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Isolation of broad-host-range bacteriophages against food- and patient-derived Shiga toxin-producing *Escherichia coli*

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ABSTRACT

This study aimed to isolate bacteriophages specific to Shiga toxin-producing *Escherichia coli* (*E. coli*) strains, particularly EHEC O157:H7, in order to develop a collection of phages against different *E. coli* pathotypes isolated from northeast of Iran. Eighteen samples were screened without any preliminary enrichment and also with small scale enrichment using *E. coli* 12900, which did not result in the phage recovery. Seven samples were prepared with an extensive enrichment. Of them, 5 samples produced plaques. Eventually, seven phages out of thirteen isolated phages were selected for phage host range investigation. Results of the spotting host range assay demonstrated that 22 pathogenic *E. coli* strains and isolates (54%) were susceptible to at least one of the phages. Phage Ecol-MHD1 was polyvalent against *E. coli* and *Salmonella* isolates. The other phages were specific to *E. coli* pathotypes. In conclusion, the phages isolated in this study can be suggested as preventive or therapeutic candidates against foodborne *E. coli* infections in humans.

Keywords

Shiga toxin-producing E. coli (STEC), Bacteriophage, Host range, Enrichment

Abbreviations

STEC: Shiga toxin-producing *Escherichia coli*
EHEC: Enterohemorrhagic *Escherichia coli*
ABR: Antibiotic-resistant

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Introduction

Escherichia coli (*E. coli*) is the most frequently reported cause of foodborne illnesses (~111 million) worldwide [1]. In 2017, Shiga toxin (Stx)-producing *E. coli* (STEC; also known as verotoxin-producing *E. coli*) caused 2050 cases of infections in the United States [2] and 6,073 cases of infections and 20 deaths in Europe [3].

Pathogenic *E. coli* strains are categorized into six diarrheagenic pathotypes [4]. Of those, STEC can lead to severe gastrointestinal infections, including hemorrhagic colitis or even life-threatening complication of hemolytic uremic syndrome (HUS) [5,6]. The subset of STEC, which is highly pathogenic in humans and has the potential to cause HUS, is named enterohemorrhagic *E. coli* (EHEC) [6]. EHEC outbreaks are linked to the consumption of raw or undercooked contaminated foods such as ground meat products, milk and cheeses, and vegetables and sprouts [7].

Although, there are numerous natural and chemical preservatives or treatments for combating foodborne pathogens, the important thing to watch for is that foodborne illness outbreaks are still occurring and even causing human death [8]. On the other hand, broad-spectrum antibiotics and chemical interventions that are commonly used for treating or preventing infections could lead to antibiotic resistance or side effects on human health [9–11]. These factors intensify a need for an alternative antimicrobial technique for controlling foodborne pathogens or treating their associated diseases. Bacteriophages are biological tools that specifically target pathogens. They are safe and non-toxic to human cells and do not influence the quality and organoleptic properties of foods [12,13]. Therefore, phages can be possible alternatives to antibiotics [14,15] and chemical food preservatives [13,16]. Recently, many studies have been conducted on phage-mediated biocontrol of pathogens and phage therapy. These reports emphasize the significance of isolating novel phages, as well as the determination of phage organismal properties, especially phage host range [17]. Specifying the range of targeted bacteria is a primary requirement for phage biocontrol and therapy, and also for developing efficient phage cocktails that could infect a desirable spectrum of bacteria [15,18]. Although commercial phage preparations against *E. coli* have been approved for food safety applications in the United States and Europe [19,20] and registered as therapy in Russia [21], Georgia, and Poland [22], further phage preparations for reduction of foodborne zoonoses are urgently needed in the developing countries. In the developing countries, not only the prevalence of infectious diseases is higher [23], but also some of the common infections such as those caused by diarrheagenic *E. coli* in children demonstrate high or absolute resistance to current antibiotics [24,25].

This study aimed to isolate bacteriophages specific to STEC strains, in particular, EHEC O157:H7 and to determine their host range for developing a collection of phages against pathogenic *E. coli* strains isolated from northeast of Iran. Additionally, in terms of phage isolation, the influence of methodology on the yield of phage recovery has been reported. The current study could eventually lead to the creation of a bacteriophage cocktail that would be a potential antibacterial against pathogenic *E. coli* isolates for safeguard in food chain or therapy.

