterial infections. This study focuses on the isolation and characterization of bacteriophages targeting *Escherichia coli* (E. coli), a pathogen responsible for various infections and a major contributor to antibiotic resistance. The primary objective was to identify and analyze bacteriophages with lytic activity against E. coli, assessing their host interaction dynamics and therapeutic potential. During this study Sewage sample was taken from hospital trash, while clinical samples of *E. coli* was obtained from the Ayub Teaching Hospital. One new native bacteriophage against *E.coli* was identified and described, they were assigned the scientific name ECP1. Since ECP1 was able to lyse two out of three but was unable to infect bacteria from other species, it was determined that the phage had a particular host range for *E. coli*. The separated phage demonstrated viability at pH values between 3 and 9 and up to 50°C. ECP1 demonstrated superior bacterial reduction capabilities by preventing and reducing the initial bacterial inoculum count during 24 hours of observation. ECP1 may be viable options for treating *E. coli*, given their superior bacterial growth reduction, phage titer, pH, thermal stability, and host range. This study underscores the significance of bacteriophage therapy as a viable strategy to address antibiotic resistance. The findings pave the way for further research into phage-based treatments, offering a sustainable and targeted approach to bacterial infections. Future investigations will focus on optimizing phage formulations and assessing their efficacy in clinical settings.

INTRODUCTION

A gram-negative bacillus identified as *Escherichia coli* (*E. coli*) is the cause of several diarrheal diseases, such as dysentery and traveler's diarrhea (Ullah et al., 2025). The most prevalent pathogen that causes simple cystitis is *E. coli,* which also causes bacteremia, pneumonia, and infections of the abdomen, such as spontaneous bacterial peritonitis. *E. coli*-related illnesses place a heavy strain on individuals and the healthcare system, thus early detection and effective treatment are essential (Khalil *et al.,* 2018). This exercise goes over the many types of *E. coli* that may infect humans, demonstrates how to recognize and treat these infections, and emphasizes the need of the interprofessional team in treating patients with this sickness (Liu *et al.,* 2018). In addition to being a component of commensal gut flora, *E. coli* may be observed on hospital and long-term care floors. In this environment, *E. coli*, the most prevalent gram-negative bacterium in the human gastrointestinal system, is not virulent. But when *E. coli* is discovered outside of the digestive system, it can lead to a number of illnesses, including pneumonia, bacteremia, peritonitis, and urinary tract infections (UTI) (Palmela *et al.,* 2018).

The human pathotypes of diarrheagenic *E. coli* (DEC) are distinguished from non-pathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) by virulence factors in *E. coli* genomes and phenotypic characteristics. Antibiotics have prevented multiple fatalities and decreased the frequency of illnesses among millions of individuals worldwide in recent years (Gomes *et al.,* 2016). For the majority of patients, antibiotics are not advised as the initial line of therapy for diarrheal illness brought on by *E. coli* because of the negative side effects and correlation with antibiotic resistance. People with severe illnesses (e.g., more than six stools per day, fever, dehydration requiring hospitalization, diarrhea lasting more than seven days, or bloody diarrhea) may benefit from antibiotics (Blaser, 2011). The International Society

of Travel Medicine (ISTM) and the Infectious Diseases Society of America (IDSA) presently prescribe ciprofloxacin, azithromycin, and rifaximin to treat diarrheal illness caused by *E. coli* (Pawłowska & Sobieszczańska, 2017). However, the increase of drug-resistant strains in recent years has called into doubt the spectacular advantages of antimicrobials in lowering rates of morbidity and death. This issue is particularly common in underdeveloped nations for a number of reasons. A global health concern is the establishment and quick spread of carbapenem and extended-spectrum cephalosporin resistance in Enterobacteriaceae. Furthermore, *E. Coli* that is resistant to antibiotics is growing and posing a serious risk to human health worldwide (Poirel *et al.,* 2018).

