academicJournals

Vol. 7(21), pp. 2637-2645, 21 May, 2013
DOI: 10.5897/AJMR2013.5417
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Prevalence and characterization of Shiga toxin O157 and non-O157 enterohemorrhagic *Escherichia coli* isolated from different sources in Ismailia, Egypt

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Accepted 3 May, 2013

Shiga toxin producing *Escherichia coli* (STEC) is recognized as important food borne pathogen, responsible for sporadic cases of serious outbreaks worldwide. The morbidity and mortality associated with several recent outbreaks due to STEC have highlighted the threat this organism poses to public health. This study was conducted to identify and characterize the virulence traits and antibiotic resistance of enterohaemorrhagic *E. coli* from different sources, between September 2008 and October 2009. A total of 384 samples from human, animal and environmental sources were collected from different locations in Ismailia, Egypt. *E. coli* isolates (n = 283) were identified by conventional microbiology culture and were phenotypically characterized using biochemical and motility tests. Multiplex PCR (mPCR) was applied for the detection of virulence genes (*stx1*, *stx2*, *eaeA* and EHEC *hlyA*). From the overall number of *E. coli* isolates, 31.4% (89/283) were isolated from stools of humans with diarrhea, 17.3% (49/283) from stools of sheep, cattle and chicken with diarrhea, 16.6% (47/283) from urine of humans with urinary tract infection, 17.3% (49/283) from water, 6.4% (18/283) from sea-food, 6% (17/283) from processed meat products, 3.9% (11/283) from dairy products and 1.1% (3/283) from poultry products (liver). The antibiotic resistance patterns showed that the isolates carried multi-drug resistance (MDR) phenotype to at least four antibiotics belonging to different classes: Erythromycin (E), gentamicin (CN), cefazolin (CZ), thiampinicol (TP), vancomycin (VA), ciprofloxacin (CIP) and ampicillin (AM). Shiga toxin genes were identified in 10 (3.5%) suspected Enterohaemorrhagic *E. coli* isolates by mPCR. Serotyping of these 10 isolates demonstrated five different serogroups (O157, O158, O114, O125 and O26): three human isolates (serogroups O157, O158), four animal isolates (serogroups O114, O26), two isolates from meat products (serogroups O125, O158) and one isolate from water (serogroup O114). This study identified STEC O157 from human cases with diarrhea, and demonstrated that meats and water could be contaminated with more than one STEC serotype. This is a concern due to their potential to cause human infections.

Key words: Hemorrhagic Escherichia coli, Shiga toxin, non-O157, O157, diarrhea, food, Egypt.

INTRODUCTION

Shiga toxin producing *Escherichia coli* (STEC) is the most important recently emerged group of foodborne pathogens (Abong'o et al., 2009). It can cause severe gastrointestinal disease, including fatal infections, and is

being detected with increasing frequency worldwide. Transmission occurs usually through consumption of undercooked meat, unpasteurized dairy products and vegetables or water contaminated by faeces of carriers (Dunn et al., 2004) primarily because Shiga toxin-producing *E. coli* (STEC) is found to be part of the normal intestinal flora of animals (Ezawa et al., 2004). Person-to-person transmission has also been documented (Karmali, 1989; Paton and Paton, 1998). Although several investigations have been carried out in the laboratory to detect these organisms in recent years, this group remains one of the most difficult to detect. STEC strains do not only produce potent cytotoxins (verotoxins) but have also acquired the ability to adhere to the intestinal mucosa in an intimate fashion (O'Brien and Holmes, 1987; Hoffman 1993; Bettelheim, 1996). All STEC strains produce STEC-specific, plasmid-mediated hemolysin encoded by *hlyA* gene (Schmidt et al., 1994) and at least one Shiga-like toxin (encoded by *stx*1 or *stx*2) (O'Brien and Holmes, 1987). Many produce intimin, a 97-kDa attachment- and effacement protein, that is encoded by *eaeA* gene (Louie et al., 1993). The present study was aimed at investigating the prevalence of shiga-toxin producing *E.coli* strains from different human and environmental sources in one of the governorates in Egypt. The virulence characterisitcs and antimicrobial susceptibility of isolates were also studied.

MATERIALS AND METHODS

Sample collection

A total of 384 samples were collected from different sources in Ismailia governorate from September 2008 through October 2009. Sampling included various types of food, water, stool from diarrheal human and animal cases, and urine from patients with urinary tract infection (UTI).