Results

Preparation without enrichment and with small scale enrichment

A screening for STEC-infecting phages was performed on filtrates of 18 samples (out of 25; group A in Table 3) directly and following enriching 100 µl of them in combination with *E. coli* O157:H7 ATCC 12900. Neither of the methods led to positive results, and none of 18 samples lysed the lawn of *E. coli* 12900, and field- and patient-derived isolates by either spotting assay or double agar overlay plaque assay.

Preparation with extensive enrichment

Screening of *E. coli*-infecting phages in the remaining samples (7 out of 25 samples; group B in Table 3) was conducted following enriching 30 ml of each sample in combination with *E. coli* O157:H7 ATCC 12900, and three pathogenic food- and patient-derived isolates. According to the results of spot assay in preliminary screening, five samples (septic wastewater of a veterinary clinic, city aqueduct, mixed minced meat, cow cartilage, and vegetables) out of seven (71%) lysed at least one of the tested bacteria. Negative samples were beef and fat that were collected from a cattle slaughterhouse.

Isolation and purification of bacteriophages

Filtrates taken from complete clear spots in preliminary screening were applied for isolating STEC-infecting phages by using the most susceptible hosts based on the spot assay results.

By applying different hosts in double agar overlay plaque assay, 13 STEC-infecting phages recovered from five positive samples. Considering the stability, titer, and clarity of plaques, seven phages (Ecol-MHD1 to Ecol-MHD7) were selected for propagation and further phage host range investigation. Septic wastewater of a veterinary clinic was the richest source that

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yielded six phages, including Ecol-MHD4 to MHD7, followed by city aqueduct that resulted in recovering four phages, including Ecol-MHD1 and Ecol-MHD2. Ecol-MHD3 was isolated from minced meat. Plaque sizes ranged from pinpoint to 3.5 mm, as shown in Figure 1. After phage propagation for expanding the phage titer, the final titer of phage suspensions reached 10^8 to 10^9 PFU/ml.

Determination of phage host range

Spotting host range assay was applied for the exploration of the host spectrum of phages. The results (Figure 2) demonstrated that 22 pathogenic *E. coli* strains and isolates (54%) were susceptible to at least one of the phages. As shown in Figure 2, phage *Ecol*-MHD1 was polyvalent and caused lysis on the lawn of 15 *E. coli*, including different pathotypes and also 3 *Salmonella* isolates. The other six phages were specific to *E. coli* pathotypes. Phage Ecol-MHD4 showed a broad spectrum of lytic activity against 15 isolates, followed by Ecol-MHD7 (13 hosts), Ecol-MHD2 (8 hosts), and Ecol-MHD6 (6 hosts), respectively. The narrowest host range belonged to Ecol-MHD3 and Ecol-MHD5, which were only specific to their original host (STEC m145). Whereas, both latter phages in comparison with other isolated phages, produced the largest plaques (~3.5 mm) (Figure 1-A). The phage cocktail could lyse the lawn of 20 *E. coli* strains and one *Salmonella* isolates. Among the phages, only Ecol-MHD2 and Ecol-MHD7 were effective on *E. coli* O157:H7 NCTC12900. The heat-map of host ranges is shown in Figure 2.

Discussion

Developing antibiotic resistance is one of the global concerns in recent years. Bacteriophages could be promising alternatives to antibiotics and food antibacterial agents. Therefore, investigation of novel bacteriophages that are efficient on antibiotic-resistant pathogens is an urgent need, particularly in the developing countries [23]. In our study, we could not isolate any STEC-specific phages from various samples (Table 3, group A) by plating them without enrichment and with small scale enrichment. Similar to our results, Oot et al. [26] could not isolate any *E. coli* O157-infecting phages out of 60 samples by using direct plating without enrichment. These results demonstrated that the levels of O157:H7-infecting phages in environmental samples could be extremely low [26,27]. However, there are some reports to approve the isolation of *E. coli*-infecting phages by applying direct plating protocol, from sewage effluent, wastewater [28,29], and stool samples [30] using other *E. coli* strains instead of 12900 as the indicator host.

