Global Veterinaria 3 (5): 354-362, 2009
ISSN 1992-6197

Using Molecular Techniques for Characterization of *Escherichia coli* Isolated from Water Sources in Egypt

J. EL-Jakee, E.I. Moussa, Kh. F. Mohamed and G. Mohamed

Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: Failure to understand the importance of water quality exposes animals and human to the risk of diseases. Microbial contamination reminds a critical risk factor in drinking water in many parts of the world. Fifty water samples were investigated to detect the occurrence of coliforms. All *E. coli* isolates were serotyped and screened for virulence genes (*hly, fliCh7*, *stx1, stx2* and *eae* genes). The results showed that 90 % of the collected water samples were positive for coliforms. The highest coliforms detection rate was recorded among water samples collected from canals followed by drinking underground water, River Nile, agricultural drain, untreated sewage water, treated sewage water and well samples respectively. The predominant *E. coli* serotype isolated from the examined water samples was O128:K67 followed by O157: K^-, O111:K58 and O55:K59 respectively. *E. coli* strains isolated from water sources were characterized by PCR and showed that 8 isolates carried *stx1* gene (verocytotoxin 1) and 4 possessed *stx2* gene (verocytotoxin 2). Intimin *(eae)*, chromosomal flagellin type H7 of *E. coli* (*fliCh7)* and enterohemolysin *(hly),* virulence genes were detected in 21.4, 21.4 and 28.6 % of the isolates respectively. It could be concluded that water may be an important reservoir for *E. coli* infection and the risks of contracting enterotoxigenic (ETEC) and or enterohemorrhagic *E. coli* (EHEC) infections from contaminated water have been clearly established.

Key words: *E. coli* · water · O157 · virulence genes · PCR

INTRODUCTION

Water plays a significant role for the sound health of every person and is essential for plant life. About 75% of the earth's crust is covered with water and the human body comprises approximately 70 % of water [1]. Therefore, water is the most urgent for life and essential for good health of human beings. In Europe and America, much attention has been paid to the problem of water purity [1]. The people of developing countries are attacked by water-borne diseases than in developed countries people. Fecal pollution in water system is expected to original from human and non-human sources and multiple pollution control measures may be necessary to meet the requirement of the Clean Water Act and its amendments [2]. *E. coli* is one of the U.S. Environmental Protection Agency (EPA) recommended indicator organisms for freshwater systems and is a sensitive measure of fecal pollution since it is common to almost all warm-blooded animals, including human [3]. Both ETEC and EHEC infections have been associated with the ingestion of food or water contaminated with these organisms [4]. *E. coli* O157: H7 is a food-borne pathogen that has emerged as a major cause of hemorrhagic colitis and is transmitted to humans by food and water. It can cause hemolytic uremic syndrome (HUS) mainly by secretion of shiga toxins encoded by the genes *stx1* and/or *stx2* and variants [5]. The virulence mechanisms that characterize *E. coli* are genetically coded for chromosomal, plasmid and bacteriophage DNAs and include heat-labile (LTI, LTIIa and LTIIb) and heat-stable (STI and STII) toxins, Vero toxin types 1,2 and 2e (VT!,VT2 and VT2e, respectively), cytotoxin necrotizing factor (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (Eagg) and enteroinvasive mechanism (Einv) [6]. With the advantage of PCR, it has become possible to identify these genes in bacterial isolates, offering the possibility of rapid diagnosis of the mechanism operating in specific *E. coli* infections. So we investigated *E. coli* contamination of water samples and all *E. coli* isolated were serotyped and screened for *hly*, *fliCh7*, *stx1, stx2* and *eae* genes.

Corresponding Author: Dr. Jakeen K. A. El-Jakee, Department of Microbiology,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

MATERIALS AND METHODS

Water Samples: In this study, 50 water samples were collected as shown in Table (1) from March to June 2007 at early morning from agricultural drains, canals, drinking underground water, treated sewage water, untreated sewage water and wells from different sites in Egypt. They were investigated for *E. coli*. The water samples were collected as described by Duelge and Unruh [7]. Each sample was labeled to show serial number, place of water, type of water as well as time and date of collection. Examination of the water samples was completed within 24 hours after collection using Standard Total Coliform Multiple-Tube (MPN) Fermentation Techniques [8].

