

Use of *Escherichia coli* bacteriophage to eliminate Shiga toxin-producing *Escherichia coli* without inducing toxin production

Maher SALEH¹, Abdulkarim HALLAK¹, Aseem ALBAKER^{1*}

¹*Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Hama University, Hama, Syria*

***Correspondence:** aseem7g41@gmail.com

ORCIDs:

Maher SALEH: <https://orcid.org/0009-0006-8058-3630>

Abdulkarim HALLAK : <https://orcid.org/0009-0008-5486-1056>

Aseem ALBAKER : <https://orcid.org/0009-0000-8136-4057>

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) pose a serious health risk, particularly in hemolytic uremic syndrome (HUS) cases, where antibiotic administration may exacerbate the condition by triggering toxin release. This study aimed to investigate the ability of the antibiotic enrofloxacin to induce Shiga toxin production in STEC, and the ability of bacteriophage to eliminate them without inducing toxin secretion.

Material and Methods: This study was conducted using bacteriophage isolated from the environment on enterohemorrhagic *Escherichia coli* (EHEC) isolated from calves. The synergy between enrofloxacin and bacteriophage was studied using the chessboard strip method, and the level of Shiga toxin secretion was measured using ELISA.

Results: The results showed a synergistic efficacy between the antibiotic enrofloxacin and bacteriophages, and a strong ability of enrofloxacin to induce toxin production. The results also showed that bacteriophage alone or in synergy with enrofloxacin were able to eliminate bacteria without inducing toxin secretion.

Conclusion: We conclude from this the possibility of using bacteriophage as a potential safe alternative in eliminating STEC infection.

Key words: Bacteriophage, Diarrhea, Enrofloxacin, HUS, Shiga toxin, STEC.

1. Introduction

Diarrhea is a common clinical condition with multiple causes, and based on its duration it is classified into acute and chronic. where acute diarrhea lasts up to two weeks, while chronic diarrhea lasts for more than four weeks. it has many infectious and non-infectious causes, and one of the most important infectious causes of diarrhea is EHEC [1].

Ruminants are considered the main reservoir of enterohemorrhagic *E. coli* of serotype O157:H7 and often show no clinical signs of infection, yet they cause life-threatening infection in humans, especially children [2].

The pathogenicity of STEC serotypes is primarily attributed to Shiga toxin (also known as Shiga-like toxin or verotoxin), which is encoded by *stx* genes. This toxin serves as the key virulence determinant responsible for the clinical manifestations observed in STEC infections, *Stx* is produced by STEC in the intestinal tract and subsequently enters the bloodstream, as it can cause systemic damage, and The kidneys exhibit particular sensitivity to *Stx* toxicity, attributed to the high concentration of its specific receptors in renal tissues[3].

Shiga toxins bind to intestinal cells, cross the brush border, and damage the vascular network of the intestinal mucosa, causing hemorrhagic colitis. Once the toxin reaches the bloodstream, its pentameric B subunit binds to globotriaosylceramide (Gb3Cer), which is well expressed on the microvascular endothelium. Upon binding to Gb3Cer, Shiga toxins are internalized via endocytosis and transported via the retrograde pathway to the Golgi apparatus, thus reaching the endoplasmic reticulum (ER). At this stage, the enzymatic A subunit is released into the cytoplasm, interfering

with protein synthesis and leading to apoptosis, endoplasmic reticulum stress, inflammation, and further cellular damage [4].

The development of hemolytic uremic syndrome in children occurs as a result of intestinal infection with Shiga toxin-producing bacteria, in addition to host susceptibility and other clinical factors. It is also likely that virulence factors that enhance intestinal colonization and attaching/effacing lesions increase mucosal damage and systemic toxin exposure. HUS risk is higher in younger children, likely due to increased Gb3 expression and immature inflammatory/complement regulation [5].

STEC infection is considered the most common cause of hemolytic uremic syndrome in children, which is a form of thrombotic microangiopathy (TMA) characterized by hemolysis, thrombocytopenia, and acute kidney injury [6].

Despite significant advances in scientific knowledge and medical technology in recent decades, hemolytic uremic syndrome (HUS) associated with Shiga toxin-producing *E. coli* (STEC) infection remains a major public health and individual challenge worldwide [4]. While antimicrobial chemotherapy is a common and often effective approach for many bacterial infections, its use is generally avoided in STEC infection [6]. The basis for this approach lies in the complex link between antimicrobial therapy and *stx* production. Notably, the *stx* genes are not genomic constituents but are delivered by lambdoid prophages that have integrated into the bacterial genome [7].