It has been suggested that for recovering broad-spectrum phages, which are particularly virulent against desirable bacteria, a volume of sample greater than 1 ml, a variety of host bacteria, and multiple cycles of selection [31], as well as an extensive enrichment, is necessary [27]. Therefore, an extensive enrichment by using different *E. coli* strains and isolates was applied to some of the untested raw samples (Table 3, group B). The results demonstrated that 5 samples out of 7 contained *E. coli*-infecting phages.

Samples taken from cattle fecal slurries are the richest sources for isolating *E. coli*-specific phages [32,33]. Phages in our study were recovered from wastewater, minced meat, cattle cartilage, and vegetables, with the highest concentration and variety in the septic wastewater of a veterinary clinic. In contrast with our data, in a study, no *E. coli* O157:H7-infecting phages were detected in meat and vegetables [29].

According to the results from host range assay, as shown in Figure 2, five phages were efficient on a broad spectrum of *E. coli* isolates including EHEC, STEC, EAEC, EPEC and ETEC pathotypes and were

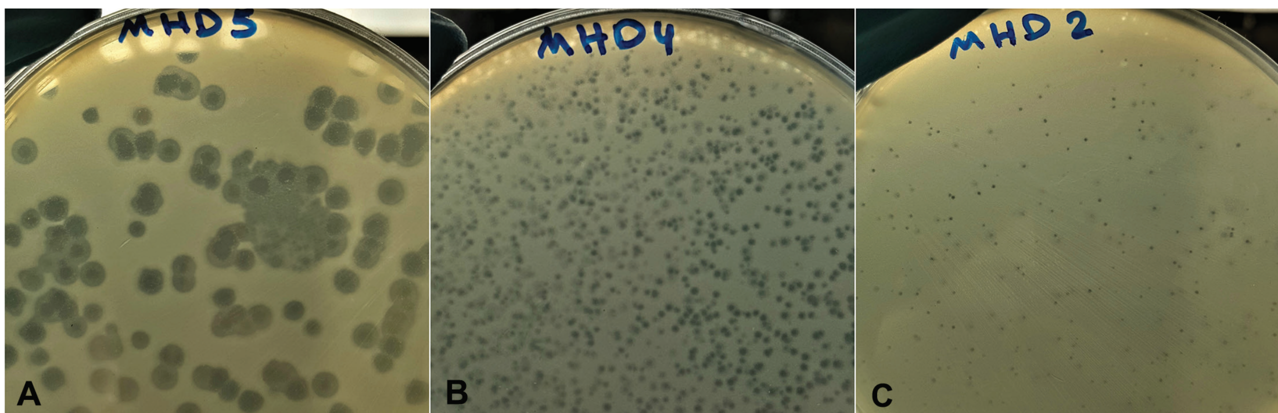

Figure 1.
Different sizes of plaques produced by some of isolated phages such as Ecol-MHD5 (A), *Ecol*-MHD4 (B) and Ecol-MHD2 (C).