Identification of the Isolates: After determination of MPN the tubes showing growth were inoculated onto MacConkey, sorbitol MacConkey and EMB agar plates (Oxoid). After 24 hr. incubation at 35°C±0.5°C for 24 hr ±2 hr. [8] suspected *E. coli* colony was detected according to Quinn *et al*.[9]. The *E. coli* isolates were subjected to serotyping by slide agglutination test [10] using standard polyvalent and monovalent *E. coli* antisera in Central Laboratories of Ministry Laboratories of Ministry of Public Health. The purified isolates were inoculated on blood agar to detect their hemolytic activity.

Table 1: Type, Source and Number of the examined water samples

Type of water*	Site of collection	No. of examined samples
Agricultural drains (7)	Baniswaf	5
	Kerdasa	2
Canal (12)	Baniswaf canal	3
	Mansoria canal	2
	Maruotia canal	2
	Gezera El Dahab	3
	Kerdasa canal	2
Drinking underground water (13)	Gezera El Dahab	3
	Kerdasa	2
	Qaloub Governorate	3
	Tanta	1
	El Zagazig	3
	New Cairo garden	1
River Nile (8)	Gezera El Dahab	2
	Maadi River Nile	1
	Helwan River Nile	5
Treated sewage water (3)	Project for treated sewage water- NewCairo	3
Untreated sewage water (4)	Project for treated sewage water- NewCairo	4
Wells (3)	Ein El sera	1
	El khyala well	1
	Gezera El Dahab	1

*Temperatures were about 30-35 ºC.

Table 2: Oligonucleotide primers sequence used for amplification of DNA recovered from water samples and *E. coli* isolates [12]

Primer designation	Specificity	Sequence (5'- 3')	Amplified product size (bp)	Temp.*
eaeA- F	Intimin	5'-GTG GCG AAT ACT GGC GAG ACT-3' 59.4 ºC (melt temp)*	890	52 ºC
eaeA-R		5'-CCC CAT TCT TTT TCA CCG TCG-3' 56.5 ºC (melt temp)		
stx1-f	Shigatoxin1	5'-ACA CTG GAT GAT CTC AGT GG-3' 54 ºC (melt temp)	614	47 ºC
stx1-R		5'-CTG AAT CCC CCT CCA TTA TG-3' 52.5 ºC (melt temp)		
stx2-F	Shigatoxin2	5'-CCA TGA CAA CGG ACA GCA GTT-3' 57.9 ºC (melt temp)	779	54 ºC
stx2-R		5'-CCT GTC AAC CGG TGA GCA GCA CTT TG-3' 63.8 ºC (melt temp)		
hlyA-F	hemolysin	5'-ACG ATG TGG TTT ATT CTG GA-3' 51.3 ºC (melt temp)	165	45-46 ºC
hlyA-R		5'-CTT CAC GTG ACC ATA CAT AT-3' 49.4 ºC (melt temp)		
fliCh7-F	Flagellar antigen	5'-GCG CTG TCG AGT TCT ATC GAG C-3' 59.9 ºC (melt temp)	625	55 ºC
fliCh7-R		5'-CAA CGG TGA CTT TAT GCG CAT TCC-3' 58.9 ºC (melt temp)		

* Integrated DNA technologies database analysis (http//eu.idtdna.com/analyzer/applications/oligoanalyzer/Default.aspx?c=EU)

Characterization of *E. coli* Strains by PCR: Specific oligonucleotide primers for five virulence genes *eae, stx1, stx2, fliCh7* and *hlyA* were selected on the base of published sequences with a Gene.Amp.PCR system 2400.The sequence, specificities, the primer combination, the length of the amplified products and the annealing temperature were summarized in Table (2).

DNA from the isolates was extracted [11]. The amplified reaction was performed [12] in 50ul volumes in micro amplification tubes (PCR tubes) and the reaction mixture consisted of 1µl (200ng) of extracted DNA template from bacterial cultures, 5 µl 10× PCR buffer (Bioflux), 1µl dNTPs (40µM) (Bioflux), 1µl Ampli Tag DNA polymerase (Bioflux), 1µl (50 pmol) from each primer pairs (each primer pair was used separately) and the volume of the reaction mixture was completed to 50µl using double distilled water. Then 40µl paraffin oil (Sigma) was added. *E. coli* ATCC 35150 serotype O157:H7 reference strain kindly supplied from Department of Microbiology Faculty of Veterinary Medicine, Cairo University was used for quality assurance and PCR specificity testing. M15 set of 100bp DNA ladder with stain (Sib Enzyme) was used as molecular marker.

