Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Characterization of verotoxigenic *E.coli* and enteropathogenic *E.coli* isolated from infants with diarrhea in combination with antimicrobial resistance pattern in Minia, Egypt

Shimaa Salah Hassan Abd El Gany[1], Gamal Fadl Mahmoud Gad[2], Suzan Omar Mousa[3], Reham Ali Ibrahem[2*]

[1] Department of Microbiology and Immunology, Faculty of Pharmacy, Deraya University, 61111 New Minia, Egypt
[2] Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt
[3] Department of Pediatric, Children's University Hospital, Faculty of Medicine, Minia University, 61519 Minia, Egypt

Received: February 16, 2020; revised: March 1, 2020; accepted: March 3, 2020

Abstract

Diarrheal disease is considered a significant health problem, principally in developing countries as it is one of the leading causes of morbidity and mortality especially in infants. Studies found that STES and EPEC strains are among the most predominant causative agents in acute diarrhea. The aim of the study was to investigate the presence of certain virulence factors and antibiotic resistances of STEC and EPEC originating from infants with diarrhea in Egypt.

A total of 200 infants of different ages suffering from diarrhea between August 2017 and February 2019 were registered in this study. Standard cultural and biochemical methods were used to isolate *E. coli* species while; PCR was applied for the detection of virulence genes (*stx*1, *stx*2, *eae* and *pfb* A) for STEC and EPEC isolates, respectively. Eighty nine (44.5 %) *E. coli* strains were isolated from 200 stool samples. A total of 40 (44.9 %) strains were identified as STEC, and 1(1.12 %) strain was atypical EPEC. Fourteen of the STEC serotypes were O157:H7. Susceptibilities of 40 STEC and 1 aEPEC isolates were determined for ten antimicrobial drugs. STEC O157:H7 strains and STEC Non-O157:H7 exhibited the highest resistance against cefazolin and no resistance against imipnem and Amikacin, respectively while, the isolated strain of aEPEC was sensitive to all the studied antibiotics except cefazolin. Results showed that STEC is one of the major causes of diarrhea that showed resistant to commonly used antibiotics so representatives must pay great attention to this matter in order to increase the health of the community.

Key words
stx1, stx2, STEC, EPEC, E. coli

1. Introduction

Diarrheal disease is a major worldwide problem, particularly in the developing countries [1]. Diarrheagenic *Escherichia coli* are one of the most significant etiologic agents of diarrhea that causing high morbidity and mortality, typically among children [2]. *Escherichia coli* (*E. coli*) strains belonging to enterobacteriac*eae* family are Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobic organisms which found as normal habitant of digestive tract in humans and warm blooded animals. Various strains of this species have been classified to different pathotypes on the basis of the pathogenesis and virulence factors [3].

Intestinal pathological types are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and Shiga toxin-producing *E. coli* (STEC). *E. coli* strains that possess shiga toxins are called STEC, verocytotoxin-producing E. coli (VT)، or enterohemorrhagic *E. coli* (EHEC) [4].

Enteropathogenic *Escherichia coli* (EPEC) are the main cause of infantile diarrhea in developing countries [5]. Mechanism and etiology of EPEC causing diarrhea is different from other virulent pathotypes of *E. coli*. The incidence of EPEC contamination is high in first six months of age [6].

The principal mechanism of EPEC pathogenesis involves an attaching and effacing (A/E) lesion resulting in intestinal microvilli damage, intimate adherence of bacteria to the intestinal epithelium and other physiological changes in the cytoskeleton at the sites of bacterial attachment.

The genes that are responsible for the production of these lesions are called intimin (*eae*) and translocated receptor (*tir*). These genes are located on a pathogenicity island recognized as the locus for enterocyte effacement (LEE), which encodes virulence factors responsible for the attaching and effacing lesions.

The characterization of typical EPEC strains (tEPEC) and atypical (aEPEC) is based on harbouring *eae* and *pfb* genes ("bundle-forming pili" encoded by the EAF plasmid). The EPEC are considered atypical (aEPEC) in case of absence of the EAF plasmid, making it *eae*+ and *pfb*− and typical (tEPEC) in case of absence of the EAF plasmid, making it *eae*+ and *pfb*+ [7].

Shiga toxin-producing *Escherichia coli* (STEC) or verocytotoxin (VT)-producing *E. coli* (VTEC) strains are considered as an important foodborne pathogens responsible for

**** Correspondence: Reham Ali Ibrahem***
Tel.: *+2 01006804684****; Fax:*** *+20 862369075*
Email Address: *rehamee_micro@yahoo.com*

sporadic cases to serious outbreaks worldwide of public health interest, and associated with diseases in humans and animals. In humans, they can cause severe outbreaks of gastrointestinal illness symptoms ranging from diarrhea and hemorrhagic colitis to hemolytic uremic syndrome [8]. STEC strains causing human infections go to a large number of O: H serotypes. Most pathogenic cases have been attributed to the STEC O157 strains; the H7 flagellum is frequently but not always present on O157 isolates [9]. On the other hand, infections with non-O157 STEC types are frequently associated with severe illness in humans, VTEC strains are characterized by the gathering of two potent cytotoxins (*stx*1 and *stx*2) causing tissue damage in man and animals, called Shiga toxins because of their similarity with the toxin formed by *Shigella dysenteriae* or called verotoxins because of their action on Vero cells [10]. *Stx*-2 toxin is more toxic to cells than *Stx*-1 [11], In addition, a number of virulence genes like the intimin (*eae*) which discussed before in EPEC and the enterohemolysin (*hly*) that releases hemoglobin from the red blood cells during bacterial attack causing severe diseases [12]. EPEC and STEC are differentiated by the presence of the Shiga tox1in genes which found only in STEC [13]

Different studies showed that DEC such as STES and EPEC strains are among the most prevalent causative agents of acute diarrhea in children especially in the developing countries [14] so, the aim of the present study was investigation of STEC and EPEC as etiological agent of diarrhea in children in Minia governorate, Egypt. PCR technique was used to detect the presence of *stx*1, *stx*2, *eaeA* and *pfb A* genes. Identification of the serotype H7:O157 among STES as well as antimicrobial susceptibility of ETEC and EPEC.

2. Materials and Methods

2.1. Specimens' collection

A total of 200 stool samples were collected from children suffering from acute diarrhea before any antibiotics administration in El Minia University hospital, Egypt during the period from August 2017 to February 2019. Diarrhea was demarcated as three or more discharges within 12 hours, or just one liquid or semiliquid stool with mucus, pus or blood. A loop full of fecal sample was streaked on MacConkey agar and incubated for 24 h at 37ºC. Pink colonies was subcultured on Eosin Methylene Blue (EMB) agar. Colonies that show green metallic sheen color were isolated for a further identification as *E. coli* using various biochemical tests [15].