A total of 99 food samples were randomly collected in sterile plastic bags from different local markets. The collected foods included meat products, poultry products, sea-food products and dairy products. Twenty five grams of each sample was treated with 225 ml of modified tryptic soy broth (mTSB- DifcoLa Jolla, CA/USA) and incubated with agitation (120 rpm) for 24 h at 37°C as described by Cowan (1985) and Ethelberg (2009). After enrichment for 24 h at 37°C, aliquots of 100 µl were plated onto Eosin Methylene Blue (EMB-Difco) agar to presumptively identify isolates as Gram-negative enteric bacteria and presumptive *E. coli* (green-metallic colonies), and onto Sorbitol MacConkey agar (SMAC-Difco) to test for sorbitol non-fermenting bacteria (colorless colonies). After 18 to 24 h at 37°C, characteristic colonies from EMB agar and SMAC agar were transferred onto Tryptic Soy agar (TSA, Difco) and used for biochemical identification tests. Thirty one water samples were collected from the Ismailia freshwater canal and examined according to standard methods (APHA, 1998) isolates yielding typical green metallic colonies were inoculated into SMAC agar and incubated at 37°C for 24 h (APHA, 1998).

Fecal specimens (n=179) were collected following standard fecal specimens (n=179) were collected following standard methods (CDC, 1997, 2007). Stool specimens were collected at early stages enteric illness from patients with diarrhea before initiating antibiotic therapy. Fresh stool sample, or a rectal swab was collected using a sterile rectal swab and inoculated into Tryptic soy broth (TSB-Difco) transport medium (CDC, 2007). Within 2 h of specimen collection, samples were streaked onto SMAC agar plates supplemented with 0.05 mg/L Cefexime in order to examine the ability of the isolates to ferment sorbitol. After 24 h at 37°C, plates were examined and individual *E. coli*-like colorless colonies were selected and subjected to further identification using standard biochemical procedures (Cowan, 1985).

Urine specimens were collected from the midstream urine (MSU) of symptomatic cases with urinary tract infection (n = 76; 24 from males and 52 from females). Within two hours after collection, all urine samples were examined following standard procedures (Izenberg, 2003). Urine specimens were diluted with 5% saline solution, and 100 µl from each diluted sample was directly used to inoculate EMB agar plates using sterile 10 µl culture loops. Bacterial colonies grown were re-streaked onto Cefexime SMAC plates, and incubated for 24 h at 37°C.

Identification and biochemical characterization of isolates

Purified suspected *E. coli*-like colonies (n = 283) were identified by examining the morphology and biochemical properties of growing colonies. Gram staining was evaluated following the procedure described by Merchant and Packer (1967) and *E. coli*-like colonies were subjected to different biochemical tests, including sugar fermentation tests, indole production test, Methyl-Red and Voges-Proskauer (IMIVC) tests, following the standard methods described by Cowan (1985).

Antibiotic susceptibility tests

The whole number of *E. coli* isolates (n = 283) were tested for their susceptibility to 16 antibiotics listed in Table 1. Testing was performed on Mueller-Hinton Agar plates using the Kirby-Bauer disk diffusion technique (Jorgensen et al., 1997). The antibiotic resistance of each *E. coli* isolate was determined based on the breakpoints of the inhibition zone diameters for individual antibiotic agents and as recommended by the disk manufacturer. The results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute for antimicrobial susceptibility testing (CLSI, 2010).

Resistance to more than four antibiotics was as multidrug resistance (MDR). MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed (Chandran et al 2008). Isolates with MDRI values of more than 0.2 or 20% were considered highly resistant.

Congo red (CR) binding activity

The individual *E. coli* isolates were tested for their binding activity with Congo red dye, which is an indicator of intestinal invasion (Berkhoff and Vinal, 1986; Koneman et al., 1999; Panigarhy and Yushen, 1990). Individual *E. coli* colonies were cultured onto Congo red medium and incubated at 37°C for 24 h. Culture plates were then transferred at room temperature for additional 24 h of incubition. Growth of red colonies indicates a Congo red positive (CR+)

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Table 1. Antibiotic Resistance profile of *E. coli* (n=283) from different sources.