RESULTS

In the present study, 50 samples were investigated to detect the occurrence of coliforms among the collected water samples. It is clear that 45 out of 50 water samples were positive for coliforms (variable MPN index / ml) with an incidence of 90 % as shown in Table (3). The highest percentage of coliforms detection rate was observed among water samples collected from canals followed by drinking underground water, River Nile, agricultural drain, untreated sewage water, treated sewage water and well samples with incidence of 24, 18,16,14,8,6 and 4 % respectively.

All coliforms positive samples were investigated to *E. coli* isolation. Among the water samples collected from River Nile the percentage of *E. coli* was 50 %. While the percentage of *E. coli* was 42.9, 33.3, 33.3, 25, 25 and 7.7 among water samples collected from agricultural drain, treated sewage water, well, canal, untreated sewage water and drinking underground water samples respectively (Table 4). The predominant *E. coli* serotype isolated from the examined water samples was O128:K67 followed by O157: K^-, O111:K58 and O55:K59 with incidence of 42.9,

Table 3: Coliforms associated with the collected water samples

Type of water samples	No. of examined samples	Coliforms No.	Coliforms %	Site of collection	No. of examined sample	MPN index /100 ml
Agricultural drain	7	7	14	Baniswaf	5	>1800
						>1800
						540
						280
						1600
				Kerdasa	2	>1800
						345
Canal	12	12	24	Baniswaf	3	>1800
						175
						31
				Mansoria canal	2	>1800
						1600
				Maruotia	2	>1800
						>1800
				Gezera El Dahab	3	>1800
						>1800
						>1800
				El Zomor	1	>1800
				Kerdasa	1	310
Drinking underground water	13	9	18	Gezera El Dahab	3	>1800
						>1800
						>1800
				Kerdasa	2	>1800
						0
				Qaloub Governorate	3	910
						1600
						>1800
				Tanta	1	0
				El Zagazig	3	0
						2
						0
				New Cairo garden	1	16

Table 3: Continued

River Nile	8	8	16	Gezera El Dahab	2	>1800 >1800
				Maadi	1	110
				Helwan	5	310 1600 1600 46 910
Treated sewage water	3	3	6	New Cairo	3	>1800 >1800 1600
Untreated sewage water	4	4	8	New Cairo	4	>1800 23.2 59.4 29.4
Wells	3	2	4	Ein El sera	1	>1800
				El khyala well	1	0
				Gezera El Dahab	1	>1800
Total	50	45	90			

No.: Positive number, %: was calculated according to total number of the examined samples.

Table 4: Prevalence of *E.coli* serotypes among the examined water samples

Type of water samples	No. of examined samples	*E. coli* positive No.	%	Source of *E.coli* isolates	Serotype	No. of isolates
Agricultural drain	7	3	42.9	Baniswaf	O157:K$^-$	1
					O128:K67	1
					O 111: K58	1
Canal	12	3	25	Maruotia canal	O128: K67	2
				Kerdasa	O111:K58	1
Drinking underground water	13	1	7.7	Gezera El Dahab	O128:K67	1
River Nile	8	4	50	Gezera El Dahab	O128:K67	1
				Maadi River Nile	O157: K -	1
				Helwan River Nile	O55:K59	1
					O157: K: -	1
Treated sewage water	3	1	33.3	NewCairo	O111: K58	1
Untreated sewage water	4	1	25	NewCairo	O157: K: -	1
Wells	3	1	33.3	Gezera El Dahab	O128:K67	1
Total	50	14	28			

*percentage was calculated according to the examined water number

Table 5: Hemolytic activity of the isolated *E. coli* serotypes

Serotypes	No.	percentage	Hemolytic activity
O55:K59	1	7.1	α
O 111:K58	3	21.4	α
O128:K67	6	42.9	α
O157:K$^-$	4	28.6	α (2)& β (2)
Total	14	100	100

No.: Positive number, %: was calculated according to total number of examined samples.