2.2. Detection of virulence genes (*stx*1, *stx*2, *eae* and *bfp* A)

2.2.1. DNA extraction and PCR

Genomic DNA template for PCR were obtained from overnight identified *E. coli* cultures that were picked, then suspended in 200 ml of sterile distilled water, and boiled for 15 min and the insoluble material was removed by centrifugation for 5 min. The supernatant was collected and stored at -20 °C until testing [16].

Detection of virulence genes was done by PCR technique. Primer sequences, PCR conditions that were used and references for the primers were listed in **(Table 1)**; PCR products were separated and visualized by gel electrophoresis in 1 % agarose in Tris acetate EDTA (TAE) buffer at 100 V,

Table 1: PCR primers, conditions for amplification of virulence genes and expected lengths of PCR amplification products.

Gene	Primer sequence (5'-3')	Conditions used	Gene product	Reference
***Stx*-1 F** **R**	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	Denaturation for 5 minutes at 95°C, 35 cycles (95°C for 30 seconds, 62°C for 30 seconds and 72°C for 30 seconds), and a final extension step at 72°C for 7 minutes	180 bp	[17]
***Stx*-2 F** **R**	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	Denaturation for 5 minutes at 95°C, 35 cycles (95°C for 30 seconds, 62°C for 30 seconds and 72°C for 30 seconds), and a final extension step at 72°C for 7 minutes	255bp	[17]
***eae* A F** **R**	GTGGCGAATACTGGCGAGACT CCCCATTCTTTTTCACCGTCG	denaturation for 3 minutes at 95°C, 35 cycles (95°C for 20 seconds, 72°C for90 seconds and 72°C for 5 minutes and a final extension step at 72°C for 5 minutes	890 bp	[18]
***bfp* A F** **R**	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	denaturation for 5 minutes at 94°C, 29 cycles (94°C for 1 minute, 60°C for1 minute and 74°C for 2 minutes and a final extension step at 72°C for 7 minutes	326 bp	[19]

where a 100 bp DNA ladder was included in each run, accordingly the amplified product.

2.3. Detection of *E. coli* O157:H7

Serotyping of *E. coli* strains was performed using 2 different monospecific *E. coli* test. Rabbit antisera (H7 and O157) produced by Statens Serum Institute according to manufacturer's recommendations [20].

2.4. Antimicrobials Susceptibility testing

The identified STEC (O157 and Non O157) and EPEC were tested for their susceptibility to 10 antimicrobial agents: Ampicillin&sulbactam (SAM, 20 µg), Amoxycillin & clavulunicacid (AMC, 30 µg), Cefazolin (CZ, 30 µg), Cefuroxime (CXM, 30 µg), Cefatriaxone (CRO, 30 µg), Cefipime (FEB, 30 µg), Amikacin (AK, 30 µg), Gentamycin(CN, 10 µg), Ofloxacin (OFX, 5 µg), Imipenem (IPM, 1 µg). This was performed on Mueller–Hinton Agar using Kirby– Bauer disk diffusion technique [21]. The antibiogram of each *E. coli* isolate was determined based on the breakpoints of the inhibition zone diameters for individual antibiotic agents as recommended by the manufacturer. Obtained results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing [22].

3. Results

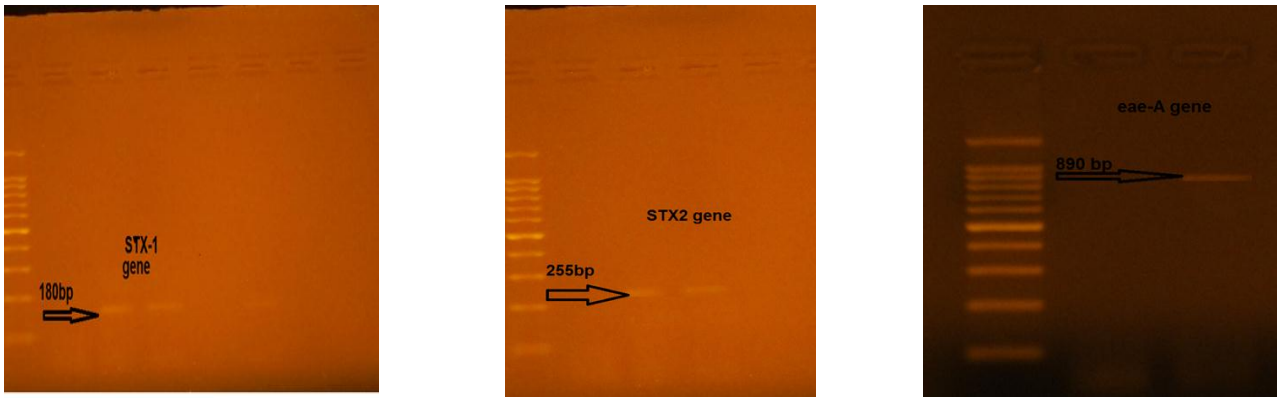
In this study, a total of 200 stool samples were collected from children of different ages suffering from acute diarrhea in in El Minia University hospital, Egypt. Among these collected stool samples. 89 (44.5 %) were confirmed as *E.coli* bacterial isolates by using various cultural and biochemical methods.

3.1. Genotypic detection of the isolated STEC and EPEC

Genomic analysis of 89 *E.coli* that were isolated from diarrheal children was detected by using PCR to detect the presence of *stx*1, *stx*2, *eae*A and *bfp*A genes. *E.coli* strains that harbored *stx* (*stx*1 and/or *stx*2) genes and were positive or negative for *eae*A were identified as STEC and strains that were negative for *stx* (*stx*1 and/or *stx*2) genes but possessed *eae*A gene were considered as EPEC, while occurrence of *bfp* A gene indicated the presence of tEPEC and its absence indicated the presence of aEPEC.

Stx1 and *stx*2 showed PCR products at 180 bp (A) and 255 bp (B) respectively, while *eae*A showed PCR products at 890 bp (C) **(Figure 1)**. No products were obtained from *bfp* A gene.

Out of total samples, 40 (44.94 %) STEC and 1 (1.11 %) aEPEC were identified which was *eae*A positive and *bfp* negative. Among STEC isolates 24 strains were *stx*1 positive, 8 strains were *stx*2 positive, 8 strains were *stx*1&*stx*2 positive and no strain was *eae* positive **(Table 2)**.