Antibiotic agent	N	%	Resistance pattern							
			Surface water	Human stool	Animal stool	UTI	Meat	Poultry	Dairy	Sea food
Number of isolates/Percentage			49 (17.3%)	89 (31%)	49 (17.3%)	47 (16.5%)	17 (6.02%)	3 (1.1%)	11 (3.9%)	18 (6.4%)
Amoxicillin/Calvulanic Acid (AMC/ 30)	141	50	4	52	36	27	11	1	2	8
Ampicillin (AM/ 10)	211	75	39	55	47	36	15	3	6	10
Cefazolin (CZ/ 30)	156	55.1	21	49	33	32	9		1	11
Ciprofloxacin (CIP/5)	148	52.1	13	53	45	20	6	1	6	11
Clindamycin (DA/2)	161	57	25	51	45	24	9	1		6
Erythromycin (E/ 15)	161	60	27	43	45	30	13	1	7	3
Gentamicin (CN /120)	119	42	24	34	29	20	9			3
Imipenem (IPM/ 10)	82	29	14	23	31	7	6			1
Nitrofurantion (F /300)	101	36	27	23	16	23	4	2	3	3
Nitrofloxacin (NOR/ 10)	118	42	23	45	33	9	4	2		2
Penicillin (P/ 10)	180	64	23	51	49	33	13		1	10
Rifampin (RA/ 5)	167	59	22	51	46	27	10	1		10
Spectinomycin (SPT/100)	113	40	31	25	31	16	6			4
Tetracycline (TE /30)	141	50	32	35	42	22	4			6
Thiampinicol (TP/ 30)	153	54	31	40	38	28	9			7
Vancomycin (VA /30)	195	69	35	46	47	35	15	1	6	10

The investigated antibiotics in the current study were as follows: Amoxicillin/Clavulanic Acid AMC 30, Ampicillin AM 10, Cefazolin CZ 30, Ciprofloxacin CIP 5, Clindamycin DA, Erythromycin E 15, Gentamicin CN 120, Imipenem IPM 20, Nitrofurantion F 300, Nitrofloxacin NOR 10, Penicillin P 10, Rifampin RA 5 , Spectinomycin SPT 100, Tetracycline TE 30, Thiampinicol TP 30 and Vancomycin VA 30.

phenotype. Different intensities in the dye uptake were scored as (+), (++) or (+++), while appearance of white colonies (*E. coli* which did not uptake the dye) indicates a Congo red negative (CR−) phenotype.

Enteroheamolysin production

Detection of hemolysin producers was performed according to the method described elsewhere (Blanco et al., 1997). Briefly, individual *E. coli* colonies were grown at 37°C for 24 h on tryptose blood agar (Difco) supplemented with 10 mM $CaCl_2$ and 5% defibrinated washed sheep blood in phosphate saline. Positive β-hemolytic reactions were indicated by complete clear zones under and around the colonies.

Detection of virulence genes

Genomic DNA

Purified genomic bacterial DNA was extracted, from overnight cultures of *E. coli* isolates after growth on TSA medium using genomic DNA mini kit (QIAGEN, QIAamp® - USA) according to the manufacturer's instructions.

The DNA templates of 283 isolates were subjected to mPCR for screening of virulence genes using specific primers as described elsewhere (Table 2). Positive controls included DNA lysate prepared from clinical specimens positive for shiga toxins *eae* and *hlyA* which are kindly, provided by US Naval Medical Research Unit #3 (NAMRU-3) laboratories, Cairo-Egypt. The production of enterohemolysin was assayed on 75 *E. coli* isolates which demonstrated a beta hemolytic phenotype. The eae gene was only assayed using DNA obtained from shiga-toxin producing strains isolates. PCRs for detection of *stx1*, *stx2*, *eae*, and *hlyA* genes were performed with commercially manufactured oligonucleotide primers. The primers used and the predicted lengths of PCR amplification products are listed in Table 2. These primers were chosen because they amplify conserved regions of the target genes and allow single-step identification of amplified DNA fragments that are visualized as stained bands after migration on agarose gel electrophoresis. PCRs were performed in 25 µl reaction

Table 2. Primer sequences of examined virulence genes and predicted lengths of PCR amplification products (Fermentas International Inc. Burlington,Ontario,Canada).