Table 6: Characterization of *E. coli* strains isolated from the water samples by PCR

E. coli serotypes	No. of examined serotypes	PCR positive for *stx1* gene		*st2* gene		*eae* gene		*hly* gene		*fliCh7*gene	
		No.	%	No.	%	No.	%	No.	%	No.	%
O55:K59	1	1	7.1	1	7.1	0	0	0	0	0	0
O111:K58	3	3	21.4	0	0	0	0	0	0	0	0
O128:K67	6	1	7.1	0	0	0	0	2	14.3	0	0
O157:K$^-$	4	3	21.4	3	21.4	3	21.4	2	14.3	3	21.4
Total	14	8	57.1	4	28.6	3	21.4	4	28.6	3	21.4

No. positive number, %: was calculated according to the total number of examined serotypes.

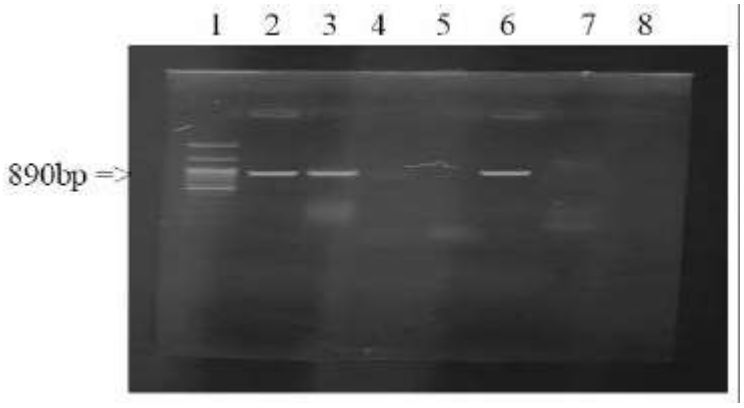

Fig. 1: PCR showing amplification of 890 bp fragment of *eae* gene.
Lane 1: marker, lane 2: positive control, lanes: 3 & 6: O157: K$^-$ isolates, lane 4: O55:K59 isolate, lane 5: O128:K67 isolate, lane 7: O111:K58 isolate and lane 8: negative control.

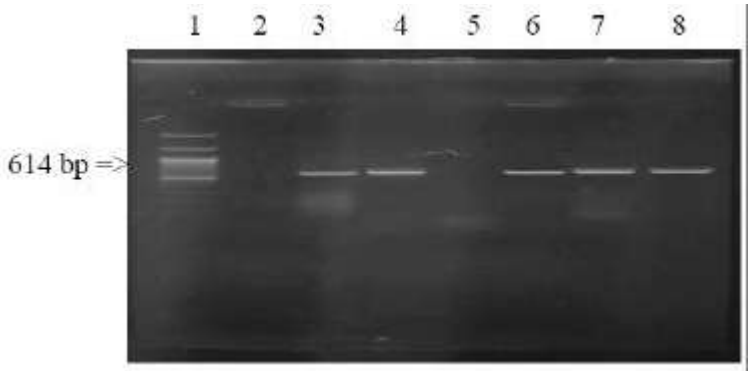

Fig. 2: PCR showing amplification of 614 bp fragment of *stx1* gene. Lane 1: marker, lane 2: negative control, lane 3: O128:K67 isolate, lane 4: O55:K59 isolate, lane 5: O128:K67 isolate (negative to *stx1*) lane 6: positive control, lane 7: O111:K58 isolate and lane 8: O157:K$^-$ isolate.

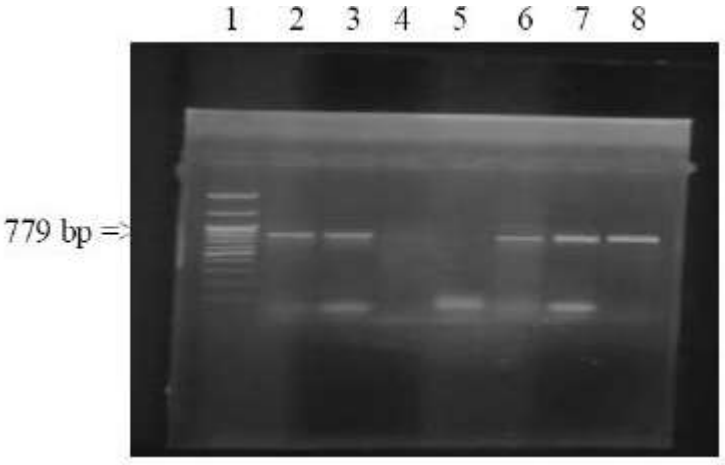

Fig. 3: PCR showing amplification of 779 bp fragment of *stx2* gene.
Lane 1: marker, lanes 2, 3 & 7: O157: K$^-$ isolates, lane 4: O111:K58 isolate
lane 5: O128:K67 isolate, lane 6: O55:K59 isolate and lane 8: positive control.