(A) **(B)** **(C)**

Figure 1: PCR detection of *Stx*1 (A), *Stx*2 (B) and *eae*A (C) genes. Lane 1, molecular size marker (100 bp Ladder); lane 3, *Stx*1 at 180 bp, *Stx*2 at 255 bp and *eae*A at 890 bp; lane 2, negative control (DNA template absent)

Table 2: Prevalence of Shigatoxigenic *E. coli* and Enteropathogenic *E-coli* among total number of *E. coli* isolates

Virulence genes	Shiga toxin producing *E.coli*				Atypical Enteropathogenic *E.coli*	
	O157		Non O157			
	No	%*	No	%*	No	%*
***Stx*1**	8	8.98	16	17.97	0	0
***Stx*2**	2	2.25	6	6.74	0	0
***Stx*1and *Stx*2**	4	4.49	4	4.49	0	0
***eae*A**	0	0	0	0	1	1.12
***pfb*A**	0	0	0	0	0	0
Total	14	15.72	26	29.2	1	1.12

*** The calculated percentage from the total no. of isolated *E. coli* strains**

3.2. Detection of O157 and non O157 STEC strains

(Table 2) shows that agglutination of *E. coli* strains with O157 and H7 antisera revealed that 14 serotypes (O157: H7). Of the 14 O157: H7, Four STEC strains were positive for both *stx*1 and *stx*2, eight strains were positive for *stx*1 only and two strains were positive for *stx*2 only while, 26 STEC did not show any agglutination with the used antisera (non O157:H7) .

Demographic data of patients are represented in **(Table 3)**. The children were classified into three different groups according to their age: (0-1), (>1-2) and (>2-5). The highest incidence of STEC and aEPEC strains was observed in male childern at the age of >2-5 years and the macroscopic analysis of the stool samples showed that 28 cases (31.46%) were watery diarrhea, 13 (14.6%) were mucoid diarrhea and no cases of bloody diarrhea. The Highest incidence of STEC was in summer and no STEC was apparent in winter. aEPEC strain was isolated in summer.

3.3. The antibiotic resistance patterns of the identified STEC and EPEC

3.3.1. The antibiotic resistance patterns of the identified STEC in terms of *stx*1, *stx*2 and both *stx*1 and *stx*2

Testing of antibiotic susceptibility of the 40 STEC isolates showed high prevalence of multi resistance to various antimicrobial agents.

3.3.2. Resistance pattern of STEC O157:H7

(Table 4) determines the resistance pattern of STEC (O157:H7) strains. Regarding the STEC (O157:H7) strains, they exhibited resistance against Cefazolin (100 %), high resistance against cefuroxime (92.85 %), cefatriaxone (85.71 %), moderate resistance against amoxicillin/clavulunic acid (57.14 %), cefipime (50 %) and ampicillin/sulbactam (42.85 %), low resistance against gentamicin (14.28 %), amikacin (7.14 %) and ofloxacin (7.14 %) while, no resistance against imipenem.

Complete resistance against cefazolin (100 %), high resistance against cefuroxime (87.5 %) and cefatriaxone (75 %) and low resistance against cefipime and amoxicillin/clavulunic acid (37.5 %), ampicillin/ sulbactam (25 %) and gentamicin (12.5 %) were detected in eight of the O157:H7 STEC strains that were positive to *stx*1 gene, while these strains were found to be completely sensitive to amikacin, imipenem and ofloxacin (100 %).

Complete resistance against cefazolin, cefuroxime, cefatriaxone and amoxicillin/clavulunic acid and partial resistance against ampicillin/ sulbactam, cefipime and ofloxacin (50 %) were detected in the two O157:H7 STEC strains that were positive to *stx*2 gene, while these strains were found to be completely sensitive to amikacin, imipenem and gentamicin (100 %).

Four O157:H7 STEC strains that were positive to both *stx*1 and *stx*2 gene were completely resistant against cefazolin, cefuroxime and cefatriaxone (100%), high resistance against ampicillin/ sulbactam, cefipime and amoxicillin/clavulunic acid (75%) and low resistance against gentamicin and amikacin (25%), while these strains were found to be completely sensitive to imipenem and ofloxacin (100%).

3.3.3. Resistance pattern of STEC (Non - O157:H7)

(Table 5) shows resistance pattern of STEC Non-O157 strains. It was noted that STEC non-O157 showed high resistance to cefazolin (88.46 %), moderate resistance to amoxicillin/clavulunic acid (53.84 %), cefuroxime (42.31 %), cefatriaxone (34.6 %) and cefipime (23.1 %).While, low resistance occurred against ampicillin/sulbactam (11.54 %), gentamicin (7.7 %) and ofloxacin (3.85 %). No resistance was observed against amikacin and imipenem (0 %)

Table 3: Distribution of STEC and aEPEC strains in accordance to season, age, gender and clinical symptoms

Characteristics	Shiga toxin producing *Escherichia Coli* (STEC)(n=40)				atypical Enteropathogenic *Escherichia Coli* (aEPEC) (n=1)		Total	
	O157 (n=14)		NON O157 (n=26)					
	No.	%*	No.	%*	No.	%*	No.	%*
Gender								
Male	4	4.49	22	24.72	0	0	26	29.21
Fem Ale	10	11.23	4	4.49	1	1.12	15	16.84
Age(year)								
0-1	3	3.37	6	6.74	0	0	9	10.11
>1-2	4	4.49	7	7.86	0	0	11	12.35
>2-5	7	7.86	13	14.6	1	1.12	21	23.59
Appearance								
Watery diarrhea	10	11.23	17	19.1	1	1.12	28	31.46
Mucoid diarrhea	4	4.49	9	10.11	0	0	13	14.6
Seasons								
Spring	5	5.62	4	4.49	0	0	9	10.11
Summer	8	8.98	20	22.47	1	1.12	29	32.58
Autumn	1	1.12	2	2.247	0	0	3	3.37
Winter	0	0	0	0	0	0	0	0

***The calculated percentage from the total number of the isolated *E.coli* strain**

Table 4: Antimicrobial drug resistance patterns in Positive O157:H7 *E. coli* strains in terms of Shiga toxins (No=14)