The life cycle of these integrated bacteriophages is closely linked to the bacterial stress response, specifically the SOS response, which can be induced by various environmental stresses, including antibiotics [8]. Therefore, it is recommended not to administer antibiotics in cases of HUS.

The dilemma of withholding antibiotics in STEC infections presents a significant challenge for clinicians. Despite the need for effective means of direct STEC control, current management of STEC infection is largely supportive, focusing on maintaining fluid and electrolyte balance and monitoring complications [9].

74 Therefore, the need has emerged to search for an alternative to antibiotic use, and the idea of using
75 bacteriophage therapy has emerged as a potential alternative, especially in cases of STEC infection.
76 Bacteriophages are viruses that have the ability to infect and kill bacteria without causing any
77 negative effects on human or animal cells [10]. Bacteriophages have high specificity in eliminating
78 bacteria and can be used to eliminate STEC without inducing the bacterial SOS stress response,
79 which is the primary cause of replication of temperate bacteriophages carrying the *stx* gene within
80 bacteria [11]. Given the importance of the topic and the urgent need to find an alternative to
81 antibiotics, especially in cases of HUS, this study came as a contribution to the search for a
82 potential alternative to antibiotics.

83 **1.1.Study objectives:**

84 This study aims to:

- 85 1) quantify the extent of Shiga toxin induction in EHEC upon enrofloxacin treatment
- 86 2) determine whether bacteriophage application can eliminate EHEC without triggering
87 toxin release.
- 88 3) evaluate the synergistic potential of a combined enrofloxacin-phage strategy for
89 enhanced bacterial killing

90 **2. Materials and methods**

91 This study was conducted in the microbiology laboratories of the Faculty of Veterinary Medicine,
92 Hama University, in the period between 15/12/2025 and 4/1/2026.

93 **2.1.Enterohemorrhagic *E. coli* isolates:**

94 Enterohemorrhagic *E. coli* (EHEC) isolates obtained from calves with diarrhea and preserved in the
95 bacterial bank of the Faculty of Veterinary Medicine were used in this study. The isolates were
96 identified using selective and differential culture media, in addition to biochemical tests. The
97 identity of the isolates and the presence of *stx1* and *eae* genes were further confirmed using

polymerase chain reaction (PCR), as previously described [12]. The isolates were then activated using nutrient broth.

2.2.Bacteriophage isolates:

Virulent *E. coli* bacteriophage were obtained from the Department of Microbiology, Faculty of Veterinary Medicine. Bacteriophage propagation was carried out by preparing 250 ml glass bottles containing 100 ml of nutrient broth supplemented with 0.05% calcium nitrate sterilized by autoclaving. An aliquot of 1 mL of the *E. coli* bacterial suspension, adjusted to the logarithmic growth phase (18 hours), was mixed with 25 mL of the bacteriophage suspension. The resulting mixture was then incubated at 37°C for 48 hours under continuous agitation. After incubation, they were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was filtered using membrane filters with pore sizes of 0.45 µm and 0.22 µm, respectively [13].

Bacteriophage titration was performed using the double agar layer technique. A series of ten-fold dilutions was performed by transferring 100 µl of bacteriophage solution to 900 µl of nutrient broth, then 100 µl from the second tube was transferred to the third tube containing 900 µl of nutrient broth, and the process was repeated until several ten-fold dilutions were obtained. Then, 300 µl of each dilution solution was mixed with 300 µl of *E. coli* solution in the logarithmic phase (18 hours old) and shaken for 10 minutes, then added to 3 ml of nutrient agar which was poured over the first agar layer, and the plates were incubated at 37°C for 24 hours. Plates with plaque counts ranging between 100-200 plaques were selected, and the bacteriophage titer was calculated in plaque forming units (PFU) [14].

2.3.Determination of minimum inhibitory concentration (MIC) of enrofloxacin:

The broth microdilution method was used to calculate the minimum inhibitory concentration. A series of dilutions of the antibiotic enrofloxacin was performed using nutrient broth at a 1:2 ratio across tubes (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 µg/ml), then EHEC at a concentration of 0.5 McFarland were added at a 1:1 ratio to achieve final concentrations of (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32

123 µg/ml). The tubes were incubated at 37°C for 18-24 hours, and the MIC was considered as the
124 lowest concentration that completely prevents visible growth (absence of turbidity) [15].