Over the past few decades, multidrug-resistant *E. coli* has been seen to arise in a number of countries. Concern over treating *E. coli* illness is growing due to the growing resistance to cephalosporins, particularly the concurrent increase in the prevalence of multidrug-resistant E. coli (Allocati *et al.,* 2013). Plasmid-borne extended-spectrum β-lactamases (ESBLs) are the main mechanism by which *E. coli* develops resistance to β-lactam antibiotics. ESBL-producing microbes have proliferated globally since the initial report in the early 1980s. ESBL genes are often transmitted on transferable plasmids that encode resistance genes. Multidrug resistant (MDR) infections are the result of commensal or fecal isolates acquiring these resistant genes (Szmolka & Nagy, 2013).

Phages that target these bacteria may be a viable therapeutic option for the treatment of ICD and could replace antibiotics(Ullah et al.). Bacteriophages, or simply "phages," are viruses that infect bacteria. When it comes to their host bacterial strains, lytic phages have strong bactericidal action (Abdelrahman et al., 2022). The lytic cycle occurs when the phage infects a cell, replicates utilizing the cell's translation and replication machinery, and then lyses the cell to release fresh phage particles into the surrounding environment. When phage concentrations are administered in enormous amounts, "lysis from without" may also happen. The fact that phages only target bacteria and cannot infect humans or other eukaryotic cells makes them extremely selective. Even within bacterial taxa, phages often only lyse strains or a minority of strains within the bacterial species, which allows for focused bacterial treatment and stands in contrast to broad-spectrum antibiotics (Dissanayake *et al.,* 2019).

MATERIALS AND METHOD

Bacterial Culture

The reference strain of *E. coli* were gathered from Ayub teaching hospital Abbottabad. For conformation on Fresh nutrient agar and the MacConkey agar plate were streaked with bacterial samples, and they were then incubated for the whole night at 37°C. The bacterium was identified using microscopy and Gram staining.

Isolation of Bacteriophages from Sewage

Sewage samples were taken from hospital waste ayub teaching hospital sewage. To isolate bacteriophages, the samples were taken to Abbottabad University Microbiology Laboratory. A previously described methodology was used to extract bacteriophages from a sewage sample (Asif *et al.,* 2018).

Isolation of Bacteriophages from Sewage

The sewage samples were centrifuged for 10 minutes at 15,000 rpm after being agitated for two minutes. For sample processing 10 mL of sterile 5 X nutrient broth, 40 mL sewage sample and one microliters of an overnight culture of *E. coli* were added as an inoculant in flask. The flask was placed in shaking incubator (120 rpm) incubated at 37°C for the overnight. The flask's contents were centrifuged for ten minutes at 10,000 rpm after incubation. The clear supernatant was collected in a fresh, sterile falcon tube and stored at 4°C. A spot test was used to detect whether bacteriophages were present in the filtrate or not (Sarowska *et al.,* 2019).

Detection of Bacteriophages in the Filtrate

After bacteriophage enrichment, existence of an *E. coli* phage was detected by a spot test following: 100 μL of an overnight-grown *E. coli* culture is spread on a nutrient agar plate for the spot test. After applying 7 μL of the filtrate contain suspect bacteriophage, the plates were allowed to dry for nearly 10 minutes. The plates were then incubated for the overnight at 37°C. Next, the plates were examined to see if bacteriophages had produced a definite lysis zone. The presence of a particular bacteriophage is indicated by the presence of the clear zone, or plaque (Ullah et al., 2023) .

Purification of Bacteriophages using a Double Layer Agar Assay

Double layer agar overlay method was used to quantify and purify bacteriophage from the lysate (filtrate that caused lysis) (Alvi *et al.,* 2020). Prior to the experiment, 100 milliliters of semisolid nutritional agar were autoclaved and placed in a conical flask in a water bath at 48°C. The lysate (1:9) was first serially diluted in microtubes up to 1/1012 using 900 μL of nutrient broths. After that, 100 μL of a fresh *E. coli* culture was added to the selected dilutions. To allow the phages to attach to the bacterial cells, the phage lysate and bacterial culture were incubated for five minutes. Following the addition of 3 mL of semisolid agar to nutritional agar plates containing the selected dilutions, the plates were incubated for the whole night at 37°C. The mixture and semisolid agar on the plate were spread out using a swirling motion. On the plates, we searched for countable plaques (30 pfu–200 pfu). Plaque forming units (pfu) on the selected plate were counted (Vinogradov *et al.,* 2002).