Sample No.	AK	CXM	SAM	OFX	CZ	AMC	FEB	CN	CRO	IPM
***Stx*1 Positive**										
173	S	R	S	S	R	S	S	S	R	S
178	S	R	S	S	R	S	S	R	R	S
146	S	R	S	S	R	S	S	S	R	S
119	S	R	R	S	R	R	R	S	R	S
120	S	R	S	S	R	S	R	S	S	S
160	S	S	S	S	R	S	S	S	S	S
143	S	R	S	S	R	R	S	S	R	S
53	S	R	R	S	R	R	R	S	R	S
***Stx*2 Positive**										
135	S	R	R	R	R	R	R	S	R	S
136	S	R	S	S	R	R	S	S	R	S
***Stx*1&*Stx*2 Positive**										
177	R	R	S	S	R	S	S	R	R	S
61	S	R	R	S	R	R	R	S	R	S
55	S	R	R	S	R	R	R	S	R	S
20	S	R	R	S	R	R	R	S	R	S
Total resistance percentage	7.14 %	92.85 %	42.85 %	7.14 %	100 %	57.14%	50 %	14.28 %	85.71 %	0 %

***The calculated percentage from the total number of the isolated STEC O157 isolates.**
Abbreviations: AK: Amikacin; CXM: Cefuroxime; SAM: Ampicillin/sulbactam; OFX: Ofloxacin; CZ: Cefazolin; AMC: Amoxicillin/clavulunic acid; FEB: Cefipime; CN: Gentamicin; CRO: Cefatriaxone; IPM: Imipenem.
S: Sensitive R: Resistant

Table 5: Antimicrobial drug resistance patterns in Positive NON-O157:H7 *E. coli* strains in terms of Shiga toxins (No =26)

Sample No.	AK	CXM	SAM	OFX	CZ	AMC	FEB	CN	CRO	IPM
***Stx*1 Positive**										
190	S	R	S	S	R	S	S	S	R	S
184	S	S	S	S	S	S	S	S	S	S
31	S	S	S	S	R	S	S	S	S	S
118	S	R	S	S	R	S	R	S	R	S
129	S	S	S	S	R	R	S	S	S	S
81	S	S	R	S	R	R	S	S	S	S
125	S	R	S	S	R	R	S	S	R	S
77	S	S	S	S	R	R	S	S	S	S
175	S	R	R	S	R	R	R	R	R	S
74	S	S	S	S	R	S	S	S	S	S
76	S	R	S	S	R	R	S	S	S	S
64	S	R	S	R	R	R	R	R	R	S
67	S	S	S	S	R	R	S	S	S	S
24	S	R	S	S	R	R	R	S	R	S
66	S	S	S	S	R	R	S	S	S	S
116	S	S	S	S	R	S	S	S	S	S
***Stx*2 Positive**										
117	S	S	S	S	R	S	S	S	S	S
183	S	S	S	S	R	S	S	S	S	S
15	S	R	S	S	R	S	S	S	R	S
1	S	S	S	S	S	R	S	S	S	S
34	S	R	S	S	R	R	R	S	R	S
33	S	R	R	S	R	R	R	S	R	S
***Stx*1& *Stx*2 Positive**										
6	S	R	S	S	R	R	S	S	S	S
130	S	S	S	S	R	S	S	S	S	S
180	S	S	S	S	R	S	S	S	S	S
191	S	S	S	S	S	S	S	S	S	S
Total resistance percentage	0%	42.31%	11.54%	3.85%	88.46%	53.84%	23.1%	7.7%	34.6%	0%

***The calculated percentage from the total number of the isolated non-O157STEC isolates.**
Abbreviations: AK: Amikacin; CXM: Cefuroxime; SAM: Ampicillin/sulbactam; OFX: Ofloxacin; CZ: Cefazolin; AMC: Amoxicillin/clavulunic acid; FEB: Cefipime; CN: Gentamicin; CRO: Cefatriaxone; IPM: Imipenem. S: Sensitive R: Resistant

Resistance against cefazolin (93.75 %), amoxicillin/clavulunic acid (62.5 %) cefuroxime (43.75 %), cefatriaxone (37.5 %) and cefipime (25%) and low resistance against ampicillin/ sulbactam and gentamicin (12.5%) and ofloxacin (6.25 %) were detected in sixteen of the non- O157:H7 STEC strains that were positive to *stx*1 gene, while these strains were found to be completely sensitive to amikacin, imipenem (100 %).

High resistance against cefazolin (83.3 %), moderate resistance against cefuroxime, cefatriaxone and amoxicillin/clavulunic acid (50 %) and cefipime (33.3 %) and low resistance against ampicillin/sulbactam (16.6 %) were detected in six non-O157:H7 STEC strains that were positive to *stx*2 gene, while these strains were found to be completely sensitive to amikacin, imipenem, gentamicin and ofloxacin (100%).

Four non-O157:H7 STEC strains that were positive to both *stx*1 and *stx*2 gene were highly resistant against cefazolin (75 %) followed by amoxicillin/clavulunic acid and cefuroxime (25 %), while these strains were found to be completely sensitive to amikacin, imipenem, ofloxacin, gentamicin ampicillin/ sulbactam, cefipime, cefatriaxone (100 %)

3.3.4. Resistance pattern of aEPEC

It was detected that the only isolated strain of aEPEC was sensitive to all the studied antibiotics except cefazolin (100%) **(Table 6)**.

4. Discussion

Diarrhea is one of the main causes for morbidity and mortality in the developing countries. Pathogenic *E. coli* strains are one of the most important pathogens that lead to diarrhea. However, nonpathogenic strains of *E. coli* are normally presented in the intestinal tract of man and other warm-blooded animals [23]

The present study investigated the incidence of STEC (O157 and non O157), EPEC and their antibiotics susceptibilities. Although diseases caused by STEC are low if compared with salmonellosis for instance, STEC is considered as an important organism because of the serious health problems that may follow infection. Disease symptoms may begin from mild diarrhea to severe bloody diarrhea [hemorrhagic colitis (HC)] especially in children (1-4 years) [24]. Other reports found that EPEC are associated with outbreaks of infantile diarrhea among children in developing countries [25]. These previous causes paid our attention to make this study.

The current study showed a percentage of 44.94 % (40/89) STEC, these results were compatible with Sudershan et al [26] whom showed that the prevalence of STEC accounted for 30 %. Abbasi et al [27] showed that 15% of the isolated *Escherichia coli* were STEC which disagreed with our results.

Lower results detected by Pourakbari et al [28] and Jafari et al [29] whom reported that the prevalence of STEC strains in children with diarrhea in Tahran and Iraq, was 17 % and 18.9 %, respectively.

It is reported that most of the strains of STEC possess *stx*2, some of them possess *stx*1 and *stx*2 and a little possess only *stx*1 [30]. These virulence genes are the two most significant virulence factors for humans. Studies had reported that *stx*2 was a more important virulence factor than *stx*1, which was associated with human diseases [31].