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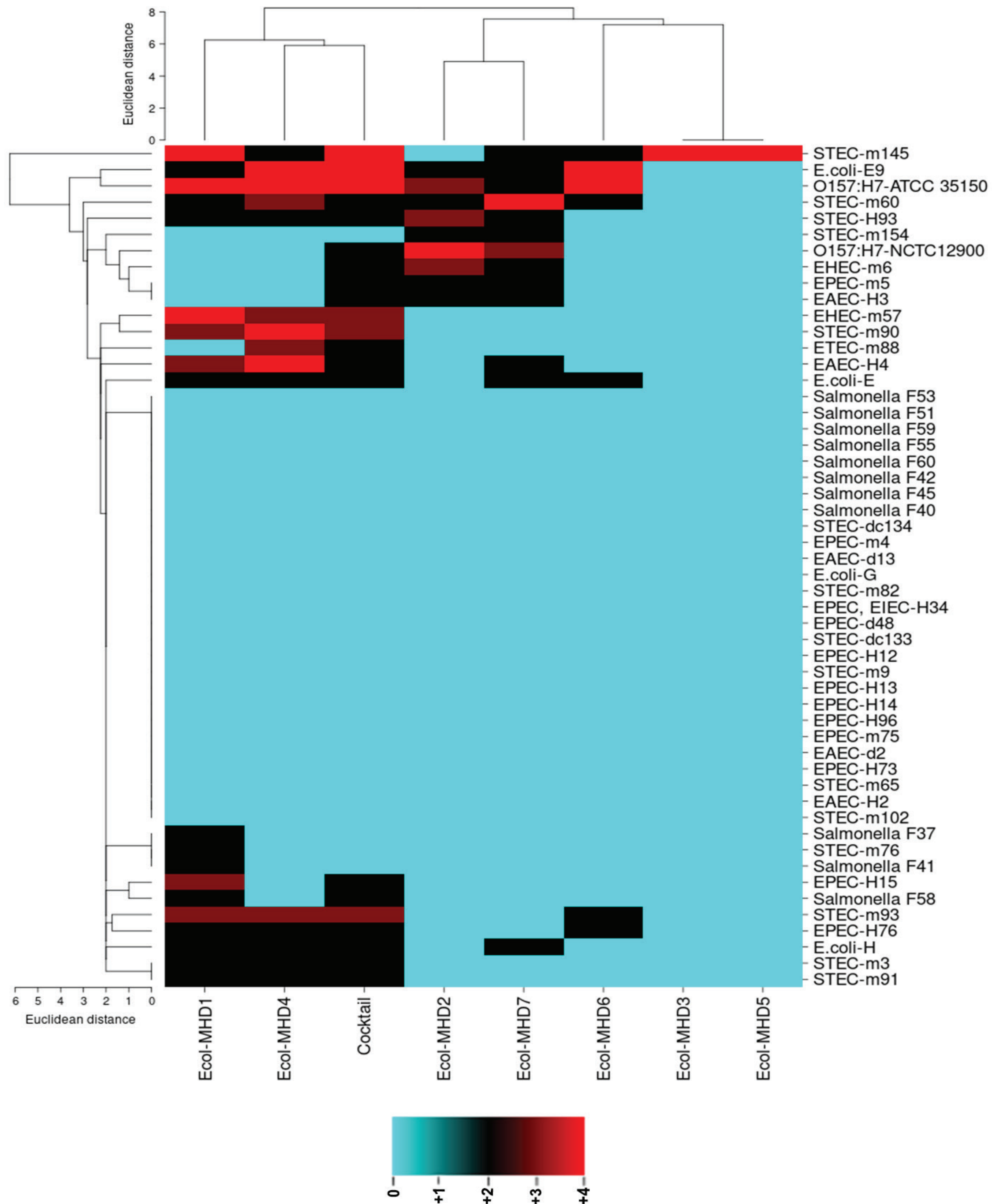

Figure 2.
Heat-map of host range of isolated phages; phages are displayed on the x-axis and bacterial isolates and strains on the y-axis. The degree of lysed lawn scored from +4 to 0. +4: complete clearing, +3: clearing throughout but with faintly hazy background, +2: substantial turbidity throughout the cleared zone, +1: a few individual plaques, 0: no clearing – but a spot may be seen where the pipette tip touched the agar.

highly infective for *E. coli* O157:H7 35150 (presence of eaeA, stx1, and stx2 genes), while only two could infect *E. coli* O157:H7 12900 (stx negative). This implies that the prevalence of *E. coli* O157:H7-specific phages could be higher than if *E. coli* 12900 was the only strain using in phage isolation procedures. In agreement with our findings, in a study conducted by Oot et al. [26] , samples that were negative by using *E. coli* 12900 for phage isolation, yielded 93% phage recovery by changing the indicator host.

Phage Ecol-MHD1 was polyvalent against a variety of field- and patient-derived pathogenic *E. coli* (33%) and three *Salmonella* isolates (out of 11), as shown in Figure 2.

Many phages specific to *E. coli* [34–40], and polyvalent phages against *E. coli* and *Salmonella* strains

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[33,41,42] have been previously reported. However, the risk of developing EHEC complications such as HUS depends on virulence factors of EHEC strains as well as host and environmental factors [6]. In view of this fact, the regional prevalence of pathogens and the emergence of antibiotic resistance should be taken into account for evolving the phage biocontrol approach. To our knowledge, this study was the first attempt in Iran to investigate effective phages against pathogenic food- and patient-derived *E. coli* and *Salmonella* isolates from northeast of Iran.