125 **2.4.Calculation of synergy between *E. coli* bacteriophage and the antibiotic enrofloxacin:**

126 EHEC bacteriophage were tested using the chessboard strip method with three replicates to study
127 synergy. *E. coli* bacteriophage were mixed with the antibiotic enrofloxacin at increasing
128 concentrations of both bacteriophage and antibiotics and applied to *E. coli* at a count of 10⁶
129 CFU/mL using microtiter plates in nutrient broth medium. The synergistic effect was calculated by
130 reading the plates by measuring optical density using an ELISA reader at a wavelength of 600 nm.
131 *E. coli* bacteriophage were enumerated in the plate wells according to [14]. The fractional inhibitory
132 concentration index (FICI) was calculated according to Alexyuk et al., [16] as follows:

$$133 \text{ FICI} = \text{MIC}_{\text{A+Ph}} / \text{MIC}_{\text{A}} + \text{MIC}_{\text{Ph+A}} / \text{MIC}_{\text{Ph}}$$

134 Where:

135 MIC_{A+Ph}: Minimum inhibitory concentration (MIC) of the antibiotic when used with the
136 bacteriophage.

137 MIC_A: Minimum inhibitory concentration (MIC) of the antibiotic when used alone.

138 MIC_{Ph+A}: Minimum inhibitory concentration (MIC) of the bacteriophage when used with the
139 antibiotic.

140 MIC_{Ph}: Minimum inhibitory concentration (MIC) of the bacteriophage when used alone.

141 The result is interpreted as:

142 FICI ≤ 0.5: Synergy

143 0.5 < FICI ≤ 1: Additive effect

144 1 < FICI ≤ 2: No interaction

145 FICI > 2: Antagonism

146 **2.5.Experimental design:**

147 EHEC aged 10 hours at a count of 10^5 bacterial cells calibrated using a spectrophotometer at
148 OD620 after washing were added to sterile 5 ml tubes containing nutrient broth with 6 replicates,
149 divided as follows:

150 Group 1 (Control): Contained EHEC only

151 Group 2: Contained EHEC and enrofloxacin at a concentration of 4 $\mu\text{g/ml}$

152 Group 3: Contained EHEC and bacteriophage at a count of 10^7 PFU/ml

153 Group 4: Contained EHEC and enrofloxacin at a concentration of 2 $\mu\text{g/ml}$ (1/2 MIC) and
154 bacteriophage at a count of 10^7 PFU/ml

155 The tubes were incubated at 37°C.

156 **2.6.Measurement of Shiga toxin concentration:**

157 Shiga toxin production was estimated after 2, 3, 5, and 8 hours. 1 ml was transferred to new tubes,
158 then centrifuged at 10,000 rpm for 10 minutes, and the supernatant was filtered using membrane
159 filters with pore sizes of 0.45 μm and 0.22 μm , respectively. The filtrate was diluted 1:10, and
160 Shiga toxin was detected using the RIDASCREEN® Verotoxin ELISA kit (R-Biopharm AG,
161 Germany). To determine the toxin concentration in the samples, Recombinant *Stx₁B* protein (Yaxin
162 Biotechnology, China) was used as a positive control and to draw a standard curve for determining
163 unknown concentrations in the samples by performing a series of dilutions at different controlled
164 concentrations. The measurement was performed at an absorbance of 450 nm.

165 **2.7.Statistical analysis:**

166 Microsoft Excel 2019 and GraphPad Prism (version 8.2.1) software were used for data processing,
167 charting, and performing statistical comparison between groups.

168 **3. Results**

169 The results of this study, shown in Figure (1), showed a synergistic efficacy between the
170 antibiotic enrofloxacin and *E. coli* bacteriophage, as the minimum inhibitory concentration of the
171 combination was 0.5 $\mu\text{g/ml}$ for enrofloxacin and 6 log₁₀ PFU/ml for bacteriophage, while the

minimum inhibitory concentration of enrofloxacin alone was 4 µg/ml. By calculating the count of *E. coli* bacteriophage, we find that the bacteriophage titer takes a convex curve shape, peaking and then declining with increasing antibiotic concentration, except for the column containing the concentration of 7 log₁₀ PFU/ml, where the decline continued with increasing antibiotic concentration, as shown in Figure (2). By calculating the fractional inhibitory concentration index (FICI), we find that there is a synergistic effect between the antibiotic enrofloxacin and bacteriophage, as the FICI was 0.225.

