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Isolation, molecular characterization, and therapeutic evaluation of bacteriophages targeting shiga toxin-producing *Escherichia coli* (STEC)

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen responsible for severe gastrointestinal infections in humans and animals. The present study aimed to isolate and characterize bacteriophages targeting STEC and evaluate their potential for bacterial control. Twenty-two E. coli isolates were revived and confirmed using cultural, biochemical, and molecular methods. Molecular analysis showed that 86.36% of isolates carried the *stx1* (Shiga toxin 1) gene and 50% carried the *eae* (*Escherichia coli* attaching and effacing) gene, while *stx2* was not detected. Thirteen bacteriophages (Ecp1–Ecp13) were isolated from farm environments and hospital sewage, of which twelve exhibited lytic activity against STEC isolates. Five phages showing broader lytic spectra were further characterized. Transmission electron microscopy identified them as members of the order Caudovirales, belonging to the families Podoviridae and Siphoviridae. The phages remained stable across temperatures from −20°C to 37°C and within pH 4–9, with optimal stability at 4°C and pH 7.5 Time-kill analysis demonstrated rapid bacterial reduction by phage Ecp10, and its application in experimentally contaminated milk significantly reduced STEC counts compared to untreated controls. These findings highlight the potential of bacteriophages as natural and targeted biocontrol agents for reducing STEC contamination in food systems.

Keywords: Bacteriophage, Environment, Food safety, Milk, Stability, STEC, Sewage

Escherichia coli, a ubiquitous bacterial species commensal to humans and warm-blooded animals, turns pathogenic under specific conditions such as compromised immunity, prolonged antibiotic use, or disruption of gastrointestinal barriers. Major pathogenic strains include enteropathogenic (EPEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), diffusely adherent (DAEC), and Shiga toxin-producing (STEC), with STEC most strongly associated with severe foodborne outbreaks (Sarowska *et al.* 2019). In humans, STEC infections can cause illnesses ranging from diarrhoea to hemorrhagic colitis and hemolytic uremic syndrome (HUS). In animals, STEC is linked to edema disease in pigs and dysentery in calves (Algammal *et al.* 2020). Ruminants, particularly cattle, serve as reservoirs and “super shedders” due to colonization at the recto-anal junction (Gonzalez *et al.* 2020, Mir *et al.* 2020). Milk and dairy products, rich in nutrients, provide an ideal medium for bacterial growth (Owusu-Kwarteng *et al.* 2020). Contamination occurs mainly via fecal contact during milking, though intra-mammary infections cannot be excluded. Consumption of raw milk and derivatives like yoghurt, cheese, and cream is, therefore, a major risk factor. Other sources include undercooked beef, sprouted seeds, and fresh produce. STEC spreads mainly via fecal-oral contact, contaminated environments, vertical transmission, and vectors such as flies or wildlife (Mir *et al.* 2024; Sajeena and Kalyanikutty 2014) with outbreaks also linked to wild animal contamination (Kim *et al.* 2020). The infectious dose is low, often fewer than 100 microorganisms (Newell *et al.* 2018). STEC serotype O157 ranks as the fourth leading global cause of diarrhoea (Poyil *et al.* 2022).

Antibiotic therapy is controversial since it may enhance Shiga toxin production via prophage induction (Hwang *et al.* 2021). Stress-induced activation of lysogenic lambdoid phages carrying stx genes results in toxin release and horizontal transfer (Pinto *et al.* 2020). Thus, alternative interventions are needed. Bacteriophages offer advantages including host specificity, biofilm disruption, safety in food applications, and reduced resistance pressure (Polaska and Sokołowska 2019; Rehman *et al.* 2019, Fauconnier, 2019). Several FDA-approved products such as EcoShield™, PhageGuard LTM, ListShield™, and SalmoFresh™ are Generally Recognized as Safe (GRAS) (O’Sullivan *et al.* 2019, Pinto *et al.* 2020). However, their application

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in milk remains underexplored. This study evaluates the lytic potential of STEC phages in milk to strengthen food protection and safety standards.