In conclusion, the phages isolated in this study, in particular Ecol-MHD1, MHD4, MHD7, MHD2, and MHD6, can be suggested as potential candidates for phage biocontrol approach and therapy. They could be used in phage cocktails or combination with other techniques as preventive or therapeutic agents against foodborne E. *coli* infections in humans.

Material and methods

Bacterial strains

A total of 52 bacterial strains and isolates, including two standard EHEC, 39 pathogenic *E. coli*, and 11 *Salmonella* isolates used in this study were provided by a simultaneous study (unpublished study). Strains and isolates, their pathotypes, origins, and antibiotic resistance profiles are shown in Tables 1 and 2.

Sampling

25 samples with a variety of sources from wastewater and cow feces to meat and vegetables (Table 3) were collected to be screened for STEC-infecting phages. Of them, 18 samples were prepared both without enrichment and with enriching a small volume of filtrates and categorized as group A in Table 3. While the remaining samples (n=7) were prepared with an extensive enrichment and categorized as group B (Table 3). Sampling occurred during spring and summer in Mashhad and its suburbs.

Sample preparation for bacteriophage screening

Sample preparation procedures are briefly explained as follow:

1. **Without enrichment.** In order to isolate diverse phages with no biases, samples in group A were prepared without enrichment for direct plating. Initial preparation was accomplished, as was described by Gill and Hyman's [43] with some modifications. Briefly, liquid samples (30 ml) were centrifugated directly, while solid samples (5 ml) centrifugated following soaking in 30 ml sodium chloride-magnesium sulfate (SM) buffer (5.8 g NaCl, 2.0 g $MgSO_4$ x 7 H_2O, 50 ml 1 M Tris (Merck, MSD, Darmstadt, Germany) pH 7.5, filled up with distilled water to 1000 ml) and overnight incubation in the refrigerator. The centrifugation was conducted initially for 20 min at 3500 rpm in order to sediment large particles and debris, and then 2 ml of supernatants were centrifuged (Sigma 1-14, GmbH, Germany) for 10 min at 13000 rpm. Supernatants were then filtered through 0.22-micrometer membrane filters (MS® PES, Membrane Solutions, LLC, USA), and filtrates were stored in the refrigerator until further use.

2. **Small scale enrichment.** 100 µl of filtrates from group A samples was added to 5 ml LB broth (Merck, MSD, Darmstadt, Germany) containing 1mM $CaCl_2$ (Merck, MSD, Darmstadt, Germany) and 100 µl overnight culture of *E. coli* O157:H7 NCTC 12900. The suspensions were incubated in an incubator (GFL 30131, mbH, Burgwedel, Germany) shaking at 50 rpm for 24 h at 37 °C. Then they were centrifuged for 10 min at 13000 rpm and afterward filtered through 0.22-micrometer membrane filters. Filtrates stored at 4 °C and used for further phage screening.

3. **Extensive enrichment.** In group B, samples were enriched following the methodology of Van Twest and Kropinski [44] with some modifications. In brief, liquid samples (50 ml) were centrifugated for 30 min at 3500 rpm, and supernatants (30 ml) were mixed with double strength LB broth (30 ml) supplemented with 1mM $CaCl_2$. The mixture was then inoculated with 100 µl of overnight culture of desired bacterial strains including *E. coli* O157:H7 12900, E9 (Hospital isolate), m145 (antibiotic-resistant (ABR) STEC), and m6 (ABR EHEC) and incubated for 48 – 72 h at 37 °C while shaking at 50 rpm. Solid samples (10 ml) were added to SM buffer (40 ml) and kept overnight in the refrigerator. They were then centrifuged for one hour at 3500 rpm, and the supernatants were enriched in the same manner. After the incubation period, all samples were individually filtered through 0.22-micrometer membrane filters, and filtrates were stored at 4 °C until further use.