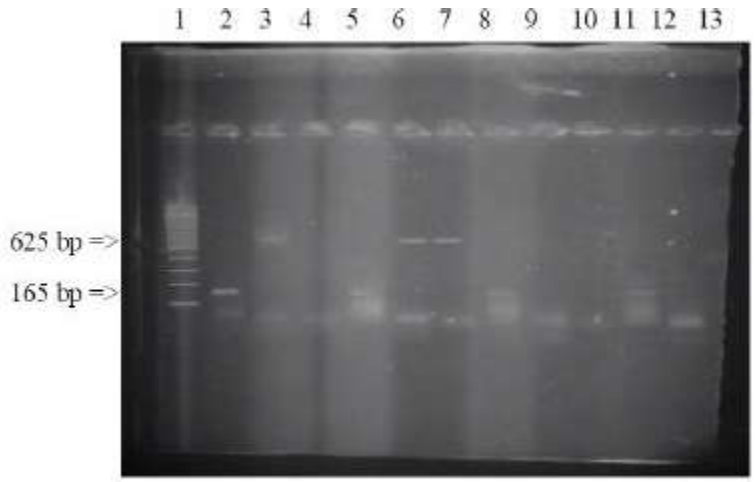

Fig. 4: PCR showing amplification of 165 6p fragment of *hly* gene and 625 bp fragment of *fliCh7*gene.DNA in lanes 2, 5, 8 and 11 were analyzed to *hly* gene, while lanes 3, 6 and 7 were analyzes for *fliCh7* gene. Lane 1: marker, lanes 2 & 8: *hly* gene positive (O157:K$^-$ isolates), lanes 5 & 11: *hly* gene positive of O128:K67 isolates, lanes 3, 6 & 7: *fliCh7* gene positive of O157:K$^-$ isolates, lanes 4, 9, 10 and 12: negative isolates for both *hly* and *fliCh7*genes (O128:K67, O55:K59, O111: K58 and O128:K67 isolates respectively). Lane 13 no DNA.

28.6, 21.4 and 7.1 % respectively (Table 5). All *E. coli* isolates displayed atypical enterohemolytic phenotype with small hemolytic zones along the sides of bacterial growth on blood agar plates after overnight incubation at 37°C (Table 5).

The present work have established the relationship between serotypes and virulence genes of *E. coli* isolated from water samples to know if the water possess the same serotypes and virulence factor profiles that shiga toxigenic *E. coli* (STEC) strains which cause human infection. Fourteen *E. coli* strains isolated from water sources were characterized in the present study by PCR and showed that 8 (57.1 %) isolates carried *stx1* gene and 4 (28.6 %) possessed *stx2* gene. Intimin *(eae)*, *fliCh7* and *hly,* virulence genes were detected in 3 (21.4 %), in 3 (21.4 %) and in 4 (28.6 %) of the isolates respectively (Table 6 and Figures 1- 4).

DISCUSSION

WHO estimates that 80 % of all sickness in the world can be attributable to inadequate potable water supplies and poor sanitation [1]. In the case of water-borne disease, outbreaks attributed to the ingestion of *E. coli* O157:H7 contaminated water from the public water system have been reported [13]. As contamination of water by STEC and / or ETEC is possible, a rapid and reliable method for the simultaneous monitoring of these *E. coli* cells in water is important. *E. coli* could serve as a more specified indicator for fecal contamination of freshwater. Therefore, the probable number method was used for the detection and enumeration of *E. coli* in water samples [14]. As standard methods in the coliforms detection, cultivation on the deoxycholate agar plate and most probable number method by brilliant green lactose bile (BGLB)-lactose broth (LB) have been used.