MATERIALS AND METHODS

Bacterial isolates: In this study, a total of 22 Shiga toxin-producing *Escherichia coli* (STEC) isolates of bovine origin and an additional 44 non-STEC *E. coli* isolates were obtained from the repository of the Division of Veterinary Bacteriology and Mycology, ICAR-IVRI, Bareilly, Uttar Pradesh. These isolates, were revived from glycerol stocks and subjected to detailed morphological, cultural, and biochemical characterization to confirm their identity. Colonies were examined on selective media, Gram staining was performed, and biochemical assays were carried out according to standard bacteriological protocols.

To ascertain their virulence potential, molecular characterization of the *E. coli* isolates was carried out by targeting the genes encoding Shiga toxins (*stx1* and *stx2*) as well as the intimin gene (*eae*). PCR amplification using specific primers (Table 1) allowed screening of these virulence-associated determinants, thereby confirming the pathogenic potential of the isolates used for subsequent phage susceptibility assays.

Isolation, purification, and propagation of bacteriophages: Sewage, known to be a rich source of bacteriophages due to continuous bacterial shedding from humans and animals, was chosen as the phage source. A total of 23 sewage samples were collected from different ecological niches: poultry farms (n=6), swine farms (n=6), cattle farms (n=6), and hospitals (n=5) in Bareilly. Each sample was enriched in Brain Heart Infusion (BHI) broth supplemented with *E. coli* ATCC 2469 indicator strain, followed by overnight incubation at 37°C.

After enrichment, samples were centrifuged at 8000 rpm for 10 minutes at 4°C to remove bacterial debris. The supernatants were passed through 0.22 µm syringe filters to obtain Bacteria-Free Filtrates (BFFs). The presence of lytic phages in these filtrates was initially screened using the spot test against the indicator strain. Double agar overlay plaque assays were then employed to confirm the lytic nature of the phages, quantify their titers, and obtain individual plaques for purification.

Each phage isolate was purified through 3–4 consecutive passages, ensuring the selection of homogeneous phage populations. For large-scale propagation, NZCYM broth supplemented with $CaCl_2$ and $MgSO_4$ was inoculated with phages and incubated for 24 hours. Following cultivation, lysates were clarified by centrifugation and filtration, and the resulting phage suspensions were preserved at 4°C for further characterization.

Determination of lytic spectrum: The host range of isolated STEC phages was determined by spot testing against all 66 *E. coli* isolates (22 STEC and 44 non-STEC). Bacterial lawns were prepared, and phage suspensions (5 µl) were spotted. After overnight incubation at 37°C, the plates were examined for clear lytic zones, indicative of phage activity. The proportion of susceptible isolates provided an estimate of the lytic spectrum of each phage.

Morphological characterization by TEM: For detailed morphological characterization, phages showing the broadest lytic spectra were concentrated using the PEG-NaCl precipitation method (Zhu *et al.* 2008). Samples were adsorbed onto carbon-coated copper grids, negatively stained with uranyl acetate, and examined under a JEM 1011 Transmission Electron Microscope (JEOL, Japan). The structural features, such as head diameter and tail morphology, allowed classification of phages into their respective families within the order *Caudovirales*.

Phage stability assays: To determine thermal stability, phage suspensions (10^7–10^9 pfu/ml) were incubated at -20°C, 4°C, 37°C, 55°C, and 85°C for one hour. Survivability was assessed by plaque assay. For pH stability, SM buffer was adjusted to pH values ranging from 2 to 9 using appropriate buffer systems. Phages were incubated in these buffers for one hour, neutralized, and assayed by the double agar overlay method.

Time–Kill Assay: The phage demonstrating the highest lytic range was selected for time–kill assays. Bacterial cultures (~10^8 CFU/ml) were infected with phage suspensions at multiplicities of infection (MOIs) of 1, 0.1, 0.01, and 0.001. Optical density at 620 nm was recorded at hourly intervals for 4 hours, enabling assessment of phage-induced bacterial growth suppression dynamics.