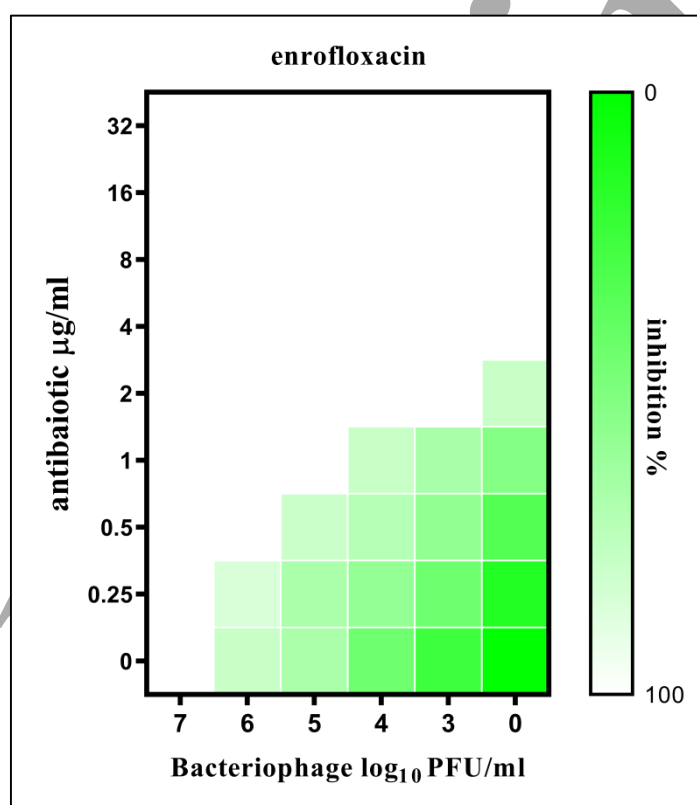


Figure (1): Synergistic effect between the antibiotic enrofloxacin and bacteriophage

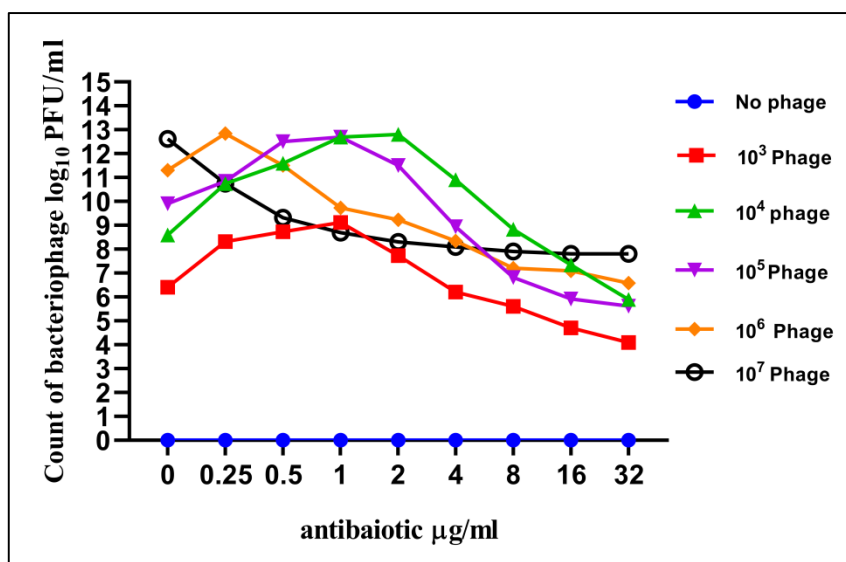


Figure (2): Bacteriophage count upon synergy with the antibiotic enrofloxacin

The results of Shiga toxin concentration estimation in the medium also show significant differences at the level ($p \leq 0.05$) between the different groups. The results of the study showed that exposing Shiga toxin-secreting *E. coli* to the antibiotic enrofloxacin increases Shiga toxin secretion, whether alone as in group 2. The results also showed that bacteriophage treatment alone led to the elimination of *E. coli* without inducing toxin secretion, as low concentrations of Shiga toxin were observed in group 3 compared to groups 1 and 2. As for bacteriophage treatment in synergy with the antibiotic enrofloxacin, it was the lowest of all study groups after 4 hours of treatment, as a low level of Shiga toxin concentration was shown, as illustrated in Table (1).