For phage purification, plates with distinctive plaque were selected. Phage was extracted off a plaque by lightly touching its surface with a sterile micropipette tip. The tip was put in a test tube containing 1 mL of fresh E. coli strain and 10 mL of nutrient broth in order to propagate the phage. Following a 24-hour culture at 37°C, the plaque became apparent and was purified. Up to 10 repeat of the purification procedure were performed. The titer of lysate solutions was calculated using the following formula.

Titter (*pfu*/mL) = plaque (*pfu*) dilution number x phage (mL) volume added to plate.

Characterization of Bacteriophages Host Range Determination

Spot testing was used to determine the phage host range. Following the addition of 100 µl of each *E. coli* bacterial culture, five microliters of each phage dilution were spotted in the middle of the plate. Plaques were observed on the plates at the appropriate titer of phage dilutions; thus, the plates were incubated at 37 °C for overnight for the identification the plaque (Khan Mirzaei & Nilsson, 2015).

Determination of Thermal Stability of Bacteriophages

The temperature stability of the bacteriophages is important since it provides an indication for phage storage and transportation. For an hour, aliquots of known titers of *E. coli* strains were incubated at 4, 25, 37, 50, and 60°C to evaluate the isolated bacteriophages' thermal stability. The bacteriophage titer after incubation was measured using the double layer agar overlay method (Yuan *et al.,* 2021).

Determination of pH Stability of Bacteriophages

The pH of the medium was adjusted using HCl and NaOH. The pH was measured using a pH paper. Aliquots of known phage titers were incubated at pH 2–10 for an hour in order to determine the pH stability of the isolated bacteriophages. After incubation, the bacteriophage titer was measured using the double layer agar overlay method (Yuan *et al.,* 2021).

Long Term Storage Stability

Bacteriophages were kept in nutrient broth at 4°C, 25°C, 37°C, 50°C and 60°C for three months. Before and after storage, the titer of each bacteriophage was measured using the double layer agar technique (Tovkach *et al.,* 2012).

Bacterial Growth Reduction

In vitro phage treatment is another name for phage-mediated inhibition of bacterial growth. This experiment evaluated the ability of bacteriophages to suppress bacterial growth. The decrease in bacterial growth caused by the bacteriophages was tested using a previously established method. Eight-hour-old *E. coli* cultures were introduced to three flasks with 50 mL of broth. The first flask was used as a control, whereas the second and third flasks, respectively, contained bacteria and bacteriophages. To determine the growth decrease, optical density at 600 nm (O.D. 600) was measured. The reduced growth was contrasted with the control using a graph. The independent experiment was carried out three times (Du *et al.,* 2009).

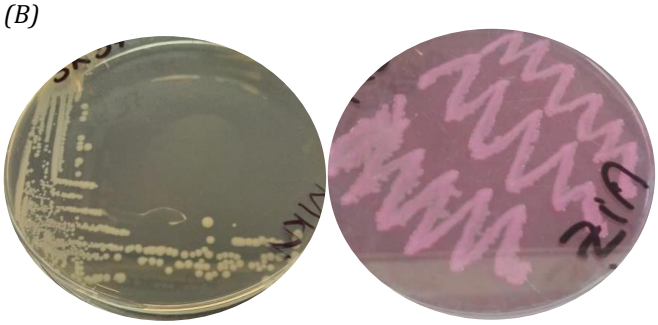
RESULTS

Bacterial growth on different media

E.coli is a rod-shaped, facultatively anaerobic, encapsulated, lactose-fermenting, gram-negative bacteria. Lactose fermentation caused the *E.coli* colonies to become pink on MacConkey agar medium. On nutrient agar, however, colonies were large and pale. Colonies were mucoid on both of these media.

Figure 1

Morphology of E. Coli on Nutrient Agar (A) and Macconkey (B)



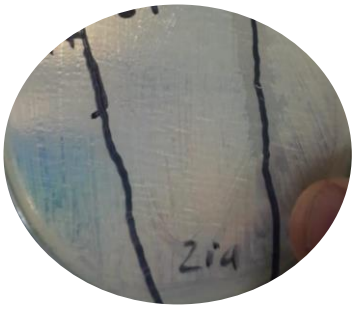
A B

Spot test for the detection of *E.colis* pecific bacteriophages

Bacteriophages against *E.colis* strain EC9 were detected in one of three sewage samples. ECP1 was the name of the bacteriophage that was acquired from DHQ Shangla ECP1 phage have high activity against *E.coli* strain (EC9) show in (figure2).