Phage activity in spiked milk samples: To simulate real-world applications, the bacteriolytic efficacy of the most potent phage was evaluated in milk artificially inoculated with STEC. Milk samples (2 ml) were inoculated with ~10^8 CFU/ml STEC, treated with phages at varying MOIs (1, 0.1, 0.01, 0.001), and incubated at 37°C. At 2-hour intervals up to 10 hours, samples were serially diluted and plated on Nutrient Agar to quantify viable bacterial counts.

Statistical analysis: All experiments were performed in triplicate. Data were analyzed using SPSS 20 software.

Table 1. List of primers used in the study

Primer code	Primer sequence 5' to 3'	Product size	Reference
stx1 F	CAG TTA ATG TGG TGG CGA AGG	348bp	Fard *et al.* 2005
stx1 R	CAC CAG ACA ATG TAA CCG CTG		
stx2 F	ATC CTA TTC CCG GGA GTT TAC G	584bp	Fard *et al.* 2005
stx2 R	GCG TCA TCG TAT ACA CAG GAG C		
eae F	TCA ATG CAG TTC CGT TAT CAG TT	482bp	Vidal *et al.* 2005
eae R	GTA AAG TCC GTT ACC CCA ACC TG		

One-way ANOVA followed by Tukey's test was applied, with statistical significance considered at $p < 0.05$.

RESULTS AND DISCUSSION

Revival and characterization of E. coli isolates: All isolates displayed typical morphological, cultural, and biochemical features of E. coli, including colonies with a metallic sheen and greenish rim on EMB agar, and pink staining on Gram's stain. Molecular characterization of STEC isolates (n = 22) revealed that 86.36% were positive for the *stx1* gene and 50% for the *eae* gene, while none carried *stx2* alone or in combination of *stx1* and *stx2*. The presence of these virulence genes was consistent with earlier reports linking STEC to diarrheagenic infections in both humans and animals (Algammal *et al.* 2020). The absence of *stx2* in the isolates may be indicative of regional strain variation or host-specific adaptation, as several studies have reported heterogeneity in the distribution of Shiga toxin genes among STEC isolates from animal reservoirs (García *et al.* 2022).

Isolation, purification, and titration of bacteriophages: A total of thirteen bacteriophages (Ecp1–Ecp13) were isolated from poultry, swine, cattle farms, and hospital sewage, of which twelve showed lytic activity against STEC isolates. Sewage and farm effluents are well-recognized reservoirs of enteric phages due to fecal contamination, and similar studies have successfully isolated bacteriophages from such environments (Necel *et al.* 2020; Korf *et al.* 2019). Recent metagenomic studies have further confirmed presence of a large diversity of enteric bacteriophages capable of infecting pathogenic E. coli in wastewater ecosystems (Chevallereau *et al.* 2022). The plaques observed were clear, with diameters ranging from 1–2 mm (Fig. 1), consistent with earlier findings (Yazdi *et al.* 2020, Montso *et al.* 2019), though other studies have reported broader size ranges of 0.7–3.5 mm (Sjahriani *et al.* 2021, Topka *et al.* 2019). Plaque morphology is influenced by agar concentration, incubation conditions, and the host's growth phase (Hyman 2019), but plaque size is also considered an inherent trait of the phage itself. Titration using the double agar overlay method revealed phage concentrations of 10^5–10^9 pfu/ml. The phage titers obtained in this study (10^5–10^9 PFU/ml) were comparable to those reported for STEC-targeting bacteriophages in previous investigations (Moye *et al.* 2018).

Lytic spectrum determination by spot test: Phages exhibited lytic activity against subsets of E. coli isolates (Fig. 2). Five phages—Ecp3, Ecp4, Ecp5, Ecp9, and Ecp10—demonstrated higher lytic activity (22.72–33.33%) and were selected for further characterization. Broad host range phages are particularly valuable for therapeutic and food safety applications because they can target multiple strains of pathogenic bacteria, thereby increasing treatment efficacy and reducing the likelihood of resistance development (Gordillo Altamirano & Barr, 2019, Hesse *et al.* 2023).