Table (1): Shiga toxin concentration in the different study treatments

Group	Toxin concentration (ng/ml)			
	2 hours	4 hours	6 hours	8 hours
Group 1	$0.5^a \pm 0.2$	$3.2^a \pm 1.3$	$4.8^a \pm 1.4$	$6.36^a \pm 1.9$
Group 2	$3.72^b \pm 1.1$	$7.46^b \pm 2.3$	$10.74^b \pm 3.2$	$16.83^b \pm 3.7$

Group 3	0.4 ^a ± 0.2	2.12 ^c ± 0.7	2.74 ^c ± 1.1	2.86 ^c ± 1.9
Group 4	0.9 ^a ± 0.8	1.68 ^c ± 0.7	1.92 ^d ± 0.6	2.04 ^d ± 0.9

Different letters a, b, c, d indicate significant differences at the level of $p \leq 0.05$

4. Discussion

Infection with hemolytic uremic syndrome (HUS) resulting from infection with Shiga toxin-producing *E. coli* represents one of the most serious complications of this bacterial infection. The therapeutic challenge lies in the mechanism of bacterial toxin secretion that causes this syndrome, as the use of chemicals or antibiotics is contraindicated in these cases, since the use of these substances may lead to exacerbation of the infection and deterioration of the patient's health condition [17].

Bacteriophage therapy has emerged as a promising alternative to antibiotics, as its mechanism of bacterial killing occurs without stimulating the bacterial SOS response and thus without stimulating Shiga toxin production, which stops the infection cycle and breaks the toxin production cycle. The bacterial SOS response is a system for repairing partial damage to bacterial cell DNA. Antibiotics, especially agents that damage DNA such as fluoroquinolones, are strong inducers of the SOS response [18].

Antibiotics inadvertently induce the SOS response, leading to several concerning outcomes. This system is activated by the RecA protein, which recognizes damage sites in DNA by exploring single-stranded regions, thus leading to self-cleavage of the LexA transcriptional repressor, resulting in activation of SOS response genes that ultimately lead to DNA repair and mutations. Additionally, activated RecA protein stimulates the self-cleavage of the repressor protein of the temperate bacteriophage encoding *stx* genes, thus removing repression from bacteriophage genes, leading to induction of the bacteriophage lytic cycle and excision of its genes from the genome, exiting into the cytoplasm to form circular DNA to begin expressing its genes to form bacteriophage proteins, including *stx* genes, resulting in increased transcription and expression of *stx* genes, thus increasing Shiga toxin production that accumulates in the periplasmic space [19]. At the end of the bacteriophage lytic cycle, it leads to expression of cell wall lytic genes, resulting in bacterial cell lysis and release of replicated bacteriophages, along with large amounts of Shiga toxin.

The danger of using antibiotics in HUS cases of STEC infection has been demonstrated in both laboratory studies and clinical observations [9].

The results of a study by Van Hoorde et al [20] showed that applying ciprofloxacin to STEC resulted in a significant increase in Shiga toxin *stx*₂ secretion, reaching 41 times the toxin secretion without antibiotic treatment. A study by Matsushiro et al [21] also showed that subinhibitory concentrations of norfloxacin induce bacteriophages carrying Shiga toxin genes in *E. coli* O157:H7 to enter the lytic cycle, leading to a 100- to 1000-fold increase in toxin gene expression, duplication of *stx* gene copies, and production of large amounts of toxins (*stx*₂ was 10 times higher than *stx*₁). In a study by Bielaszewska et al [22], ciprofloxacin caused significant induction of bacteriophages carrying *stx*₂ Shiga toxin genes, leading to increased toxin gene transcription in EHEC O104:H4 strain. These effects were observed at all tested subinhibitory concentrations (1/2 MIC to 1/16 MIC) and peaked at 1/2 MIC. Given these results, the researchers recommended avoiding the use of ciprofloxacin in patients with diarrhea suspected to be STEC infection because it increases the development of hemolytic uremic syndrome. In a study by Rehman et al [23] that examined the effect of antibiotics on inducing *stx*₁ and *stx*₂ Shiga toxin genes in 15 clinical isolates of STEC, variation in the intensity of the effect was observed according to the type of antibiotic. Tigecycline caused the highest increase in toxin gene expression, reaching an 8-fold increase compared to untreated isolates, while ciprofloxacin stimulated toxin production with an increase of up to 6.5-fold. In contrast, fosfomycin showed weak induction not exceeding two-fold, while meropenem had the least effect overall. The study results also indicated that antibiotic-resistant bacterial isolates were more prone to increased toxin production compared to sensitive isolates. In a study by Maurer et al [24], enrofloxacin was applied to *E. coli* O157:H7, and the results showed that antibiotic treatment led to a three-fold increase in toxin secretion in enrofloxacin-resistant bacteria compared to non-resistant strains after antibiotic induction.