Figure 2

Detection of Bacteriophages through Spot Test ECP1

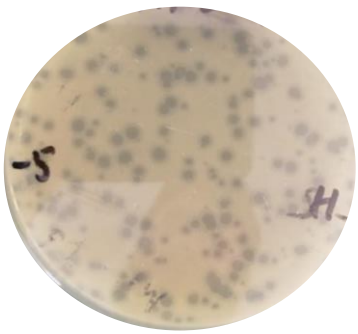


Isolated Bacteriophages Produced Clear Transparent Plaques

On a double layer agar plate, the isolated phage produced a circular, clear plaque that was antagonistic to *Ecoli*. The plaque is composed of two circular layers, with an outer circle around the inner, completely transparent center. The existence of this hazy layer surrounding plaque is a sign that bacteriophages are producing the depolymerase enzyme. ECP1 phage had a diameter of 2 mm. The halo surrounding the plaque indicates that the bacterial host cell was decapsulated by soluble enzymes such depolymerase, which was produced by phages ECP1.

Figure 3

Purified Bacteriophage of ECP1

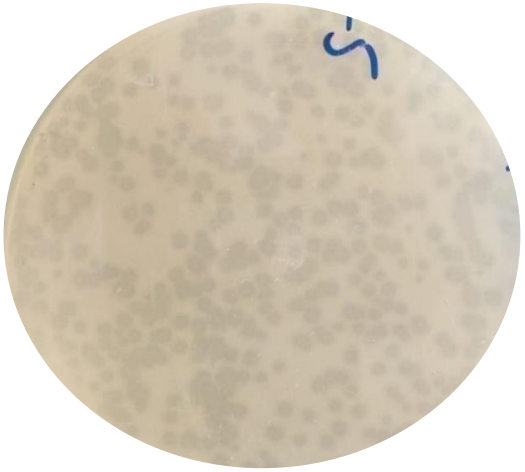


Isolated Phages Have High Titer

The isolated phages' lytic behavior and promise as a therapeutic agent are demonstrated by the translucent, clear plaques. ECP1 titers was reported to be 4×10^9 pfu/mL, following a 24-hour phage propagation period in liquid culture.

Figure 4

Titer of Purified Bacteriophage of ECP1



Isolated Bacteriophages were Found to have Narrow Spectrum

It was discovered that the isolated *E.coli* ECP1 phages was very strain-specific. While no infectivity was seen for the other examined genera, ECP1 bacteriophage was able to infect and create a lytic zone against isolates of *E.coli*. According to the findings of the host range specificity test, ECP1 was able to infect 2 out of 3 *E.* coli but not bacteria from other genera (*E. coli*, *S. typhi*, *P. aeruginosa*).

Table 1

Host Range Spectrum of ECP1 Bacteriophages against Various Bacterial Cultures

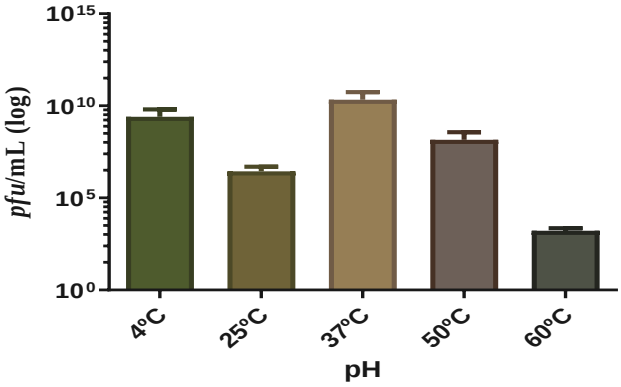
Bacterial culture	Spot test ECP1	Source
E.coli 1	+	Clinical source
E.coli 2	+	Clinical source
E.coli 3	-	Clinical source
K. pneumoniae 4	-	Clinical source
P. Aeruginosa 5	-	Clinical source
Salmoniella spp 6	-	Clinical source

The Isolated Bacteriophages were Found Thermally Stable

The stability of bacteriophages is significantly impacted by temperature. It impacts adhesion, penetration, and proliferation in all aspects of phage replication. It was discovered that the ECP1 phage remained stable at 50ºC without experiencing any titer changes. For ECP1, there was no drop in phage titer at 4 or 37ºC, however there was a 1-fold drop at 25 and 50ºC (Figure 4). At 60ºC, the ECP1 titer decreased 7 times (Figure 5). Since ECP1 phage was stable at 25°C and 37°C, respectively, and since the average human body temperature is 37°C, these phages may be readily used if they were to be utilized as a therapeutic agent. Moreover, these phages may be transported without the need for specific heating conditions.