Target gene	Oligonucleotide sequence (5'- 3')	Product length (bases)	Cycle condition
stx	F -CAACACTGGATGATCTCAG R-CCCCCTCAACTGCTAATA	349 bp	96°C 5 min, 95°C 1min, 55°C 1min, 72°C 1min for 30 cycles, 72°C 7min
stx	F-ATCAGTCGTCACTCACTGGT RCTGCTGCTGTCACAGTGACAAA	110 bp	96°C 5 min, 95°C 1min, 55°C 1min, 72°C 1min for 30 cycles, 72°C 7min
hly	F-ACGATGTGGTTTATTCTGGA R-CTTCACGTGACCATACATAT	165 bp	96°C 5 min, 95°C 1min, 48°C 1min, 72°C 1min for 30 cycles, 72°C 7min
eae	F-GTGGCGAATACTGGCGAGACT R-CCCCATTCTTTTTCACCGTCG	890 bp	96°C 5 min, 95°C 1min, 55°C 1min, 72°C 1min for 30 cycles, 72°C 7min

*stx*1:shiga toxin 1, *stx*2:shiga toxin 2, *hlyA*:enterohemolysin, *eae*: intimin

mixtures containing 2 µl of template DNA, 5 µl of 5 x PCR buffer, 4 µl of a 2.5 mM mixture of deoxynucleoside triphosphates, 2 µl of 25 mM $MgCl_2$, 0.25 µl of 5U of Ampli Taq Gold DNA polymerase per µl, and 2 µl of a 20 µM concentration of each primer. The thermo-cycling conditions were done in a Gene Amp PCR system 9700 (AB Applied Biosystems). PCR products (5 µl) were loaded onto 2% agarose gels and run at 120 mV for 30 min. A molecular marker (1-kb DNA ladder; Gibco/BRL) was run concurrently. The DNA bands were visualized and photographed under UV light after the gel was stained with ethidium bromide.

Serotyping

Serotyping for the somatic "O" antigen of *E.coli* isolates propagated on 18-hour Nutrient broth cultures at 37°C was performed commercially in the Clinical Microbiology Reference Unit-Central Health Laboratories (Abdien Cairo, Egypt). A slide agglutination test (Koneman et al., 1999) was used employing 8 diagnostic polyvalent and 43 corresponding monovalent anti-*E. coli* sera provided by Denka Seiken, Chuo-ku/ Japan.

RESULTS

Prevalence

A total of 283 *E. coli* isolates were recovered from 384 samples collected from different sources in Ismailia. The prevalence of *E. coli* among the different sources is demonstrated in Table 3. *E. coli* was isolated with higher frequency from human stools (31.4%), surface water (17.3%), animal stools (17.3%), human urine (16.5%), seafood products (6.4%) and processed meat products (6.1%), while a lesser frequency was found from dairy products (3.9%) and poultry products (1.1%). Almost 70% (n=194) of the *E. coli* isolates demonstrated clear invasive phenotype on CR agar while 26% (n=75) showed beta-hemolytic patterns on blood agar. The invasive pattern phenotype was in isolates from diarrheic animals (86%), seafood (83%) meat products (82%), urinary tract infection (UTI) (77%) and diarrheic patients (73%). In addition, high percentages of beta hemolytic phenotype were recorded in *E. coli* isolates originating from food products: poultry (66%), processed meat (47%) and seafood (33%). Twenty two percent (61/269) of isolates shared a common phenotype with respect to the presence of both invasive pattern on CR and β-hemolytic activity. Of these 61 isolates, 14 originated from human stool, 16 from animal stool, 13 from UTI, 6 from water, 10 from meat products, 1 from poultry and 1 from seafood. Most of the *E. coli* isolates with combined CR and β-hemolytic phenotype characteristics originated from clinical samples from patients with diarrhea and urinary tract infection.

Antibiotic resistance phenotype

The antibiotic resistance patterns of the 283 *E. coli* isolates are shown in Table 1. The results demonstrated that 75% of the isolates were resistant to AM/ 10, 69% to VA /30, 64% to P/ 10 and 60% to E/ 15. Almost 50% of the tested isolates were AMC/ 30 resistant. Fewer isolates were found to be resistant to NOR/ 10 (36%) and IPM/10 (28%). The antibiogram profile of *E coli* strains carrying different virulence genes is demonstrated in Table 4. Most strains demonstrated multi-drug resistance index (MDRI), that is, they were resistant to at least four antibiotics belonging to different classes: E 15, CN 120, CZ 30, TP 30, VA 30, CIP5 and AM10. Strains belonging to the O157 serogroup were sensitive to CIP and NOR, whereas 30% of strains belonging to non-O157 serogroups were resistant to CIP and 100% were resistant to at least three antibiotics.