Our results showed that among 40 STEC isolates, 24 strains harbored *stx*1 (26.96 %), 8 strains harbored *stx*2 (8.988 %), 8 strains harbored stx1 and stx2 (8.988 %) and no strain harbored *eae*A (intimin gene) so we can say that there was no enterohemorrhagic strain of *E. coli* (EHEC) as the EHEC possess intimin (*eae*) gene with both stx1 and stx2.

Aly, Essam and Amin [32] isolated only one strain of EHEC which harbored intimin, *stx*1and *stx*2 genes together from clinical cases in Cairo, Egypt and this result differed completely from findings of the present work.

Some reports suggested that there was an exciting occurrence in developing countries in which EHEC was isolated much less frequently than other diarrheagenic *Escherichia coli* strains such as ETEC or EPEC [1]

Production of intimin gene was not essential for pathogenesis because a number of sporadic cases of HUS caused by eae-negative O157 STEC [33]. Das and his assisstants [34] determined that majority of human STEC isolates lacked the virulence gene (*eae*). A study done by Auda [35] revealed that there was no STEC isolates carry eae-A gene. These results agreed with our findings.

Bonyadian et al [36] stated that among 58 STEC, 16 strain were positive for stx1 (27.6 %), 4 strains were positive for stx2 (6.9 %) and 8 strains were positive for stx1 and stx2 (13.8 %) that were approximately similar to our results except for the prevalence of both stx1 and stx2 was higher than our findings.

Jafari et al [29] revealed that the percent of STEC harbored *stx*1, *stx*2, *stx*1and *stx*2 were 28.57 %, 11.7 % and 1.8 % respectively. Abbasi et al [27] found that among 15 STEC isolates, STEC

Table 6: Antimicrobial drug resistance patterns in Enteropathogenic *E. coli* strain

Sample No.	AK	CXM	SAM	OFX	CZ	AMC	FEB	CN	CRO	IPM
***eae*A Positive**										
197	S	S	S	S	R	S	S	S	S	S
Total resistance percentage	0	0	0	0	100	0	0	0	0	0

***The calculated percentage from the total number of the isolated aEPEC isolate.**

Abbreviations: AK: Amikacin; CXM: Cefuroxime; SAM: Ampicillin/sulbactam; OFX: Ofloxacin; CZ: Cefazolin; AMC: Amoxicillin/clavulunic acid; FEB: Cefipime; CN: Gentamicin; CRO: Cefatriaxone; IPM: Imipenem.

S: Sensitive R: Resistant

strains contain *stx*2 was higher than *stx*1 and this disagreed with the current study. These differences regarding secretion of *stx* genes might be attributed to the difference in geographical distribution, diet habits, seasonal changes, stress, ages and the state of the immune system.

Despite that STEC O157:H7 is the most identified cause of human gastroenteritis, there are many non-O157:H7 *E. coli* which can cause severe diarrheal diseases [37]. It was obvious by findings in Iran showing absence of the O157:H7 serotype among STEC isolates [38]. Also, certain studies in Canada, United States, Japan, England and Scotland expressed low prevalence of STEC O157:H7[39]. EL-Alfy and co- workers [40] demonstrated that non-O157 STEC comprised a significant proportion (70 %) of all detected STEC strains and KhoSrAvi et al [41] reported that non-O157:H7 *E. coli* were the major cause of pediatric infections in this region of Iran. All the previous findings were near to this study.

Sharaf and Shabana [42] reported that 17 STEC were O157 with percentage of 35.4 % and 9 STEC were non-O157 with percentage of 18.8 % that disagreed with our reports except for the prevalence of *stx*1 which was significantly higher than stx2 in O157 STEC and non O157 STEC which was similar to the current study. Onlen and his co-workers [43] stated that *stx*2 frequency was found to be higher than the *stx*1 frequency that was in contrast to these findings.

This study determined that the highest number of STECs among diarrheal cases was reported in > 2-5 year and in males. The high frequency of *E. coli* O157:H7 and non O157:H7 serotypes were detected during summer. Similar study was done by Sharaf and Shabana [42] who reported that STEC was higher in 0-4 years old and in males.

Results showed that the percentage of enteropathogenic *Escherichia coli* was 1.12%. Bahmanabadi et al [44] agreed with the current study. They identified only 7 isolates from 101 *E. coli* were EPEC.

Abbasi and co-workers [27] found that 13 strain of *E. coli* were EPEC (27 %) that disagreed with our findings. It was reported that EPEC had a major role in the incidence of infantile diarrhea in developing countries [45]. Also, Studies from different area proved the significant role of EPEC in the occurrence of endemic diarrhea [46, 47] or its important role in outbreaks [48, 49] but these previous findings and reports were different completely from our results that reported high prevalence of STEC and only one aEPEC strain. Despite that we considered as developing country, we had very low frequency of aEPEC thus might be attributed to the self-limiting nature of the disease [50]. Studies from India and other countries reported the occurrence of increased frequency of atypical EPEC from children without diarrhea [51-53] and the examined stool specimens in this study were taken from children suffering from acute diarrhea and that also might be the cause of low number of EPEC strains.

In this study, All EPEC isolates were positive for *eae* gene but negative for *bfp* and stx gene and were classified as atypical EPEC genotype (eae^{+}, bfp^{-}, stx^{-}) [54]. In our study, only one aEPEC was positive only for *eae*-A gene but negative *for bfp*-A and *stx*1&*stx*2. Bahmanabadi and his assisstants [44] identified only 7 EPEC isolates from 101 *E. coli* strains. These EPEC strains carried *eae* but did not carry *stx*1, *stx*2and *bfp*A genes and this agreed with our result. Singh et al [5] reported that atypical EPEC (*eae*+ and *bfp*-) detected only in 2 diarrheal samples (5%) which was slightly higher than our findings.

On the other hand, Blanco et al [55] found that atypical EPEC strains could be a major cause of diarrhea in Spain and proved that, in developed countries, atypical EPEC were more often isolated from patients with diarrhea (5.2 %) than typical EPEC (0.2 %).

Different results were obtained in New Delhi, India by Ghosh and Ali [56]. They isolated 296 *E. coli* strains from 296 fecal specimens from children suffering from diarrhea. Only 4.05 % samples harbored the *eae* gene and were considered EPEC. Out of 12 EPEC isolates, 25 % were classified as atypical EPEC, while 75 % were found to have the *bfp*A gene and were classified as typical EPEC.