Figure 5

Thermal stability of ECP1 bacteriophage at different temperatures

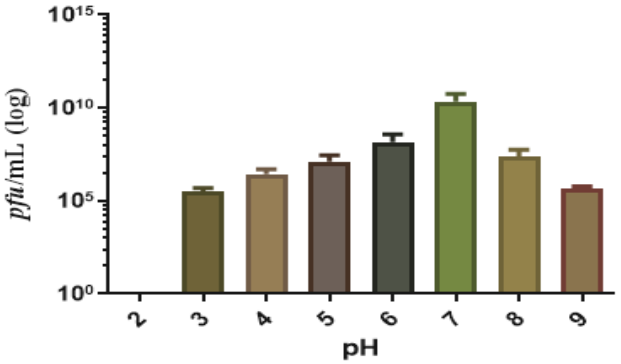


PH stability of bacteriophages

The environment's acidity and alkalinity have a crucial role in phage stability. After an hour, the ECP1phage showed resistance to the pH range of 3.0 to 9.0. It was discovered that the ideal pH range for phage ECP1 was 4–9. Additionally, at pH 9, 5, and 4, a drop of 1, 2, and 3 log was noted, respectively (Figure 6). Very few phages can withstand such a wide pH range.

Figure 6

Effect of Various pH on the Viability 0f ECP1 Bacteriophage 4°C was found best for Storage



After three months of storage, the phage ECP1 titer was determined to be steady. For phage ECP1, there was no titer drop at 4°C, 37°C but at 25°C and there was a decrease of 1log. (Table 2).

Table 2

Storage Stability of Bacteriophage at Different Temperature

Phage	Titer Before Storage	Titer After Storage		
		4°C	25°C	37°C
HI3	8×10^9 *pfu*/mL	5×10^9	7×10^8	4×10^9

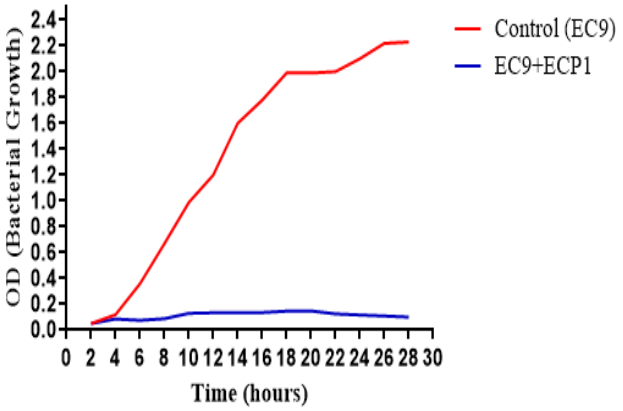
ECP1 Phage Reduced *E.coli* Growth

The bacterial growth reduction test was used to evaluate the antibacterial activity of the ECP1. Using a spectrophotometer to record the OD600 every two hours for the following twenty-four hours, the capacity of phage to inhibit bacterial growth was tracked and contrasted with the growth of the control (Figure 9). Until 24 hours of observation, inhibited and reduced the original bacterial inoculum, demonstrating remarkable bacterial reduction

abilities (Figure 10). Since ECP1 phage was extremely uncommon and no resistant mutants appeared during ECP1 infection, it is possible that the phage uses a variety of bacterial receptors to attach. Although the effectiveness of bacteriophages in reducing bacteria has not been demonstrated, bacteriophages in cocktails have the potential to hinder or limit bacterial development. ECP1 was proposed as a possible phage treatment option based on the suppression of bacterial growth, provided that the required clinical and animal model investigations are conducted.