As shown in Table (3) the MPN index / 100 ml reached to > 1800 in some samples and the highest coliforms detection rate was observed among water samples collected from canals followed by drinking underground water, River Nile, agricultural drain, untreated sewage water, treated sewage water and well samples with incidence of 24, 18,16,14,8,6 and 4 % respectively. In the most polluted part of the river, the average biological oxygen demand level exceeds 40 mg/ml and the average fecal coliforms count is greater than 10^7CFU per 100 ml [15]. Residents who live near the Ganges suffer from a high incidence of water borne diseases, including cholera and dysentery [16]. Risk factors for disease include poor sanitation and regular use of the river for personal hygiene, laundry and utensil washing.

Antibody screening indicated that 4 isolates detected from agricultural drain (Baniswaf), River Nile (Maadi & Helwan areas) and untreated sewage water were of *E. coli* serotype O157:K$^-$ as shown in Table (4). *E. coli* O157:H7

was detected by Hamner *et al.* [15] among bacteria collected from the Ganges River, identification of potentially pathogenic isolates from extensively used source water indicates that O157:H7 may be a significant but as yet under acknowledged public health concern in India. Furthermore, Gannon *et al.* [17] tested Raw river and irrigation water in the Oldman River Basin in southern Alberta for the presence of *E. coli* O157:H7 over the 2 years (2000-2001). The number of *E. coli* O157:H7 isolated from raw water peaked during the summer months, while *E. coli* O157:H7 was only isolated from 11/802 (1.35%) of raw water samples over the entire sampling season in 2000 and from 16/806 (2.05%) of the samples in 2001 [17].

As shown in Table (5) the predominant serotype isolated from the examined water samples was O128:K67 followed by O157: K^-, O111:K58 and O55:K59 with incidence of 42.9, 28.6, 21.4 and 7.1 % respectively. Infection with some non O157 STEC types, such as O26:H11 or H-, O91:H21or H-, O103:H2, O111:H, O113:H21, O117:H7, O118:H16, O121:H19, O128:H2 or H-, O145:H28 or H- and O146:H21 are frequently associated with severe illness in humans [18]. STEC organisms that cause hemorrhagic colitis (HC) and the potentially fatal hemolytic uremic syndrome (HUS) are classed as EHEC [12]. Although the majority of HUS cases in humans have been associated with the EHEC serotype O157:H7, non-O157 serotypes also cause disease and are more common than *E. coli* O157:H7 in some geographical areas, in the United States, it is estimated that 20% to 50% of STEC human infections are caused by non-O157:H7 serotypes [12]. Nevertheless, the role of other non-O157 STEC types in human disease needs further examination, O26, O103, O111,O145 and O157 are all highly associated with HUS and HC [19].

E. coli strains isolated from water sources were characterized as shown in Table (6) and Figures (1- 4) by PCR. The 14 *E. coli* isolates belonged to 6 different seropathotypes (associations between serotypes and virulence genes), seropathotype O157:K^- *stx1, stx2, eae, fliCh7* (3 isolates) was the common followed by O111:K58 *stx1* (3 isolates), O157: K^- *stx1, stx2 eae, fliCh7, hly* (2 isolates), O55:K59 *stx1, stx2* (1 isolate), O128: K67 *stx1, hly* (1 isolate) and O128:K67 *hly* (1 isolate). The presence of varies type of virulence gene of *E. coli* in water isolates was detected by Ram and Shanker [20] using multiplex PCR selected *E. coli* gene sequences: *stx1*, *stx2*, *hlyA*, *chuA, eae*, *lacZ*, *lamB* and *fimA*. PCR simulation of these assays showed PCR products for *stx2* (248bp) *stx1* (102 bp), *lacZ* (228bp) and *lamB* (86 bp) in multiplex #1 and *eae* (200bp), *chuA* (147 bp), *hlyA* (141bp) and *fimA* (79 bp) in multiplex #2, respectively. PCR analysis of 49 *E. coli* O157:H7 and 209 non-O157 isolated by Oporto *et al*. [21] showed a different distribution of virulence genes, all *E. coli* O157:H7 were *stx* (2) gene-positive*, eaeA* was detected in 95.9 % and the toxigenic profile *stx* (2) / *eaeA/E-hlyA* was present in 75.5 % of the isolates. Among the non-O157 STEC, prevalence of *eaeA* was significantly lower (5.3 %) and *E-hlyA* was present in 50.2 % of the isolates but only sporadically associated with *eaeA* [21]. Combined presence of *stx2* and *eae* in STEC has been identified as a risk factor for HUS [15]. STEC strains causing human gastrointestinal disease are referred to as EHEC and serogroups O157, O111, O26, O113 and O103 are responsible for many outbreaks and sporadic cases of HUS and HC [22].