Sample screening for STEC-infecting bacteriophages

All filtrates were initially screened for the presence of phage by applying spotting assay, as described by Akhtar et al. [45] with some modifications. For this purpose, several bacterial strains and isolates including *E. coli* O157:H7 NCTC 12900 and ACTC 35150, m3 (ABR STEC), m6 (ABR STEC), m57 (ABR EHEC), m60 (STEC), m90 (ABR STEC), m91 (ABR STEC), m145 (ABR STEC), and E9 (Hospital isolate) (presented in Table 1) were cultivated in LB broth supplemented with 1mM $CaCl_2$. 100 µl of their overnight cultures was inoculated into 5 ml molten LB overlay agar (0.4% agar; Quelab, Inc, Montréal, Canada), and the mixture was overlaid on 1.5% LB underlay agar supplemented with 1mM $CaCl_2$. The overlay agar was allowed to solidify at room temperature. Subsequently, 10 µl of each sample was spotted on the top agar and left at room temperature until the drop dried and then incubated for 24 h at 37 °C. Samples that produced clear zones or single plaques on any of the bacterial lawns were considered as positive and selected for phage isolation. Complete clear spots were picked and kept for 24 h in SM buffer in the refrigerator, and after centrifugation and microfiltration, filtrates used for further procedure of phage isolation. All negative samples were rescreened for plaque formation on the lawn of *E. coli* 12900 by using double agar overlay plaque assay.

Isolation, purification, and propagation of bacteriophages

For phage isolation and purification, positive samples were investigated by using double agar overlay plaque assay, as it was described by Kropinski et al. [46]. Briefly, 100 µl of the overnight culture of host *E. coli* isolates and 100 µl of filtrate from each positive sample was inoculated into 5 ml 0.4% Luria-Bertani (LB) overlay agar, then plated on 1.5% LB underlay agar supplemented with 2mM $CaCl_2$ and incubated at 37 °C overnight. Individual plaques were picked by a sterile needle, soaked in 1ml SM buffer, and stored in the refrigerator for 24 h. Phages were purified by centrifugation of the suspensions for 10 min at 13000 rpm followed by sterile filtration. The filtrates were plated a minimum of three times to obtain purified phages.

Phage propagation was accomplished following the methodology of Viazis et al. [33] with some modifications. In brief, 100 µl of each phage at titers of 10^4 to 10^6 PFU/ml along with 100 µl of the

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Table 1.
Characteristics of *E. coli* strains.

E. coli	Origin	Pathotype	Antibiotic resistance profile		
			Resistant	Intermediate	Susceptible
d2	raw milk	EAEC	CTX	CAZ, FEP, GEN, AMP, CHL, IPM, AZM	FOX, CIP, SXT, TET
d13	raw milk	EAEC	AMP	CTX, FEP	CAZ, FOX, CIP, GEN, SXT, TET, CHL, IPM, AZM
d48	raw milk	EPEC	CTX, CAZ, AMP	FOX, AZM	FEP, CIP, GEN, SXT, TET, CHL, IPM
dc133	cheese	STEC	CTX, AMP, IPM	CAZ, GEN, CHL, AZM	FEP, FOX, CIP, SXT, TET
dc134	cheese	STEC	CTX, CAZ, FEP, FOX, TET	IPM	CIP, GEN, SXT, AMP, CHL, AZM
m3	minced meat	STEC	FEP, FOX, SXT, AMP, TET, CHL, IPM	CTX, CAZ, GEN	CIP, AZM
m4	lamb	EPEC	CTX, CAZ, CIP, SXT, AMP, TET, CHL	FOX, GEN, IPM	FEP, AZM
m5	minced meat	EPEC	CIP, GEN, SXT, AMP, TET	CTX, CAZ	FEP, FOX, CHL, IPM, AZM
m6	lamb	EHEC	CTX, CIP, SXT, AMP, TET	CAZ, GEN	FEP, FOX, CHL, IPM, AZM
m9	minced meat	STEC	CTX, CIP, GEN, SXT, AMP, TET, AZM	CAZ, FEP	FOX, CHL, IPM
m57	lamb	EHEC	FEP, FOX, SXT, AMP, TET, CHL, IPM	CTX, CAZ, GEN	CIP, AZM
m60	lamb	STEC	-	AMP	CTX, CAZ, FEP, FOX, CIP, GEN, SXT, TET, CHL, IPM, AZM
m65	lamb	STEC	CTX, CAZ, FEP, GEN, SXT, AMP, TET, CHL, AZM	-	FOX, CIP, IPM
m75	minced meat	EPEC	CTX, CIP, SXT, AMP, TET	GEN, IPM, AZM	CAZ, FEP, FOX, CHL
m76	minced meat	STEC	CTX, CAZ	GEN, AMP, CHL, AZM	FEP, FOX, CIP, SXT, TET, IPM
m90	lamb	STEC	TET, CHL	CAZ	CTX, FEP, FOX, CIP, GEN, SXT, AMP, IPM, AZM
m91	lamb	STEC	CTX, AMP, TET	CAZ, GEN, CHL	FEP, FOX, CIP, SXT, IPM, AZM
m93	lamb	STEC	TET	CAZ	CTX, FEP, FOX, CIP, GEN, SXT, AMP, CHL, IPM, AZM
m102	lamb	STEC	SXT, AMP, TET	CTX, CAZ, FOX, AZM	FEP, CIP, GEN, CHL, IPM
m145	lamb	STEC	CTX, CIP, SXT, TET, CHL, AZM	CAZ, FEP	FOX, GEN, AMP, IPM
m154	minced meat	STEC	CTX, CAZ	FOX, AMP	FEP, CIP, GEN, SXT, TET, CHL, IPM, AZM
H2	Hospital*	EAEC	CTX, CAZ, FOX, CIP, SXT, AMP, TET, IPM, AZM	-	FEP, GEN, CHL