Figure 7

Bacterial Growth Reduction Potential of ZI3 Bacteriophage



DISCUSSION

E. coli belongs to the family Enterobacteriaceae. The bacteria *E.coli* is encapsulated and facultative anaerobic. Gram-negative rods feature a unique polysaccharide capsule (CPS) and can be organized singly, in pairs, or in short chains. Furthermore, this bacterium is responsible for a significant percentage of community-acquired diseases worldwide. High rates of morbidity and mortality, along with the potential for metastatic spread, are characteristics of these illnesses (Paczosa & Mecsas, 2016). The multidrug-resistant (MDR) strain of *E. coli* is responsible for a large number of illnesses. However, it has been demonstrated that some *E.coli* species can develop genetic components and mutations that confer virulence traits and/or resistance to antibiotics, ultimately leading to the development of convergent clones called multidrug-resistant *E.coli* species. Bacteriophage treatment is one such tactic that can be used in replacement of antibiotics (Russo & Marr, 2019).

Isolating and characterizing lytic bacteriophages against *E.coli* strains is the aim of the current investigation. Sewage samples were gathered from Ayub Teaching hospital Abbottabad in order to isolate bacteriophages. One out of three sewage samples had bacteriophages that were effective against *E.coli.* The virus obtained from ayub Teaching Hospital abbottabad was known as ECP1, Alike study by Rehman *et al.,* (2015) which shows bacteriophages against *E.coli* strains were detected in six of fifteen samples from hospital sewage water. The isolated phage produced a distinct, circular plaque on a double-layer agar plate that was hostile to *E.coli*. The plaque is made up of two circular layers, with the inner, fully transparent core surrounded by an outside circle. The presence of this murky layer around plaque indicates that depolymerase enzyme is being produced by bacteriophages. The diameters of ECP1 was 2 and 3 mm. The halo around the plaque shows that soluble enzymes such depolymerase, which are produced by the phage ECP1 decapsulated the bacterial host cell. Alike study by Russo *et al.,* (2013) shows on double layer phage KP1 and KP5 produces transparent plaques against *E. coli* strains.

The transparent, clear plaques show the lytic nature of the isolated phages and their potential as a therapeutic agent. The ECP1 titers were found to be 4×109 pfu/mL after a 24-hour liquid culture phage propagation time. Similar study was conducted by Abedon (2011) which shows phage titer in this range for *E. coli* phage Kp1 and Kp5was 4.1×10^{8}*pfu*/mL and 5.1×10^{8}*pfu*/mL. It was discovered that the isolated *E. coli* ECP1 phages was very strain-specific. While no infectivity was seen for the other examined genera, ECP1 bacteriophages was able to infect and create a lytic zone against isolates of *E. coli*. Similar study by (Roy, 2018) demonstrates that the Klpp1 bacteriophage exclusively lysed *E. coli* species and did not infect any other bacteria. The isolated phages' spectrum of infectivity was unique to *E. coli*, which can be useful in treating infections caused by the bacteria because these phages won't harm normal microflora.

Up to 50ºC, the ECP1 phage wes shown to remain stable with no change in titer. For ECP1, a 1-fold drop in phage titer was noted at 25 and 50ºC, while no decrease was seen at 4 or 37ºC. At 60ºC, the titer of ECP1 decreased 6 times. Since ECP1 phage was stable at 25°C and 37°C, respectively, and since the average human body temperature is 37°C, these phages may be readily used if they was to be utilized as a therapeutic agent. Similar study was conducted by Gill and Abedon (2003) which shows *E.coli* phage is active in temperatures ranging from 4 to 60 degrees Celsius. After an hour, the ECP1phage showed resistance to the pH range of 3.0 to 9.0. It was discovered that the ideal pH range for phage ECP1 was 6–8. Additionally, at pH 9, 5, and 4, respectively, a drop of 1, 2, and 3 log was seen. Alike study by Abedon (2011) which shows *E. coli* best work at pH. Ranges from 5-9.