Based on the need to treat STEC infection without inducing toxin production, without inducing the SOS response, therapy with virulent bacteriophages (lytic cycle) has been proposed. The bacteriophage binds to a specific receptor on the bacterial surface and injects its own DNA, which carries genes encoding its proteins, to take over the bacterial cell and use it to synthesize its proteins. At the end of protein synthesis, maturation, and assembly, genes on the bacteriophage DNA are expressed to produce enzymes that lyse the bacterial cell wall and release nascent bacteriophages to infect other bacterial cells [22].

Armstrong [7] reported that the use of virulent bacteriophages kills bacteria without inducing the SOS response, representing a safe alternative to antibiotics. In a study by Cho et al., [11], the ability of virulent bacteriophages to combat Shiga toxin-producing *E. coli* (STEC) infection without inducing toxin production was evaluated. The results showed that virulent bacteriophages reduced bacterial counts by 3 log₁₀ within two hours, at a level similar to ciprofloxacin. While ciprofloxacin increased *stx*₂ gene expression and induced the SOS response, virulent bacteriophages did not induce toxin gene expression or its production at the protein level as detected by Western blot. Vero cell toxicity tests also showed that toxin levels in the bacteriophage-treated group were 1.2-1.3 times lower compared to the ciprofloxacin-treated group.

A study by Howard-Varona et al [25] addressed the possibility of using bacteriophage therapy to eliminate Shiga toxin-producing *E. coli* O157:H7 in vitro without causing the increase in toxin production typically associated with antibiotics. This was tested by adding two bacteriophages, p000v and p000y, to STEC cultures. The results showed a significant decrease in *stx2a* gene copy number after 7.5 hours of treatment. While copies increased 8-fold in untreated samples, they decreased to 0.3 and 0.4 of their initial values in samples treated with p000v and p000y bacteriophages, respectively.

The ability of virulent bacteriophages to eliminate STEC without inducing toxin production is attributed to their rapid lytic cycle and cell lysis, which differs fundamentally from antibiotic-mediated killing. Bacterial cells undergo rapid lysis mediated by bacteriophage-encoded endolysins, as the process of injecting bacteriophage genetic material into bacteria, taking over the cell, and beginning bacteriophage protein production is faster than activating the SOS response responsible for temperate bacteriophage entry into the lytic cycle, thus preventing activation of this cycle and eliminating bacteria with minimal amounts of bacteriophage-encoded toxin [18].

It is also believed that the synergy between bacteriophages and antibiotics is due to the inability of bacteria to develop resistance to both simultaneously. When bacteria are exposed to bacteriophages or antibiotics, they quickly begin to form resistance, primarily by hiding receptors on the bacterial cell wall. Therefore, the half-inhibitory concentration of the antibiotic induces bacteria to change their cell wall structure to develop resistance against it, which allows for further bacteriophage infection and bacterial elimination.

5. Conclusions:

We conclude from this study that bacteriophage are a potentially safe alternative to antibiotics, partially or completely, especially in cases of infection with *E. coli* that cause HUS.

Acknowledgments:

AI tools were used to assist with language refinement only, and were not used for data analysis or interpretation of results.

Authors' contributions:

A.ALBAKER and A. HALLAK performed the laboratory experiment, while M. SALEH performed the statistical analysis and wrote the research.

Conflict of interest:

The authors declare no conflict of interest.

Funding:

The main funder of this research is the University of Hama, which provided the laboratories in which the research was conducted.