Virulence genes

Ten (10) shiga toxin producing strains were identified by mPCR. Three of these (EC255:O157, EC306:O158, EC322:O114) were positive for both *stx1* and *stx2* while the remaining 7 isolates (EC150:O125, EC0111:O26, EC294:O158, EC357:O114, EC291:O26, EC158:O158, EC94:O157) were found positive only for *stx1* (Table 4).

Table 3. *E coli* isolates by source, hemolytic activity, Congo red indicator phenotype, and virulence genes.

Source	Sample type	Number of samples (N=384)	Isolated *E. coli* (N=283)	Prevalence of *E. coli* (%)	Beta hemolysis (N=75)	Congo red indicator (N=194)	Virulence genes			
							*stx*1 *hly*A	*stx*1 /2	*stx*1 +eae	*stx*1/2+ *hly*
Surface water	Fresh canals	31	49	17.3	7 (14.2)%	19 (39)%			1	
Human stool	Male	37	38	13.41	5 (13)%	27 (71%)				
	Female	27	20	7.1	8 (40%)	19 (95%)				
	Children	43	31	10.92	7 (22.5%)	19 (61.2%)	1			
Human urine	Male	24	8	2.82	5 (62.5%)	8 (100%)	1			
	Female	52	39	13.7	9 (23%)	28 (71%)	1			1
Animal stool	Cattle	33	14	4.9	5 (35.7%)	14 (100%)			1	
	Calves	20	5	1.78	2 (40%)	3 (60%)	1			
	Sheep	9	20	7.1	5 (25%)	15 (75%)		1		
	Chickens	9	10	3.55	4 (40%)	10 (100%)	1		1	
Meat products	Minced	11	3	1.1	3 (100%)	2 (66.6%)				
	Steaks	3	6	2.12	1 (16.6%)	6 (100%)				
	Sausage	8	2	0.7	2 (100%)	1(50%)	1			
	Kofta	6	2	0.7	1 (50%)	2 (100%)		1		
	Burger	8	0	0	0	0				
	Luncheon	8	4	1.4	1 (25%)	3 (75%)				
	Liver	4	0	0	0	0				
Poultry products	Livers	6	3	1.1	2 (66%)	2 (66.6%)				
	Lamps	8	0	0	0	0				
Sea food	Oysters	9	7	2.5	2 (28.5%)	6 (85.7%)				
	Calamari	7	4	1.4	2 (28.5%)	4 (100%)				
	Bivalves	7	7	2.5	2 (28.5%)	5 (71.4%)				
Dairy products	Raw milk	6	4	1.4	1 (25%)	1 (25%)				
	Yogurt	4	4	1.4	0	0				
	Cheese	4	3	1.1	1 (33%)	0				
Total		384	283							

Congo red (CR) binding activity indicated that 194 *E. coli* isolates (70%) shown clear invasive phenotype on CR agar while 75 isolates (26.6%) shown beta hemolytic phenotype in blood agar. Twenty two percent (61/269) of isolates shared a common phenotype with respect to the presence of both invasive pattern on CR agar and β-hemolytic activity. Of these 61 isolates, 14 originated from human stool, 16 from animal stool, 13 from UTI , six from surface water, 10 from meat products , one from poultry and 1 from sea food.

The 10 shiga toxin producing strains were tested for the presence of the *eae* virulence gene, as an indicator of intestinal invasion. Three shiga toxin producing strains were positive for the *eae* gene with a 890-bp PCR product. In addition, PCR testing of the 75 beta hemolytic *E. coli* isolates yielded three *hlyA*-positive strains with a PCR product of 165-bp.

Serotyping

Serotyping of the ten STEC strains revealed five serotypes (O157, O158, O114, O125 and O26) (Table 4): three human isolates (serotypes O157, O158), four animal isolates (serotypes O114, O26), two isolates from meat products (serotypes O125, O158) and one isolate from water (serotypes O114). Three STEC strains, belonging to three serotypes, were positive for both *stx1* and *stx2.* These strains were isolated from a urine sample of a female with urinary tract infection (serotypes O157), a stool sample from infected sheep (serotypes O114) and from a meat product (serotypes O158).