ECP1 antibacterial activity shown a remarkable capacity to suppress and reduce the initial bacterial inoculum after a 28-hour observation period. The phage may be able to attach itself to a large number of bacterial receptors since these phages are uncommon and there are no resistance mutations present during ECP1 infection. Bacteriophages may prevent or lessen bacterial development in mixtures, but their ability to limit bacterial growth on their own has not been shown. After the required clinical and animal model tests, it is proposed that ECP1 would be a suitable option for phage therapy based on the reduction in bacterial growth. To evaluate ECP1, the bacterial growth reduction test was employed. Using a spectrophotometer, the OD600 was measured every two hours for the next twenty-four hours in order to track phage capacity to inhibit bacterial growth and compare it with the control's growth. ECP1 stopped the growth of bacteria for sixteen hours. It has been demonstrated that phages that can prevent *K. pneumonia* from growing might stop the bacterial growth for up to 16 hours (Lin *et al.,* 2017).

CONCLUSION

Multidrug-resistant bacterial pathogen infections and their consequences are turning into a serious health emergency. It becomes challenging to treat the common infections. Although there are numerous options for alternative therapy techniques, bacteriophages are a powerful substitute. It is possible to separate the bacteriophages from the natural habitat of bacteria. Following the required animal model and clinical trials, bacteriophages ECP1 appear to be a strong contender for phage therapy due to them in vitro effectiveness in inhibiting bacterial growth, stability across a wide pH and temperature range, and long-term storage stability without the use of chemicals.