Data availability:

All data are provided in the revised manuscript.

References

- 1) Jaaz WS, Kadham EJ. Identification of virulence factors genes (*eaeA*) and (*bpfA*) of Enteropathogenic Escherichia coli strains isolated from children with diarrhea in Kerbala city. Perinat J. 2026;34(1):96-102. <https://doi.org/10.57239/prn.26.03410013>

- 2) Bibbal D, Ruiz P, Sapountzis P, Mazuy-Cruchaudet C, Loukiadis E, Auvray F, et al. Persistent circulation of enterohemorrhagic *Escherichia coli* (EHEC) O157: H7 in cattle farms: Characterization of enterohemorrhagic *Escherichia coli* O157: H7 strains and fecal microbial communities of bovine shedders and non-shedders. *Front Vet Sci.* 2022;9:852475. <https://doi.org/10.3389/fvets.2022.852475>
- 3) Tamburini G, Daprai L, Gazzola A, Rossetti D, Tomassini E, Dilio G, et al. Epidemiology of Bloody Diarrhea, Shiga Toxin-producing *Escherichia coli* and Hemolytic Uremic Syndrome in Children. *Pediatr Infect Dis J.* 2026;10-1097. <https://journals.lww.com/pidj/pages/currenttoc.aspx>
- 4) Dato L, Mancuso MC, Daprai L, Ria T, Rossetti D, Callegaro A, Ardissino G. Bloody diarrhea, STEC infection, and HUS in the molecular microbiology era. *Pediatr Nephrol.* 2026;41(4):973-980. <https://doi.org/10.1007/s00467-025-06930-y>
- 5) Hanke J, Wardzyńska B, Bendykowska MJ, Lipińska-Opalka A. From Shiga toxin-producing *Escherichia coli* infection to haemolytic uraemic syndrome—bacterial, host and iatrogenic factors in children. *Paediatr Fam Med.* 2026;22(1):14-25. <https://doi.org/10.15557/PiMR.2026.0003>
- 6) Sevilla P, Martínez A, Nuevo DV, Córdoba JG, Camarena JJ. Impact of antibiotic treatment on hemolytic uremic syndrome risk in *Escherichia coli* O157 infections: a comprehensive systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis.* 2025;44(8):1763-1778. <https://doi.org/10.1007/s10096-025-05145-1>
- 7) Armstrong GD. Virulent Bacteriophages for Controlling Shiga Toxin-Producing *Escherichia coli* (STEC) Without Inducing Toxin Production. *J Infect Dis.* 2026;233(1):1-2. <https://doi.org/10.1093/infdis/jiaf036>
- 8) Alconcher LF, Balestracci A, Lucarelli LI, Bronfen S, Meni Battaglia L. Improving hemolytic uremic syndrome diagnosis in children: the role of serology for Shiga toxin-

producing *Escherichia coli* detection. *Pediatr Nephrol.* 2026;1-9.
<https://doi.org/10.1007/s00467-025-07128-y>

9) Freedman SB, Xie J, Neufeld MS. Shiga toxin-producing *Escherichia coli* infection, antibiotics, and risk of developing hemolytic uremic syndrome: a meta-analysis. *Clin Infect Dis.* 2016;62(10):1251-1258. <https://doi.org/10.1093/cid/ciw099>

10) Faizal A, Niu YD. Inhibition of Shiga toxin-producing *Escherichia coli* O157: H7 attachment to human intestinal cells by single or combined lytic bacteriophages. *Can J Microbiol.* 2026;72:1-13. <https://doi.org/10.1139/cjm-2025-0172>

11) Cho E, Kim J, Won T, Ryu S, Jeon B. Virulent bacteriophages for controlling Shiga toxin-producing *Escherichia coli* without inducing toxin production. *J Infect Dis.* 2026;233(1):87-95. <https://doi.org/10.1093/infdis/jiaf035>

12) Albaker AK, Saleh M, Alsaleh A. Detection of virulence factors of *Escherichia Coli* strains isolated from calves diarrhea and their resistance to antibiotics in Hama Governorate, Syria. *Open Vet J.* 2025;15(12):6237.
<http://dx.doi.org/10.5455/OVJ.2025.v15.i12.8>

13) Fikadu A, Amankwah S, Alemu B, Alemu Y, Naga A, Tekle E, et al. Isolation and phenotypic characterization of virulent bacteriophages against multidrug-resistant *Escherichia coli* and its phage-resistant variant from sewage sources. *Infect Drug Resist.* 2024;17:293-303. <https://doi.org/10.2147/IDR.S441085>.

14) Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and in vitro evaluation of bacteriophages against MDR bacterial isolates from septic wound infections. *PLoS One.* 2017;12(7):1-16. <https://doi.org/10.1371/journal.pone.0179245>.

15) Kavanagh A, Ramu S, Gong Y, Cooper MA, Blaskovich MA. Effects of microplate type and broth additives on microdilution MIC susceptibility assays. *Antimicrob Agents Chemother.* 2019;63(1):10-1128. <https://doi.org/10.1128/aac.01760-18>

- 16) Alexyuk PG, Bogoyavlenskii AP, Akanova KS, Moldakhanov YS, Kerimov TT, Sokolova NS, Berezin VE, Alexyuk MS. Isolation of Lytic Bacteriophages of *Escherichia coli* and Their Combined Use with Antibiotics Against the Causative Agents of Colibacillosis in Calves. *Vet Sci.* 2025;12:817. <https://doi.org/10.3390/vetsci12090817>
- 17) Liu Y, Thaker H, Wang C, Xu Z, Dong M. Diagnosis and treatment for Shiga toxin-producing *Escherichia coli* associated hemolytic uremic syndrome. *Toxins.* 2022;15(1):10. <https://doi.org/10.3390/toxins15010010>
- 18) Sahu R, Singh AK, Kumar A, Singh K, Kumar P. Bacteriophages concept and applications: A review on phage therapy. *Curr Pharm Biotechnol.* 2023;24(10):1245-1264. <https://doi.org/10.2174/1389201024666221104142457>
- 19) Rostøl JT, Chmielowska C, Marina A, Penadés JR. Revisiting the life cycle of temperate phages. *Nat Rev Microbiol.* 2026;1-13. <https://doi.org/10.1038/s41579-026-01318-7>
- 20) Van Hoorde S, Vereecke N, Sperling D, He X, Vanbeylen E, Van Denberghe E, et al. External factors influence intrinsic differences in Stx2e production by Porcine Shiga Toxin-producing *Escherichia coli* strains. *PLoS Pathog.* 2025;21(10):e1013616. <https://doi.org/10.1371/journal.ppat.1013616>
- 21) Matsushiro A, Sato K, Miyamoto H, Yamamura T, Honda T. Induction of prophages of enterohemorrhagic *Escherichia coli* O157:H7 with norfloxacin. *J Bacteriol.* 1999;181(7):2257-2260. <https://doi.org/10.1128/jb.181.7.2257-2260.1999>
- 22) Bielaszewska M, Idelevich EA, Zhang W, Bauwens A, Schaumburg F, Mellmann A, et al. Effects of antibiotics on Shiga toxin 2 production and bacteriophage induction by epidemic *Escherichia coli* O104: H4 strain. *Antimicrob Agents Chemother.* 2012;56(6):3277-3282. <https://doi.org/10.1128/aac.06315-11>

- 23) Rehman A, Andleeb S, Ullah SR, Mustafa Z, Gul D, Mehmood K. Antibiotic-mediated expression analysis of Shiga toxin 1 and 2 in multi-drug-resistant Shiga toxigenic *Escherichia coli*. *Folia Microbiol.* 2021;66(5):809-817. <https://doi.org/10.1007/s12223-021-00882-0>
- 24) Maurer C, Meunier D, Madec JY. Shiga Toxin Stx2 Production Is Promoted by Enrofloxacin in Experimental In Vitro–Selected Mutants of *Escherichia coli* O157: H7 Resistant to Fluoroquinolones. *Foodborne Pathog Dis.* 2009;6(2):257-259. <https://doi.org/10.1089/fpd.2008.0165>
- 25) Howard-Varona C, Vik DR, Solonenko NE, Li YF, Gazitua MC, Chittick L, Samiec JK, Jensen AE, Anderson P, Howard-Varona A, Kinkhabwala AA, Abedon ST, Sullivan MB. Fighting shiga-toxin producing *Escherichia coli* O157:H7 with virulent bacteriophages. *Antibiotics.* 2018;7(4):101. <https://doi.org/10.3390/antibiotics7040101>