DISCUSSION

STEC can cause diarrhea, bloody diarrhea and hemorrhagic colitis in humans. STEC infections also frequently result in hemolytic-uremic syndrome (HUS), a life-threatening condition characterized by hemolytic anemia, thrombocytopenia and renal failure (Tarr et al., 2005). Transmission of STEC occurs through contaminated foods, such as ground beef, through contaminated water and by person-to-person spread (Steinmuller et al., 2006).

In some countries, O157:H7 is the most common serotype of STEC (Gould, 2009) and is the serotype most often associated with HUS (Voetsch et al., 2004). Approximately 150 non-O157 STEC serotypes also cause diarrheal disease (European Food Safety Authority, 2010). Some studies suggest that non-O157 STEC infections result in milder disease compared to O157 infections (Brooks et al., 2005). Most of the medically significant reports on O157 STEC are published in developed countries, and only a handful of reports are

Table 4. Antibiogram, serotypes and origin of STEC strains by genetic and phenotypic characteristics

Isolate origin	Positive genes	Serotype	Resistance Profile	Non-resistance profile	Multi-drug resistance index MDRI (%)
EC94: Child stool	*Stx 1*	O157	Am 10, P 10, SPT, AMC 30, IPM 20, CN 120, CZ 30, VA 30	F 300, TE 30, CIP 5, NOR 10-TP 30 DA 2,	50
EC306: Meat Kofta)	*Stx 1* *Stx2*	O158	Am 10, E 15, P 10, TE 30, RA 5, DA 2, AMC 30, IPM 20, CN 120, NOR 10, CZ 30, TP 30, VA 30	CIP 5	81.25
EC294: Calf stool	*Stx 1*	O158	Am10, E15, P10, SPT, F 300, TE 30, CIP 5 , TP 30	RA 5, DA 2, AMC 30, CN 120, NOR 10 , CZ 30	50
EC158: Urine (Male)	*Stx 1*	O158	Am 10, E 15, P 10, TE 30, RA 5, DA 2, AMC 30, CN 120, CIP 5, NOR 10, CZ 30, TP 30, VA 30	SPT, F 300, IPM 20, RA 5	81.25
EC322: Sheep (stool)	*Stx 1* *Stx2*	O114	E 15, SPT, F 300, TE 30, IPM 20, CN 120, CIP 5, NOR10, TP 30, VA 30	Am 10, RA 5, DA 2, AMC 30	56.25
EC357: Raw water	*Stx 1* *eae*	O114	Am10, E15, SPT, F 300, TE 30, RA 5, DA 2, CN 120, TP 30 , VA 30	CIP 5, NOR 10	62.5
EC150: Meat (sausage)	*Stx 1*	O125	Am 10, E 15, P 10, TE 30, RA 5, DA 2, CN 120, NOR 10, CZ 30, TP 30,VA 30	IPM 20, SPT , F300	68.75
EC0111: Chicken (stool)	*Stx 1* *eae*	O26	Am10, E15, P10, SPT , F300 TE 30, RA 5, DA 2, AMC 30, CIP 5	IPM 20, TP 30, VA 30	62.5
EC291: Cattle (stool)	*Stx 1* *eae*	O26	Am 10, SPT, CN 120, VA 30	E 15, F 300, RA 5, DA 2, AMC 30, IPM 20, NOR 10, CZ 30, TP 30	25

available from developing countries with nearly no data available on non-O157 STEC from countries in North Africa and the Middle East. The aims of the present study were to identify the frequencies of O157 and non-O157 STEC among different human, animal and environmental sources and to characterize the genetic background and

virulence traits of suspected STEC isolates.