REFERENCES

1. Abdelrahman, F., Rezk, N., Fayez, M. S., Abdelmoteleb, M., Atteya, R., Elhadidy, M., & El-Shibiny, A. (2022). Isolation, characterization, and genomic analysis of three novel E. coli bacteriophages that effectively infect E. coli O18. *Microorganisms*, *10*(3), 589. https://doi.org/10.3390/microorganisms10030589
2. Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: an overview. *International journal of environmental research and public health*, *10*(12), 6235-6254. https://doi.org/10.3390/ijerph10126235
3. Alvi, I. A., Asif, M., Tabassum, R., Aslam, R., Abbas, Z., & ur Rehman, S. (2020). RLP, a bacteriophage of the family Podoviridae, rescues mice from bacteremia caused by multi-drug-resistant Pseudomonas aeruginosa. *Arch. Virol*, *165*(6), 1289-1297. https://doi.org/10.1007/s00705-020-04601-x
4. Asif, M., Alvi, I. A., & Rehman, S. U. (2018). Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect. Drug Resist.*, *11*, 1249. https://doi.org/10.2147/idr.s166750
5. Blaser, M. (2011). Stop the killing of beneficial bacteria. *nature*, *476*(7361), 393-394. https://doi.org/10.1038/476393a
6. Dissanayake, U., Ukhanova, M., Moye, Z. D., Sulakvelidze, A., & Mai, V. (2019). Bacteriophages reduce pathogenic Escherichia coli counts in mice without distorting gut microbiota. *Frontiers in Microbiology*, *10*, 1984. https://doi.org/10.3389/fmicb.2019.01984
7. Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B.-J., & Jiang, S. (2009). The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat. Rev. Microbiol.*, *7*(3), 226-236. https://doi.org/10.1038/nrmicro2090
8. Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., Ferreira, L. C., & Martinez, M. B. (2016). Diarrheagenic escherichia coli. *Brazilian journal of microbiology*, *47*(Suppl. 1), 3-30. https://doi.org/10.1016/j.bjm.2016.10.015
9. Khalil, I. A., Troeger, C., Blacker, B. F., Rao, P. C., Brown, A., Atherly, D. E., Brewer, T. G., Engmann, C. M., Houpt, E. R., & Kang, G. (2018). Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990–2016. *The Lancet infectious diseases*, *18*(11), 1229-1240. https://doi.org/10.1016/s1473-3099(18)30475-4
10. Khan Mirzaei, M., & Nilsson, A. S. (2015). Isolation of phages for phage therapy: a comparison of spot tests and efficiency of plating analyses for determination of host range and efficacy. *PloS one*, *10*(3), e0118557. https://doi.org/10.1371/journal.pone.0118557
11. Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World journal of gastrointestinal pharmacology and therapeutics*, *8*(3), 162. https://doi.org/10.4292/wjgpt.v8.i3.162
12. Liu, C. M., Stegger, M., Aziz, M., Johnson, T. J., Waits, K., Nordstrom, L., Gauld, L., Weaver, B., Rolland, D., & Statham, S. (2018). Escherichia coli ST131-H 22 as a foodborne uropathogen. *MBio*, *9*(4), 10.1128/mbio. 00470-00418. https://doi.org/10.1128/mbio.00470-18
13. Paczosa, M. K., & Mecsas, J. (2016). Klebsiella pneumoniae: going on the offense with a strong defense. *Microbiology and molecular biology reviews*, *80*(3), 629-661. https://doi.org/10.1128/mmbr.00078-15
14. Palmela, C., Chevarin, C., Xu, Z., Torres, J., Sevrin, G., Hirten, R., Barnich, N., Ng, S. C., & Colombel, J.-F. (2018). Adherent-invasive Escherichia coli in inflammatory bowel disease. *Gut*, *67*(3), 574-587. https://doi.org/10.1136/gutjnl-2017-314903
15. Pawłowska, B., & Sobieszczańska, B. M. (2017). Intestinal epithelial barrier: The target for pathogenic Escherichia coli. *Advances in Clinical & Experimental Medicine*, *26*(9). https://doi.org/10.17219/acem/64883
16. Poirel, L., Madec, J.-Y., Lupo, A., Schink, A.-K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial resistance in Escherichia coli. *Microbiology Spectrum*, *6*(4), 10.1128/microbiolspec. arba-0026-2017. https://doi.org/10.1128/microbiolspec.arba-0026-2017
17. Roy, A. (2018). *Isolation and characterization of bacteriophage from environmental water samples specific for Klebsiella pneumoniae* BRAC Univeristy].
18. Russo, T. A., & Marr, C. M. (2019). Hypervirulent klebsiella pneumoniae. *Clinical microbiology reviews*, *32*(3), 10.1128/cmr. 00001-00019. https://doi.org/10.1128/cmr.00001-19
19. Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., & Choroszy-Krol, I. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. *Gut pathogens*, *11*, 1-16. https://doi.org/10.1186/s13099-019-0290-0
20. Szmolka, A., & Nagy, B. (2013). Multidrug resistant commensal Escherichia coli in animals and its impact for public health. *Frontiers in Microbiology*, *4*, 258. https://doi.org/10.3389/fmicb.2013.00258
21. Tovkach, F., Zhuminska, G., & Kushkina, A. (2012). Long-term preservation of unstable bacteriophages of enterobacteria. *Мікробіологічний журнал*(74,№ 2), 60-66.
22. Ullah, F., Ahmad, S. S., Khan, M. A., & Moon, S. Bacteriophage Therapy against Antimicrobial Resistant Crisis. https://doi.org/10.59653/jhsmt.v2i01.318
23. Ullah, Z., Ahmad, M., Sajjad, W., Ullah, R., Ali, A. A., Ali, F. Z., Waqar, A., & Zaid, Z. (2025). Molecular epidemiology of staphylococcus aureus isolated from mice. A study of antibiotic resistance and genetic diversity using 16s-rrnaa gene sequencing.
24. Ullah, Z., Alvi, I. A., Niaz, Z., Ullah, I., Ullah, A., & Rehman, S. U. (2023). Reducing Multidrug-Resistant (MDR) Klebsiella pneumoniae via the Efficient Use of Bacteriophages ZI3 and HI3. *Current Trends in OMICS*, *4*(1), 17-35. https://doi.org/10.32350/cto.41.02
25. Vinogradov, E., Frirdich, E., MacLean, L. L., Perry, M. B., Petersen, B. O., Duus, J. Ø., & Whitfield, C. (2002). Structures of lipopolysaccharides from Klebsiella pneumoniae: Elucidation of the structure of the linkage region between

core and polysaccharide O chain and identification of the residues at the non-reducing termini of the Ochains. *J. Biol. Chem.*, *277*(28), 25070-25081. https://doi.org/10.1074/jbc.m202683200

26. Yuan, X., Zhang, S., Wang, J., Li, C., Li, N., Yu, S., Kong, L., Zeng, H., Yang, G., & Huang, Y. (2021). Isolation and characterization of a novel Escherichia coli Kayfunavirus phage DY1. *Virus Research*, *293*, 198274. https://doi.org/10.1016/j.virusres.2020.198274