Another report in Iran that performed by Bouzari et al [38], Out of 102 *Escherichia coli* isolates that obtained from fecal samples collected from patients (children and adults) with acute diarrhea in a number of Iranian provinces, 52 strains of *Escherichia coli* were identified to harbor STEC 26 (50 %), EPEC 13 (25 %) and EHEC 13 (25 %). This finding were nearly similar to our results regarding the higher prevalence of STEC than EPEC and the absence of tEPEC but differed in the finding of EHEC as no EHEC were found in our cases.

Our study revealed that the only detected aEPEC strain among diarrheal cases was reported in > 2-5 year and in female. aEPEC strain was detected during summer. Similar findings showed that the higher incidence of EPEC strains is in the summer [57]. Also, this phenomenon agrees with the findings in other studies [58, 59]. On the other hand, Bouzari et al [38] showed a high incidence of EPEC isolates during autumn.

The findings of this study and other previous reports recommend that geographical region and time of sampling are the most significant measures in epidemiology of diarrhea in children in developing countries.

Highest rate of infection was observed in the age >2-5 years. This may be owing to feeding children breast milk till the age of 1 to 2 years from mothers that has been reported to contain high levels of immunoglobulin A (IgA) antibodies [60].

The antibiotic susceptibility for STEC (O157 and non O157) showed high resistance to cefazolin and amoxicillin/ clavulunic acid, moderate resistance to cefuroxime, cefatriaxone and cefipime, low resistance to ampicillin/sulbactam (11.54%), gentamicin (7.7%) and ofloxacin (3.58%) and no resistance to amikacin and imipenem.

The resistance pattern of non O157 STEC to ampicillin was 9.2 % which was close to this current study [61]. Sharaf and Shabana [42] reported that non O157 STEC indicated slightly low resistance to gentamicin 10.3 % which agreed with this result except for the resistance to ampicillin (23.7 %) which was higher than our findings. From the 14 isolates of O157 STEC, all strains were resistant to cefazolin, 92.85 % were resistant to cefuroxime and 57.14% were resistant to amoxicillin/clavulunic

acid. Findings of this work agreed with a study made in Nigeria showed that *E. coli* O157 isolates obtained from hospitalized children exhibited 100 % resistance to cefuroxime and 67 % resistance to amoxicillin/clavulunic (Augmentin) [62]. Another study was nearly close to this work, revealed that more than 60% of the O157:H7 *E. coli* isolates were resistant to amoxicillin [26].

The current study demonstrated that aEPEC was completely sensitive to all tested antibiotics except cefazolin which agreed with that obtained by Sharaf and Shabana [42]. They reported that atypical EPEC isolates showed less resistance to all antimicrobial drugs.

5. Conclusion

This study found that STEC is the most commonly identified *E. coli* strain in our study and STEC non-O157:H7 pose an increasing risk in public health that is not less important than STEC O157: H7 . To end the increasing rate of resistance, we should avoid the indiscriminate use of antibiotics. We should increase awareness to proper sanitation programs. Supplementary studies can be specified to evaluate the role of the educational programs on reduction of the infections caused by diarrheagenic *E. coli.*

In the next future, studying the role of other diarrheagenic *E. coli* in infantile diarrhea will be done due to lack of funding.

Acknowledgement

The authors are grateful to Pediatrics department, Faculty of medicine, Minia University. Great thanks to the technicians, employees and workers of Microbiology and Immunology Department, Faculty of Pharmacy, Minia University. Also, we would like to thank doctor staff and patients of Minia University Hospital for their generous help and kindness.

References

[1] S. Vilchez, D. Reyes, M. Paniagua, F. Bucardo, R. Mollby, A. Weintraub, Prevalence of diarrhoeagenic Escherichia coli in children from Leon, Nicaragua, *Journal of medical microbiology*, 58 (2009) 630-637.

[2] A. Jafari, M.M. Aslani, S. Bouzari, Enteroaggregative Escherichia coli, a heterogenous, underestimated and under-diagnosed E. coli pathotype in Iran, *Gastroenterology and hepatology from bed to bench*, 6 (2013) 71-79.

[3] M.A. Croxen, R.J. Law, R. Scholz, K.M. Keeney, M. Wlodarska, B.B. Finlay, Recent advances in understanding enteric pathogenic Escherichia coli, *Clinical microbiology reviews*, 26 (2013) 822-880.

[4] D.M. Lozer, T.B. Souza, M.V. Monfardini, F. Vicentini, S.S. Kitagawa, I.C. Scaletsky, L.C. Spano, Genotypic and phenotypic analysis of diarrheagenic Escherichia coli strains isolated from Brazilian children living in low socioeconomic level communities, *BMC infectious diseases*, 13 (2013) 418.

[5] T. Singh, S. Das, V.G. Ramachandran, D. Shah, R. Saha, S.A. Dar, A. Rai, Typical & atypical enteropathogenic Escherichia coli in diarrhoea & their role as carrier in children under five, *The Indian journal of medical research*, 145 (2017) 551-557.

[6] K.Z. Hasan, P. Pathela, K. Alam, G. Podder, S.M. Faruque, E. Roy, A.K. Haque, R. Haque, M.J. Albert, A.K. Siddique, R.B. Sack, Aetiology of diarrhoea in a birth cohort of children aged 0-2 year(s) in rural Mirzapur, Bangladesh, *Journal of health, population, and nutrition*, 24 (2006) 25-35.

[7] L.R. Trabulsi, R. Keller, T.A. Tardelli Gomes, Typical and atypical enteropathogenic Escherichia coli, *Emerging infectious diseases*, 8 (2002) 508-513.

[8] K. Preussel, M. Hohle, K. Stark, D. Werber, Shiga toxin-producing Escherichia coli O157 is more likely to lead to hospitalization and death than non-O157 serogroups--except O104, *PloS one*, 8 (2013) e78180.

[9] C.N. Ateba, C.C. Bezuidenhout, Characterisation of Escherichia coli O157 strains from humans, cattle and pigs in the North-West Province, South Africa, *Int J Food Microbiol*, 128 (2008) 181-188.

[10] Y.J. Jeong, S.K. Park, S.J. Yoon, Y.J. Park, M.S. Lee, Experimental In Vivo Models of Bacterial Shiga Toxin-Associated Hemolytic Uremic Syndrome, *Journal of microbiology and biotechnology*, 28 (2018) 1413-1425.

[11] M.A. Rivero, J.A. Passucci, E.M. Rodriguez, A.E. Parma, Role and clinical course of verotoxigenic Escherichia coli infections in childhood acute diarrhoea in Argentina, *Journal of medical microbiology*, 59 (2010) 345-352.