In the current study, 283 *E. coli* isolates were recovered from 384 samples collected from different sources in Ismailia, one of the largest governorates located in the North-east of Egypt, on the West Bank of the Suez canal. The highest isolation frequencies of *E. coli* among the different sources were from human stool (31.4%), surface water and animal stools (17.3%), human urine (16.5%), and processed meat products (6.1%), with lower isolation frequencies from dairy products (3.9%) and poultry products (1.1%). The current study assayed for the presence of virulence genes in the 283 *E. coli* isolates and for their *in vitro* invasive properties. Findings showed that 70% of the isolates demonstrated an invasive phenotype on CR agar while 26% displayed beta-hemolytic patterns on blood agar. These percentages were similar to those obtained by other groups (Abhilasha et al., 2001; Hussin 1998; Sahaly, 1995) who found that profiles positive for the Congo red test and hemolysis were widely spread among *E. coli* strains isolated from diseased animals. In the current study, most positive CR and EHL isolates were obtained from clinical samples from patients with diarrhea and urinary tract infections, indicating the potential of these isolates to cause disease. This corroborates with previous findings (Hoda, 2009) that emphasized the role of hemolysin-producing *E. coli* in the increase of pathogenic potential and the critical role these organisms play in causing extra-intestinal infection. An invasive pattern phenotype was recorded in isolates from a considerably high percentage of diarrheic animals (86%), seafood (83%), meat products (82%), UTI (77%) and diarrheic patients (73%), whereas the beta hemolytic phenotype was widely spread among isolates originating from diverse food products (66% from poultry, 47 % from processed meat and 33% from seafood). These highly prevalent virulence phenotypes may reflect that the majority of the isolated *E. coli* possess the potential to cause disease.

The antibiotic resistance profile of the 283 *E. coli* isolates indicated that antibiotic resistance is quite common among the *E. coli* isolates: 75% of the isolates were resistant to AM followed by VA (69%), P (64%), E (60%), while 55% demonstrated resistance to CIP and 50% resistance to tetracycline. In addition, 50% of the tested isolates were resistant to AMC which was mainly isolated from human stool (n = 52), animal stool (n=36), UTI (n = 27), meat products (n = 11), seafood (n = 8), water (n = 2), dairy products (n = 2) and poultry (n = 1). Almost 93% of the isolated *E. coli* were MDR as they were resistant to between 4 and 13 of the tested antibiotics; this pattern was more pronounced among isolates from clinical samples and meat products as all of these isolates were resistant to AMC/ 30, AM/ 10, E/ 15, P/ 10, RA/ 5, TE /30 and VA /30. The MDR phenotypes among clinical samples represent a major health threat and suggest that some of these drugs may be of limited use. The detection of EHEC poses a challenge for clinical microbiology laboratories. Key issues concerning their detection include the need to distinguish from commensal *E. coli* (Bennett-Wood et al., 2004, Edson et al., 2010). The currently accepted method for detection may be direct plating on SMAC or other media such as cefexime-SMAC or SMAC supplemented with cefexime and tellurite (CT-SMAC). Culture on SMAC is a simple method for screening for STEC O157, but it cannot identify non-O157 serogroups. The multiplex PCR approach for the detection of *stx1*, *stx2, eaeA* and *hlyA* genes is advantageous in rapidly detecting these virulence genes and is highly recommended.

Our study demonstrates that non-O157 STEC comprised a significant proportion (70%) of all STEC strains detected. SMAC culture was selected for detection of STEC in which 10 STEC strains primarily appeared as typical *E. coli* colonies (pink-red), and could not be differentiated from other pathogenic or non-pathogenic (commensal) *E. coli strains* which grow on the same medium. We applied PCR assay for shiga toxins detection and found the assay to be a helpful tool for the detection of STEC belonging to both O157 and non-O157 serogroups. Our data support the consideration of others in the mandated use of a Shiga toxin assay for the identification of STEC (Kehl, 2002). If only a Shiga toxin assay is used, it is important that positive samples be subcultured for STEC detection and serotyped, with the goal of identifying and curtailing potential outbreaks of STEC infection. We were able to identify molecular markers (*stx1*, *stx*2 and *hlyA*) for the 10 STEC isolates which represented 4% of the total isolates. Three strains carried both s*tx1* and *stx2*, seven strains carried *stx1* only, one strain carried *stx1, stx2* and *hlyA* and three strains carried *stx1* and *eaeA*.