Amplification of 165 bp fragment of *hly* gene was observed from the extracted DNA of 2 O157: K^- and 2 O128:K67strains while *fliCh7* gene (625 bp) was detected in three O157 strains (Figure, 4). A factor that may also affect the virulence of STEC is the enterohemolysin, also called enterohemorrhagic *E. coli* hemolysin, which is encoded by the *ehxA* gene [18]. Varela *et al.* [23] isolated STEC strains from 3 (1.5 %) children with bloody diarrhea, 1 (7 %) from a child with HUS and 4 (1.8 %) from ground beef samples, all strains were *eae* and *ehxA* positive, the serotypes found were: O157:H7 (9 strains), O26:H11 (2), O111: NM (1) and O145:HNT (1). Recently Neelam *et al*. [24] described PCR test which targets the *E. coli fliCh7* gene, that is responsible for production of the H7 component for identification of H7 in *E. coli* O157:H7 strains can be used to rapidly identify strains that possess the H7 component and to confirm *E. coli* O157:H7 isolates that show a negative H7 agglutination reaction. It could be concluded that the patterns of virulence factors carried by the majority of water strains in the present study are therefore consistent with those of strains pathogenic for humans.Identifying the major contributing sources of contamination is the critical component for accurate assessment and successful control measures. Detection of potentially pathogenic O157:H7 bacteria in the examined water samples is alarming, many poorer use the river daily for bathing and washing laundry and as a source of water for cooking. These people may be at great risk for contracting O157:H7 infection.