Fig. 1. Plaque morphology of *E. coli* phages

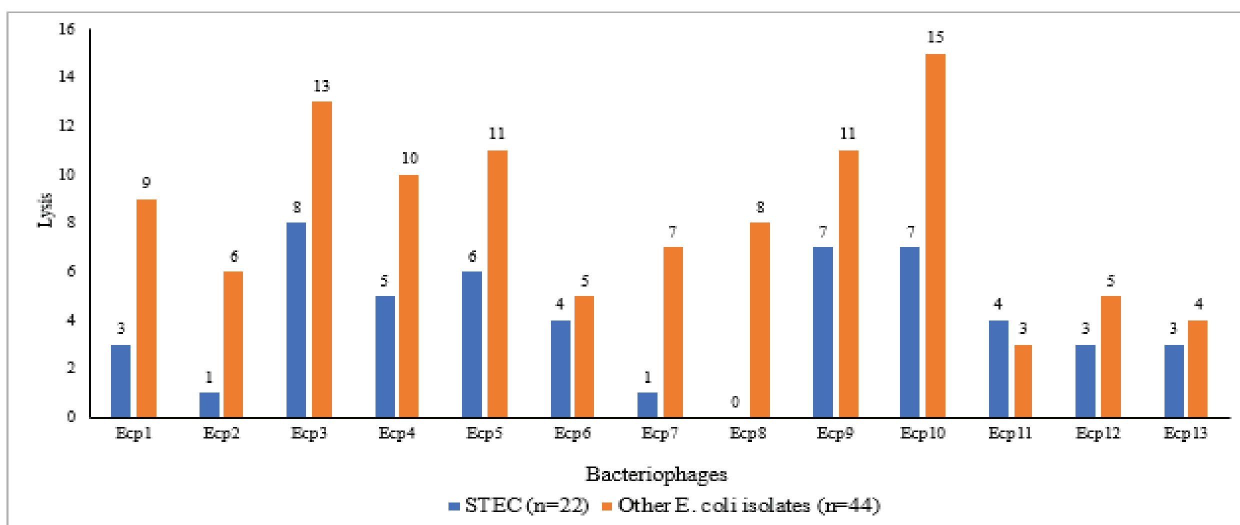

Fig. 2. Lysis profile against different *E. coli* isolates by spot test

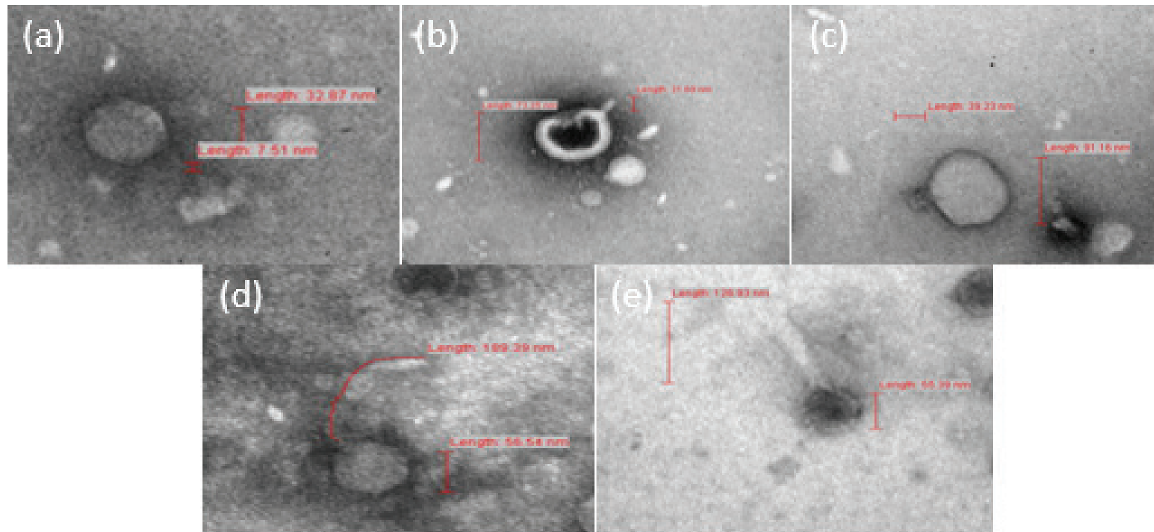

Fig. 3. TEM images showing the morphology of the individual phages: (a) Ecp3, (b) Ecp4 (c) Ecp5 (d) Ecp9 and (e) Ecp10