[12] A.L. Cookson, S.C. Taylor, J. Bennett, F. Thomson-Carter, G.T. Attwood, Serotypes and analysis of distribution of Shiga toxin producing Escherichia coli from cattle and sheep in the lower North Island, New Zealand, *N Z Vet J*, 54 (2006) 78-84.

[13] M.Y. Alikhani, S.H. Hashemi, M.M. Aslani, S. Farajnia, Prevalence and antibiotic resistance patterns of diarrheagenic Escherichia coli isolated from adolescents and adults in Hamedan, Western Iran, *Iran J Microbiol*, 5 (2013) 42-47.

[14] M. Asadi Karam, S. Bouzari, M. Oloomi, M. Aslani, A. Jafari, Phenotypic and Genotypic Characterization of Enteropathogenic Escherichia coli (EPEC) strains in Tehran, Iran, *Iran J Microbiol*, 2 (2010) 3-7.

[15] S.T. Cowan, Cowan and Steel's Manual for Identification of Medical Bacteria, 2nd ed., Cambridge University Press, Cambridge, London, 1985.

[16] M.A. Islam, A.S. Mondol, E. de Boer, R.R. Beumer, M.H. Zwietering, K.A. Talukder, A.E. Heuvelink, Prevalence and genetic characterization of shiga toxin-producing Escherichia coli isolates from slaughtered animals in Bangladesh, *Applied and environmental microbiology*, 74 (2008) 5414-5421.

[17] A.W. Paton, J.C. Paton, Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157, *Journal of clinical microbiology*, 36 (1998) 598-602.

[18] P.K. Fagan, M.A. Hornitzky, K.A. Bettelheim, S.P. Djordjevic, Detection of shiga-like toxin (stx1 and stx2), intimin (eaeA), and enterohemorrhagic Escherichia coli (EHEC) hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR, *Applied and environmental microbiology*, 65 (1999) 868-872.

[19] S.T. Gunzburg, N.G. Tornieporth, L.W. Riley, Identification of enteropathogenic Escherichia coli by PCR-based detection of the bundle-forming pilus gene, *Journal of clinical microbiology*, 33 (1995) 1375-1377.

[20] F. Orskov, I. Orskov, Serotyping of Escherichia coli: in En Methods in Microbiology, London: *Academic Press Ltd*1984.

[21] J.H. Jorgensen, J.D. Turnide, J.A. Washington, Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods In: *Manual of Clinical Microbiology*, 4th ed., ASM Press, Washington, DC, 1997.

[22] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. PA: *Clinical and Laboratory Standards Institute.* 27th M100, 2018.

[23] M. Soleimani, A. Morovvati, S.Z. Hosseini, M.R. Zolfaghari, Design of an improved multiplex PCR method for diagnosis of enterohaemoraghic E.coli and enteropathogic E.coli pathotypes, *Gastroenterology and hepatology from bed to bench*, 5 (2012) 106-111.

[24] P.I. Tarr, C.A. Gordon, W.L. Chandler, Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome, *Lancet*, 365 (2005) 1073-1086.

[25] B. Bakhshi, S. Fallahzad, M.R. Pourshafie, The occurrence of atypical enteropathogenic Escherichia coli strains among children with diarrhea in Iran, Journal of infection and chemotherapy : *official journal of the Japan Society of Chemotherapy*, 19 (2013) 615-620.

[26] R. Sudershan, R.N. Kumar, B. Kulkarni, L. Kashinath, V. Bhaskar, K. Polasa, E. coli pathotypes and their antibiotic resistance in young children with diarrhea in Hyderabad, India, Int *J Curr Microbiol App Sci*, 3 (2014) 647-654.

[27] P. Abbasi, M. Kargar, A. Doosti, J. Mardaneh, S. Ghorbani-Dalini, M.A. Dehyadegari, Characterization of Shiga-toxin producing E. coli (STEC) and enteropathogenic E. coli (EPEC) using multiplex Real-Time PCR assays for stx1, stx2, eaeA, *Iranian Journal of Microbiology*, 6 (2014) 169.

[28] B. Pourakbari, H. Heydari, S. Mahmoudi, F. Sabouni, M. Teymuri, F. Ferdosian, M. Ashtiani, S. Mamishi, Diarrhoeagenic E. coli pathotypes in children with and without diarrhoea in an Iranian referral paediatrics centre/Pathotypes Escherichia coli diarrheogenes chez des enfants souffrant ou