REFERENCES

1. Pant, P.R., 2004. Tailored media for the detection of *E. coli* and coliforms in the water sample. Journal of Tribhuvan university, 24(1): 49-54.
2. Simpson, J.M., J.W. Santo Domingo and D.J. Reasoner, 2002. Microbial source tracking: state of the science. Environmental Science Technology, 36(24): 5279-5288.
3. U.S. Environmental Protection Agency 1986. Ambient water quality criteria for bacteria EPA-440/5–84/002. Office of Water Regulation and Standards, Criteria and Standards Division, Washington, D.C.
4. Gannon, V.P., R.K. King, J.Y. Kim and E.J. Thomas, 1992. Rapid and sensitive method for detection of Shiga-like toxin producing *Escherichia coli* in ground beef using the polymerase chain reaction. Journal of Applied Environmental Microbiology, 58(12): 3809-3815.
5. Bidet, P., P. Mariani-Kurkdjian, F. Grimont, N. Brahimi, C. Courroux, P. Grimont and E. Bingen, 2005. Characterization of *E. coli* O157: H7 isolates causing haemolytic uraemic syndrome in France. Journal of Medical Microbiology, 54: 71-75.
6. Pass, M.A., R. Odedra and R.M. Batt, 2000. Multiplex PCRs for Identification of E. coli Virulence Genes. Journal of Clinical Microbiology, 38(5): 2001-2004.
7. Duelge, S. and M. Unruh, 2002. Detection of *Escherichia coli* and *Enterobacter aerogenes* in water samples collected from two sites, one site near South Shore Water Treatment Facility, the other farther from the Facility, on Lake Michigan, in Milwaukee, Wisconsin.
8. Clesceri, L.S., A.E. Greenberg and R.R. Trussell, 1998. Standard method for the examination of water and waste water, 7th Ed. American public health association. Washington, DC.
9. Quinn, P.J., B.K. Markey, M.E. Carter, W.J.C. Donnelly and F. C. Leonard, 2002. Veterinary Microbiology and Microbial Disease, 1st Edition, Blackwell Science, United Kingdom.
10. Edwards, P.R. and W.H. Ewing, 1972. Identification of *Enterobacteriaceae.* Minneapolis, Burgess Cp. Atlanta, USA (3rd Ed.).
11. Sritharan, V. and Jr. Barker, 1991. A simple method for diagnosis of *Mycobacterium tuberculosis* infection in clinical sample using PCR. Molecular Cell probes, 5: 389-395.
12. Sheng, H., M.A. Davis, H.J. Knecht, D.D. Hancock, J.V. Donkersgoed and C.J. Hovde, 2005. Characterization of a Shiga Toxin-, Intimin- and Enterotoxin Hemolysin-Producing *E. coli* ONT: H25 Strain Commonly Isolated from Healthy Cattle. Journal of Clinical Microbiology, 43(7): 3213-3220.
13. Geldreich, E.E., K.R. Fox, J.A. Goodrich, E.W. Rice, R.M. Clark and D.L. Swerdlow, 1992. Searching for a water supply connection in the Cabool, Missouri disease outbreak of *Escherichia coli* O157:H7. Water Research, 626(8): 1127-1137.
14. Chao, K.K., C.C. Chao and W.L. Chao, 2004. Evaluation of Colilert-18 for Detection of Coliforms and *Escherichia coli* in Subtropical Freshwater. Journal of Applied Environmental Microbiology, 70(2): 1242-1244.
15. Hamner, S., S.C. Broadaway, A.B. Mishra, A. Tripathi, R.K. Mushra, E. Pulcini, B.H. Pyle and T.E. Ford, 2007. Isolation of Potentially Pathogenic *Escherichia coli* O157:H7 from the Ganges River. Journal of Applied Environmental Microbiology, 73(7): 2369-2372.
16. Hamner, S., A. Tripathi, R.K. Mishra, N. Bouskill, S.C. Broadaway, B.H. Pyle and T.E. Ford, 2006. The role of water use patterns and sewage pollution in incidence of water-borne/enteric diseases along the Ganges River in Varanasi, India. International Journal of Environmental Health Research, 16(2): 113-132.
17. Gannon, V.P., T.A. Graham, S. Read, K. Ziebell, A. Muckle, J. Mori, J. Thomas, B. Selinger, I. Townshend and J. Byrne, 2004. Bacterial pathogens in rural water supplies in Southern Alberta, Canada. Journal of Toxicology Environmental Health, A 67(20-22): 1643-1653.
18. Blanco, M., J.E. Blanco, A. Mora, G. Dahbi, M.P. Alonso, E.A. González, M.I. Bernárdez and J. Blanco, 2004. Serotypes, virulence genes and Intimin types of shiga toxin (Verotoxin)-Producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (*eae*). Journal of Clinical Microbiology, 42(2): 645-651.
19. Pearce, M.C., C. Jenkins, L. Vali, A.W. Smith, H.I. Knight, T. Cheasty, H.R. Smith, G.J. Gunn, M.E.J. Woolhouse, S.G.B. Amyes and G. Frankel, 2004. Temporal Shedding Patterns and Virulence Factors of *E. coli* Serogroups O26, O103, O111, O145 and O157 in a Cohort of Beef Calves and Their Dams. Journal of Applied Environmental Microbiology, 70(3): 1708-1716.

20. Ram, S. and R. Shanker, 2005. Computing TaqMan probes for Multiplex PCR Detection of *Escherichia coli* O157 Serotypes in water. In-Silico Biology, 5: 1-6.
21. Oporto, B., J.I. Esteban, G. Aduriz, R.A. Juste and A. Hurtado, 2008. *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Public Health, 55(2): 73-81.
22. Djordjevic, S.P., V. Ramachandran, K.A. Bettelheim, B.A. Vanselow, P. Holst, G. Bailey and M.A. Hornitzky, 2004. Serotypes and virulence gene profiles of shiga toxin-producing *Escherichia coli* strains isolated from feces of pasture-fed and lot-fed sheep. Journal of Applied Environmental Microbiology, 70(7): 3910-3917.
23. Varela, G., I. Chinen, P. Gadea, E. Miliwebsky, M. Mota, S. González, G. González, M.J. Gugliada, C.C. Carbonari, G. Algorta, M. Bernadá, R. Sabelli, L. Pardo, M. Rivas and F. Schelotto, 2008. Detection and characterization of shiga toxin-producing *Escherichia coli* from clinical cases and food in Uruguay. Revista Argentina de microbiologia, 40(2): 93-100.
24. Neelam, N., F. Pina, T. Glenn, P. Kitty and C. William, 2009. Comparison of the H7 latex agglutination test with a *fliCh7* real-time PCR assay for confirmation of the H type of *Escherichia coli* O157:H7. Journal of Food Protection Publication Acceptance Date: May 11, 2009.