Morphological characterization by TEM: TEM analysis revealed that all the five selected phages belonged to the order Caudovirales (Fig. 3). Ecp3, Ecp4, and Ecp5 were identified as Podoviridae, with icosahedral heads (32.87–91.16 nm) and short non-contractile tails (7.51–39.23 nm), while Ecp9 and Ecp10 were Siphoviridae, with icosahedral heads (~55 nm) and long non-contractile tails (126.93–189.39 nm). These findings are consistent with earlier studies reporting Caudovirales as the most common tailed phages infecting enteric bacteria (Abdelrahman *et al.* 2022) Since tailed phages are evolutionarily ancient and widely distributed, their isolation in this study was expected. Recent taxonomic revisions by the International Committee on Taxonomy of Viruses (ICTV) have further confirmed the evolutionary diversity and ecological dominance of these tailed phages in microbial ecosystems (Turner *et al.* 2023).

*Effect of temperature on phage stability:*The phages remained stable between –20°C and 37°C, with maximum titers at 4°C. At 55°C, the titers significantly decreased, and complete inactivation occurred at 85°C after 60 min (Fig. 4). These findings align with earlier reports that higher temperatures denature phage proteins and nucleic acids, thereby reducing viability (Abdelrahman *et al.* 2022; Chandra *et al.* 2011). However, some studies have reported the Bacillus phages tolerate temperatures up to 80°C. Reduced stability at high temperatures may also reflect impaired host metabolism, as bacterial growth declines between 45-51°C.

*Effect of pH on phage stability:*The phages were stable across pH 4–9, with maximum stability at pH 7.5 (Fig. 4). At pH 2, all phages were completely inactivated within 60 min. These results agreed with earlier findings (Ullah *et al.* 2021), confirming that phages were most stable under neutral to slightly alkaline conditions (Ateba *et al.* 2019). Inactivation at acidic pH is likely to be due to oxidative effects on capsid proteins, leading to loss of infectivity (Abdelrahman *et al.* 2022).

Time-killing curve: Phage Ecp10 was selected for time-kill analysis. Across all MOIs, bacterial reduction was observed within 4 h. At MOIs 1 and 0.1, marked lytic activity was evident within the first hour, as OD_{620} decreased from