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H3	Hospital	EAEC	CTX, CAZ, FOX, CIP, SXT, TET, IPM	AZM	FEP, GEN, AMP, CHL
H4	Hospital	EAEC	CTX, CAZ	AMP, CHL	FEP, FOX, CIP, GEN, SXT, TET, IPM, AZM
H12	Hospital	EPEC	-	AMP	CTX, CAZ, FEP, FOX, CIP, GEN, SXT, TET, CHL, IPM, AZM
H13	Hospital	EPEC	CTX, CAZ, FEP, GEN, SXT, AMP, TET, AZM	CIP	FOX, CHL
H14	Hospital	EPEC	CTX, SXT, AMP, TET	CAZ, CHL, IPM, AZM	FEP, FOX, CIP, GEN
H15	Hospital	EPEC	-	CTX, CAZ, AMP	FEP, FOX, CIP, GEN, SXT, TET, CHL, IPM, AZM
H34	Hospital	EPEC, EIEC	-	CTX	CAZ, FEP, FOX, CIP, GEN, SXT, AMP, TET, CHL, IPM, AZM
H73	Hospital	EPEC	CTX, TET	CAZ	FEP, FOX, CIP, GEN, SXT, AMP, CHL, IPM, AZM
H76	Hospital	EPEC	CIP, AXT, AMP, TET	CTX, FOX, CHL, IPM, AZM	CAZ, FEP, GEN
H93	Hospital	STEC	-	CTX, CAZ, AMP	FEP, FOX, CIP, GEN, SXT, TET, CHL, IPM, AZM
H96	Hospital	EPEC	CTX, CAZ, FEP, GEN, SXT, AMP, TET, AZM	CHL	FOX, CIP, IPM
E9	Hospital		-	-	GEN, AMK, LVX, IPM, MEM, CIP, CAZ, NIT, CFZ
H	Cattle farm trough				
E	Cattle farm trough				
G	Cattle farm trough				
O157:H7	NCTC 12900				
O157:H7	ATCC 35150 EHEC				

*Hospital isolates taken from hospitalized children.
CTX: Cefotaxime 30, FEP: Cefepime 30, CAZ: Ceftazidime 30, FOX: Cefoxitin 30, CIP: Ciprofloxacin 5, GEN: Gentamicin 10, SXT: Trimethoprim 1.25-Sulfamethoxazole 23.75, AMP: Ampicillin 10, TET: Tetracycline 30, CHL: Chloramphenicol 30, IPM: Imipenem 10, AZM: Aztreonam 30. AMK: Amikacin 30, LVX: Levofloxacin 5, MEM: Meropenem 10, NIT: Nitrofurantoin 300. CFZ: Cefazolin 30.