It was of a particular concern to find that all the 10 STECs belonging to both O157 and non-O157 serogroups were positive for both CR and hemolysis tests, indicating that these serogroups may share common virulence characteristics. Most studies on O157 and non-O157 STEC have included mixed populations of adults and children (Brooks et al., 2005; CDC, 2005). Outbreaks of non-O157 STEC have been reported in children (Ethelberg, 2007) and in 38% of STEC infections among a pediatric cohort (Klein et al., 2002). Our results identified seven STEC strains that were isolated from clinical samples, of which four were isolated from stools of diarrheic animals and two (O157and non-O157) were from adult patients with UTIs and one (O157) was from stool of diarrheic child under 5 years of age. Data collected from multiple sites in the world showed that the highest incidence of both *E. coli* O157 infection and HUS occurred among children of up to 5 years old and that the median age of patients with HUS was 4 years (Gould, 2009); nevertheless, the incidence of non-O157 STEC infection and the clinical sequelae need to be more clearly defined (Klein et al., 2002). *E. coli* accounted for the majority of urinary tract infections in young adults and

pregnant women (Joshua et al., 2006).EHEC can cause hemolytic colitis (Johnson et al., 2006), and hemolytic uremic syndrome (HUS) which progress in some patients to renal failure (Koooopman et al., 1991). HUS is a life threatening condition especially among children and the elderly (Koooopman et al., 1991). In Nigeria, Akinduti et al. (2008) isolated O157:H7 serotype from urine samples of adult males and females suffering from severe UTI. Akinduti et al. (2008) reported a very high prevalence (46.4%) of *E. coli* 0157:H7 among tested patients; their study cited that renal diseases associated with severe UTI were mainly caused by *E. coli* O157:H7. The percentage of STEC in our clinical UTI samples is considered notably high considering the small number of UTI cases (n=47) included in the study. To the best of our knowledge, this report is the first from the country to identify non-O157 STEC from patients with UTI, which suggests the need for further community and hospital-based surveillance studies to better estimate the prevalence of STEC in this region and their impact on human disease.

Christina et al. (2011) surveyed 5,110 children for STEC at Children's Hospital in Boston and demonstrated that 50 cases (0.9%) had confirmed STEC infections; 33 were caused by O157:H7 strains and 17 were caused by non-O157:H7 strains belonging to various serotypes such as O25:H11, O26:H11, O26:H28, O111:nonmotile, O118:H16, O126:H1, O130:H11, O145:nonmotile and O146:H28. However, there were no significant differrences in disease severity between STEC infections in children caused by O157:H7 and those caused by non-O157 STEC. Our survey identified a comparable percentage in the distribution of non-O157 serogroups as those reported from Christina et al. (2011) as we identified four non-O157 serogroups (O158, O114, O125 and O26) which were isolated from clinical specimens, meat products and water.

The MDR phenotype of both O157 and non-O157 strains in the current study signifies that the specific STEC isolates are highly resistant to commonly available antibiotics, limiting the selection of treatment choice. Multidrug resistance among *E. coli* isolates has been a subject of concern worldwide (Chitnis et al., 2003, Lestari et al., 2008). Ground beef and other bovine products have often been implicated as sources of Shiga toxigenic *E. coli* (Griffin and Tauxe, 1991), along with other food products (Ackers et al., 1998). Occasional outbreaks of STEC have also been associated with drinking public water (Swerdlow et al., 1992) and swimming in contaminated water (Friedman et al., 1999).

The present investigation also revealed three non-O157 STEC strains, two originating from processed meat products and one from the main fresh water canal of Ismailia. It was unknown whether the contamination of meat or water originated from animal or human sources but conceivably, a combination of the above origins could explain the presence of STEC traits found in these isolates. We are in the process of analyzing PFGE results of all STEC strains to compare clonality and evaluate the relatedness of the isolated strains. Most of the STEC isolates in our study possess two or more virulence factors or traits (CR indicator, hemolysin production, *stx1, stx2*, *hlyA*). The presence of multiple virulence factors increases the virulence potential of microorganisms, since such factors function synergistically to overcome and defeat normal host defences. Thus, strains with more virulence factors are potentially considered as virulent pathogens (Hamuel et al., 2011). Our findings may imply that the isolates from the examined sources could be considered as potentially pathogenic.

It is of interest to demonstrate that STEC serotype O104 was not identified from the environmental sources tested in the present study; fenugreek imported from Egypt during the period 2009 to 2011 was blammed as the most likely source of STEC epidemic in Germany that resulted in 49 deaths. One of the limitations of this investtigation is that we could not identify the (H) flagellar antigens and hence determine the exact serotype for the isolated shiga toxin producing *E. coli* as it was commercially unavailable during the time-frame of our study. Additionally, since this is considered as a lab-based study, and with the small number of human disease cases in the present study (and the lack of clini-cal data), an association between disease severity and specific STEC serotypes could not be made.

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