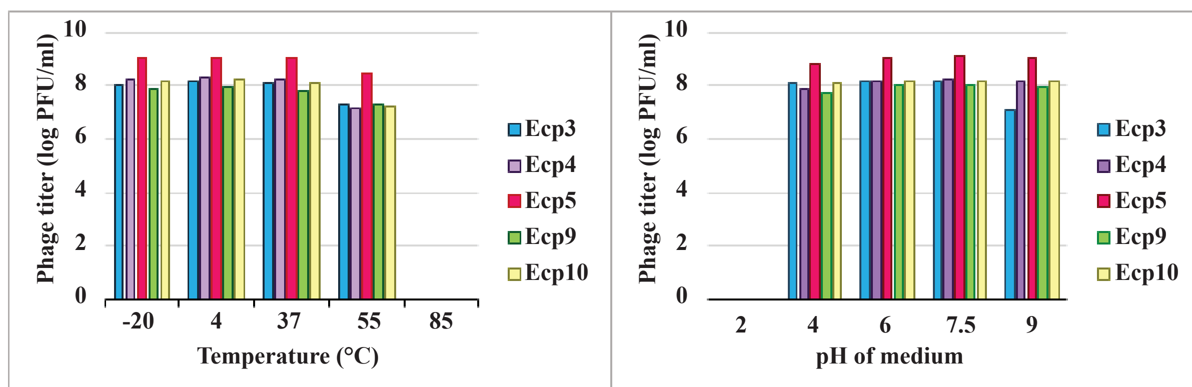

Fig. 4. Graph showing temperature and pH stability test

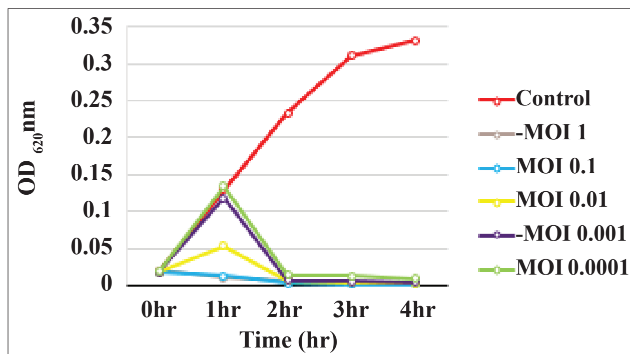

Fig. 5. Mean of OD values (±SD) of bacteria at different MOIs

0.021 ± 0.004 to 0.004 ± 0.002 and from 0.018 ± 0.001 to 0.002 ± 0.000, respectively (Fig. 5). Rapid bacteriolysis at low multiplicities of infection is an important property for phage-based antimicrobial strategies, as it indicates efficient adsorption and replication within the host bacteria (Lin *et al.* 2017, Hesse *et al.* 2023). These MOIs were considered optimal for subsequent experiments. The ability of phages to achieve rapid bacterial lysis at low MOI highlighted their potential for food decontamination applications.

Suppression of STEC isolates in milk by phage Ecp10: In spiked milk, untreated samples showed an increase in bacterial counts from 7.11 to 8.82 log CFU/ml over 6 h. In contrast, phage treatment reduced counts from 6.90 to 5.16 log CFU/ml (MOI 1) and from 6.78 to 4.95 log CFU/ml (MOI 0.1) (Table 2). These findings were consistent with Abdelsattar *et al.* (2021), who also observed strong phage-mediated suppression of bacterial populations in milk. Conversely, Grygorcewicz *et al.* (2020) reported increased efficacy at higher MOIs (50–1000), although excessively high MOIs may lead to abortive infections. The successful suppression of STEC in milk in this study supported the recent reports of effective phage-mediated decontamination against other pathogens such as P. lactis and S. Typhimurium (Phongtang *et al.* 2019).

Overall, bacteriophages represent a promising and environmentally friendly strategy for controlling foodborne pathogens such as STEC. Their ability to specifically target bacterial pathogens without disturbing beneficial microbiota makes them attractive alternatives to conventional antimicrobials. Recent advances in phage therapy, genomic characterization, and regulatory approvals for phage-based food safety products further support the potential of bacteriophages as effective biocontrol agents in food systems (Chevallereau *et al.* 2022, FAO/WHO, 2023). Nevertheless, further studies are required to evaluate the efficacy of these phages under industrial food processing conditions and to assess their effectiveness against STEC contamination in diverse food matrices.

Table 2. The average log CFU count of bacteria

	Bacterial count (log CFU/ml)					
	0 hr	2 hr	4hr	6hr.LO	8hr	10 hr
Control	7.11	7.73	8.34	8.82	9.45	9.98
MOI 1	6.90	6.38	5.73	5.16	5.72	6.23
MOI 0.1	6.78	6.13	5.54	4.95	5.61	6.27
MOI 0.01	7.04	6.65	5.91	6.43	7.19	7.68
MOI 0.001	6.91	6.42	5.76	6.26	7.05	8.17

REFERENCES

Abdelrahman F, Rezk N, Fayez M S, Abdelmoteleb M, Atteya R, Elhadidy M and 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.

Abdelsattar A S, Safwat A, Nofal R, Elsayed A, Makky S and El-Shibiny A. 2021. Isolation and characterization of bacteriophage ZCSE6 against *Salmonella* spp.: Phage application in milk. *Biologics* **1** (2): 164–76.

Algammal A M, El-Kholy A W, Riad E M, Mohamed H E, Elhaig M M, Yousef S A A, Hozzein W N and Ghobashy M O. 2020. Genes encoding the virulence and the antimicrobial resistance in enterotoxigenic and shiga-toxigenic *E. coli* isolated from diarrheic calves. *Toxins* **12** (6): 383.