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Table 2.
Salmonella isolates and their origins

Salmonella	Origin
F37	mixed minced meat
F40	mixed minced meat
F41	mixed minced meat
F42	chicken meat
F45	chicken meat
F51	chicken leg
F53	chicken meat
F55	chicken meat
F58	beef
F59	beef

exponential phase of host strain was inoculated in 10 ml LB broth supplemented with 1mM $CaCl_2$. Cultures were incubated for 72 h with shaking at 37 °C, and meanwhile, 10 ml fresh LB broth was added to them. Subsequently, cultures were centrifugated at 3000 rpm for 15 min, and supernatants were filtered through 0.22 pore-size filters and stored at 4 °C.

Determination of phage host range.

Following the methodology of Kutter [27], spot testing exploration of the host range was accomplished. 39 pathogenic *E. coli* and 11 *Salmonella* isolates, which all were isolated from various meat, milk, cheese, cattle farm trough, and human samples obtained from different zones of Mashhad (unpublished data) and two standard strains (Table 1) were tested for phage susceptibility. Briefly, bacterial strains were grown in LB broth supplemented with 1mM $CaCl_2$ for 4 hours at 37 °C to reach an OD600 of 0.4 to 0.6. Cultures were individually plated on LB underlay agar as it was described earlier for screening samples. When overlay agar solidified, 10 µl of purified phages was pipetted on the overlay agar, and plates were incubated at 37 °C overnight. A phage cocktail consists of the same aliquots of seven selected phages was also tested for the spectrum of susceptible hosts. After 24 h, the sensitivity of bacteria to phages was determined considering the degree of lysed lawn and spots were classified according to a standard system for assessing bacterial infection by phages as described by Kutter [27]. This experiment was accomplished in two repetitions. Cluster analysis performed using CIMMiner software.

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Author Contributions

Conceived and designed the experiments: Golshan Shakeri. Performed the experiments: Golshan Shakeri. Analyzed the data: Golshan Shakeri. Research space and equipment: Abdollah Jamshidi. Contributed reagents/materials/analysis tools: Golshan Shakeri, Abdollah Jamshidi, Kiarash Ghazvini. Wrote the paper: Golshan Shakeri, Abdollah Jamshidi.

Conflict of Interest

The authors declare that they have no competing interests.

Table 3.
Samples and the methods of preparation

Samples prepared without enrichment and with small scale enrichment (Group A)	Samples prepared with extensive enrichment (Group B)
Minced meat	Minced meat
- beef (n=2)	- beef (n=1)
- mixed beef and lamb (n=6)	- mixed beef and lamb (n=1)
Cattle slaughterhouse	
- Septic wastewater (n=3)	Veterinary hospital
- Effluent (n=2)	- Septic wastewater (n=1)
	City aqueduct (n=1)
Cattle feces (n=5)	Cow cartilage (n=1)
	Cow fat (n=1)
	Vegetables (n=1)
Total (n=18)	**Total (n=7)**

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Abstracts (in Persian)

جداسازی باکتریوفاژهای وسیع الطیف علیه ایزوله های باکتری اشریشیاکلای تولید کننده شیگاتوکسین جدا شده از مواد غذایی و بیماران

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چکیده

هدف از این مطالعه جداسازی باکتریوفاژهای اختصاصی STEC و جهت تولید یک مجموعه‌ی باکتریوفاژ علیه پاتوتایپ های اشریشیاکلای موجود در شمال شرق ایران بود. روشهای پلیت مستقیم و غنی سازی در حجم کم، در گروه A منجر به تشخیص فاژ نشد. در حالیکه غنی سازی کامل در گروه B، منجر به تشخیص فاژ در ۵ نمونه از مجموع ۷ نمونه شد. در ٪۵۴ باکتری های اشریشیاکلای نسبت به فاژها حساس بودند. فاژ پلی والان Ecol-MHD1، علیه اشریشیاکلای و سالمونلا مؤثر بود. در نتیجه، این فاژها برای کنترل عفونت های ناشی از اشریشیاکلای مناسب هستند.

واژگان کلیدی

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