Ateba C N and Akindolire M A. 2019. Isolation and characterization of bacteriophages with lytic activity against virulent Escherichia coli O157:H7: Potential bio-control agents. *African Journal of Biotechnology* **18** (12): 355–365.

Chandra M, Thakur S, Narang D and Saxena H M. 2011. Isolation of a bacteriophage against Salmonella Dublin and determination of its physical resistance under varied in vitro conditions. *African Journal of Microbiology Research* **5**(15): 2044-2047.

Chevallereau A, Pons BJ, van Houte S and Westra E R. 2022. Interactions between bacterial and phage communities in natural environments. *Nature Reviews Microbiology* **20**: 49–62.

Fauconnier A. 2019. Phage therapy regulation: from night to dawn. *Viruses* **11** (4): 352.

García A, Fox JG, Besser TE. 2010. Zoonotic enterohemorrhagic Escherichia coli: A One Health perspective. *ILAR Journal* **51**(3):221-232.

FAO/WHO. (2023). Shiga toxin-producing Escherichia coli (STEC) and food safety risk assessment. Food and Agriculture Organization / World Health Organization.

Gordillo Altamirano F L and Barr J J. 2019. Phage therapy in the post antibiotic era. *Clinical Microbiology Reviews* **32**(2):e00066-18.

Gonzalez G A and Cerqueira M A. 2020. Shiga toxin-producing *Escherichia coli* in the animal reservoir and food in Brazil. *Journal of Applied Microbiology* **128** (6): 1568–1582.

Grygorcewicz B, Chajęcka-Wierzchowska W, Augustyniak A, Wasak A, Stachurska X, Nawrotek P and Dołęgowska B. 2020. In-milk inactivation of *Escherichia coli* O157:H7 by the environmental lytic bacteriophage ECPS-6. *Journal of Food Safety* **40** (2): e12747.

Hesse S, Adhya S. 2019. Phage therapy in the twenty-first century: facing the decline of the antibiotic era; Is it finally time for the age of the phage? *Annual Review of Microbiology* **73**:155-174.

Hwang S B, Chelliah R, Kang J E, Rubab M, Banan-Mwine Daliri E, Elahi F and Oh D H. 2021. Role of recent therapeutic applications and the infection strategies of Shiga toxin-producing *Escherichia coli*. *Frontiers in Cellular and Infection Microbiology* **11**: 614963.

Hyman P. 2019. Phages for phage therapy: isolation,

characterization, and host range breadth. *Pharmaceuticals* **12** (1): 35.

Kim J S, Lee M S and Kim J H. 2020. Recent updates on outbreaks of Shiga toxin-producing *Escherichia coli* and its potential reservoirs. *Frontiers in Cellular and Infection Microbiology* **10**: 273.

Korf I H, Meier-Kolthoff J P, Adriaenssens E M, Kropinski A M, Nimtz M, Rohde M, van Raaij M J and Wittmann J. 2019. Still something to discover: novel insights into *Escherichia coli* phage diversity and taxonomy. *Viruses* **11** (5): 454.

Lin D M, Koskella B and Lin H C. 2017. Phage therapy: An alternative to antibiotics in the age of multidrug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics* **8**(3): 162–173.

Mir R A, Schaut R G, Looft T, Allen H K, Sharma V K and Kudva I T. 2020. Recto-anal junction (RAJ) and fecal microbiomes of cattle experimentally challenged with *Escherichia coli* O157:H7. *Frontiers in Microbiology* **11**: 522078.

Montso P K, Mlambo V and Ateba C N. 2019. Characterization of lytic bacteriophages infecting multidrug-resistant shiga toxigenic atypical *Escherichia coli* O177 isolates isolated from cattle feces. *Frontiers in Public Health* **7**: 355.

Moye Z D, Woolston J and Sulakvelidze A. 2018. Bacteriophage applications for food production and processing. *Viruses* **10**(4): 205.

Necel A, Bloch S, Nejman-Faleńczyk B, Grabski M, Topka G, Dydecka A, Kosznik-Kwaśnicka K, Grabowski Ł, Jurczak-Kurek A, Wołkowicz T and Węgrzyn G. 2020. Characterization of a bacteriophage, vB_Eco4M-7, that effectively infects many *Escherichia coli* O157 isolates. *Scientific Reports* **10** (1): 3743.

Newell D G and La Ragione R M. 2018. Enterohaemorrhagic and other Shiga toxin-producing *Escherichia coli* (STEC): Where are we now regarding diagnostics and control strategies?. *Transboundary and Emerging Diseases* **65**: 49–71.

O'Sullivan L, Bolton D, McAuliffe O and Coffey A. 2020. The use of bacteriophages to control and detect pathogens in the dairy industry. *International Journal of Dairy Technology* **73** (1): 1–11.

Owusu-Kwarteng J, Akabanda F, Agyei D and Jespersen L. 2020. Microbial safety of milk production and fermented dairy products in Africa. *Microorganisms* **8** (5): 752.

Phongtang W, Choi G P, Chukeatirote E and Ahn J. 2019. Bacteriophage control of *Salmonella Typhimurium* in milk. *Food Science and Biotechnology* **28**: 297–301.

Pinto G, Almeida C and Azeredo J. 2020. Bacteriophages to control Shiga toxin-producing *E. coli*–safety and regulatory challenges. *Critical Reviews in Biotechnology* **40** (8): 1081–1897.

Połaska M and Sokołowska B. 2019. Bacteriophages—a new hope or a huge problem in the food industry. *AIMS Microbiology* **5** (4): 324.

Połaska M and Sokołowska B. 2019. Bacteriophages—a new hope or a huge problem in the food industry. *AIMS Microbiology* **5**(4): 324.

Poyil M M, Karuppiah P, Raja S S and Sasikumar P. 2022. Isolation, extraction, and characterization of Verotoxin-producing *Escherichia coli* O157:H7 from diarrheal stool samples. *Sudan Journal of Medical Sciences* **17** (1): 116–127.

Rehman S, Ali Z, Khan M, Bostan N and Naseem S. 2019. The dawn of phage therapy. *Reviews in Medical Virology* **29**(4): e2041.

Sajeena T A M and Kalyanikutty S. 2024. Pathogenic Factors of Shiga Toxigenic Escherichia coli. *Journal of Pure and Applied Microbiology* **18**(1) :46-63.

Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G and 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.

Sjahriani T, Wasito E B and Tyasningsih W. 2021. Isolation and identification of *Escherichia coli* O157:H7 lytic bacteriophage from environment sewage. *International Journal of Food Science* **2021**(1): 7383121.

Topka G, Bloch S, Nejman-Faleńczyk B, Gąsior T, Jurczak-Kurek A, Necel A, Dydecka A, Richert M, Węgrzyn G and Węgrzyn A. 2019. Characterization of bacteriophage vB-EcoS-95, isolated from urban sewage and revealing extremely rapid lytic development. *Frontiers in Microbiology* **9** :3326.

Turner D, Kropinski AM and Adriaenssens EM. 2022. A Roadmap for Genome-Based Phage Taxonomy. *Viruses* **13**(3):506.

Ullah A, Qamash T, Khan F A, Sultan A, Ahmad S, Abbas M, Khattak M A K, Begum N, Din S U, Jamil J and Kalsoom. 2021. Characterization of a coliphage AS1 isolated from sewage effluent in Pakistan. *Brazilian Journal of Biology* **82**: e240943.

Yazdi M, Bouzari M, Ghaemi E A and Shahin K. 2020. Isolation, characterization and genomic analysis of a novel bacteriophage VB_EcoS-Golestan infecting multidrug-resistant *Escherichia coli* isolated from urinary tract infection. *Scientific Reports* **10** (1): 7690.

Zhu Z and Dimitrov D S. 2008. Construction of a large naive human phage-displayed Fab library through one-step cloning. (In) *Therapeutic Antibodies: Methods and Protocols* (pp. 129-142). Totowa, NJ: Humana Press.