non de diarrhees dans un centre pediatrique d'orientation-recours iranien, *Eastern Mediterranean Health Journal*, 19 (2013) 617.
[29] F. Jafari, L. Garcia-Gil, S. Salmanzadeh-Ahrabi, L. Shokrzadeh, M. Aslani, M. Pourhoseingholi, F. Derakhshan, M. Zali, Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children's hospitals, *Journal of infection*, 58 (2009) 21-27.
[30] M. Trevisani, R. Mancusi, G. Delle Donne, C. Bacci, L. Bassi, S. Bonardi, Detection of Shiga toxin (Stx)-producing Escherichia coli (STEC) in bovine dairy herds in Northern Italy, *Int J Food Microbiol*, 184 (2014) 45-49.
[31] S.D. Manning, A.S. Motiwala, A.C. Springman, W. Qi, D.W. Lacher, L.M. Ouellette, J.M. Mladonicky, P. Somsel, J.T. Rudrik, S.E. Dietrich, W. Zhang, B. Swaminathan, D. Alland, T.S. Whittam, Variation in virulence among clades of Escherichia coli O157:H7 associated with disease outbreaks, *Proc Natl Acad Sci U S A*, 105 (2008) 4868-4873.
[32] M.E. Aly, T.M. Essam, M.A. Amin, Genotypic Detection of Enterohaemoragic E. coli (EHEC) among Diarrheagenic Patients in Egypt, *International Journal of Microbiological Research*, 5 (2014) 61-64.
[33] M. Kargar, M. Homayoon, Prevalence of shiga toxins (stx1, stx2), eaeA and hly genes of Escherichia coli O157: H7 strains among children with acute gastroenteritis in southern of Iran, *Asian Pacific journal of tropical medicine*, 8 (2015) 24-28.
[34] S.C. Das, T. Ramamurthy, S. Ghosh, G.P. Pazhani, T. Sen, R. Singh, Molecular characterization of locus of enterocyte effacement pathogenicity island in shigatoxic Escherichia coli isolated from human & cattle in West Bengal, India, *The Indian journal of medical research*, 146 (2017) S30.
[35] D.A. Auda, PCR Detection of Intimin Gene in Shiga-Like Toxin Producing Escherichia Coli Isolated from Animal Carcasses, *Journal of Univesity of Thi-Qar*, 9 (2014) 1-8.
[36] M. Bonyadian, H. Momtaz, E. Rahimi, R. Habibian, A. Yazdani, M. Zamani, Identification & characterization of Shiga toxin-producing Escherichia coli isolates from patients with diarrhoea in Iran, *The Indian journal of medical research*, 132 (2010) 328-331.
[37] S. Purwar, S. Roy, S. Metgud, Non-O157:H7 Shiga Toxin Producing Diarrhoeagenic Escherichia coli (STEC) in Southern India: A Tinderbox for Starting Epidemic, *Journal of clinical and diagnostic research : JCDR*, 10 (2016) DC11-DC15.
[38] S. Bouzari, E. Farhang, S.M. Hosseini, M.Y. Alikhani, Prevalence and antimicrobial resistance of shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli isolated from patients with acute diarrhea, *Iran J Microbiol*, 10 (2018) 151-157.
[39] E.C. Mathusa, Y. Chen, E. Enache, L. Hontz, Non-O157 Shiga toxin-producing Escherichia coli in foods, *Journal of food protection*, 73 (2010) 1721-1736.
[40] S.M. EL-Alfy, S.F. Ahmed, S.A. Selim, M.H.A. Aziz, A.M. Zakaria, J.D. Klena, Prevalence and characterization of Shiga toxin O157 and non-O157 enterohemorrhagic Escherichia coli isolated from different sources in Ismailia, Egypt, African *Journal of Microbiology Research*, 7 (2013) 2637-2645.
[41] A.D. KhoSrAvi, S. KhAghAni, A.F. SheiKh, A. AhMADzADeh, A. ShAMSizADeh, Prevalence of Escherichia coli O157: H7 in children with bloody diarrhea referring to Abuzar teaching hospital, Ahvaz, Iran, *Journal of clinical and diagnostic research: JCDR*, 10 (2016) DC13.
[42] E.F. Sharaf, I.I. Shabana, Prevalence and molecular characterization of Shiga toxin-producing Escherichia coli isolates from human and sheep in Al-Madinah Al-Munawarah, *Infectio*, 21 (2017) 81-87.
[43] C. Onlen, N. Duran, S. Bayraktar, E. Ay, B. Ozer, The frequency of shiga-like toxin (stx1 and stx2) and EHEC-hlyA in food by multiplex PCR, *Revista Romana de Medicina de Laborator*, 25 (2017) 317-326.
[44] R. Bahmanabadi, M.B. Khalili, B. Bakhshi, M.M. Soltan-Dallal, Evaluation of the prevalence of typical and atypical enteropathogenic Escherichia coli isolated from stool specimens of patients with diarrhea admitted to Tehran Children's Hospital by the PCR Method, Feyz *Journal of Kashan University of Medical Sciences*, 22 (2018) 222-229.
[45] A.G. Torres, X. Zhou, J.B. Kaper, Adherence of diarrheagenic Escherichia coli strains to epithelial cells, *Infect Immun*, 73 (2005) 18-29.
[46] M.V. Dulguer, S.H. Fabbricotti, S.Y. Bando, C.A. Moreira-Filho, U. Fagundes-Neto, I.C. Scaletsky, Atypical enteropathogenic Escherichia coli strains: phenotypic and genetic profiling reveals a strong association between enteroaggregative E. coli heat-stable enterotoxin and diarrhea, *J Infect Dis*, 188 (2003) 1685-1694.
[47] M.A. Vieira, J.R. Andrade, L.R. Trabulsi, A.C. Rosa, A.M. Dias, S.R. Ramos, G. Frankel, T.A. Gomes, Phenotypic and genotypic characteristics of Escherichia coli strains of non-enteropathogenic E. coli (EPEC) serogroups that carry EAE and lack the EPEC adherence factor and Shiga toxin DNA probe sequences, *J Infect Dis*, 183 (2001) 762-772.
[48] J. Yatsuyanagi, S. Saito, H. Sato, Y. Miyajima, K. Amano, K. Enomoto, Characterization of enteropathogenic and enteroaggregative Escherichia coli isolated from diarrheal outbreaks, *Journal of clinical microbiology*, 40 (2002) 294-297.
[49] C. Jenkins, H.R. Smith, A.J. Lawson, G.A. Willshaw, T. Cheasty, J.G. Wheeler, D.S. Tompkins, Serotypes, intimin subtypes, and antimicrobial resistance patterns of atypical enteropathogenic Escherichia coli isolated in England from 1993 to 1996, *Eur J Clin Microbiol Infect Dis*, 25 (2006) 19-24.
[50] S. Dutta, G.P. Pazhani, J.P. Nataro, T. Ramamurthy, Heterogenic virulence in a diarrheagenic Escherichia coli: evidence for an EPEC expressing heat-labile toxin of ETEC, *Int J Med Microbiol*, 305 (2015) 47-54.
[51] V. Bueris, M.P. Sircili, C.R. Taddei, M.F. dos Santos, M.R. Franzolin, M.B. Martinez, S.R. Ferrer, M.L. Barreto, L.R. Trabulsi, Detection of diarrheagenic Escherichia coli from children with and without diarrhea in Salvador, Bahia, Brazil, Mem Inst Oswaldo Cruz, 102 (2007) 839-844.
[52] S.A. Wani, A. Nabi, I. Fayaz, I. Ahmad, Y. Nishikawa, K. Qureshi, M.A. Khan, J. Chowdhary, Investigation of diarrhoeic faecal samples for enterotoxigenic, Shiga toxin-producing and typical or atypical enteropathogenic Escherichia coli in Kashmir, India, *FEMS Microbiol Lett*, 261 (2006) 238-244.
[53] T.J. Ochoa, F. Barletta, C. Contreras, E. Mercado, New insights into the epidemiology of enteropathogenic Escherichia coli infection, Transactions of the Royal Society of Tropical Medicine and Hygiene, 102 (2008) 852-856.
[54] V.A. Shetty, S.H. Kumar, A.K. Shetty, I. Karunasagar, I. Karunasagar, Prevalence and characterization of diarrheagenic Escherichia coli isolated from adults and children in Mangalore, India, *Journal of laboratory physicians*, 4 (2012) 24.
[55] M. Blanco, J.E. Blanco, G. Dahbi, M.P. Alonso, A. Mora, M.A. Coira, C. Madrid, A. Juarez, M.I. Bernardez, E.A. Gonzalez, J. Blanco, Identification of two new intimin types in atypical enteropathogenic Escherichia coli, *Int Microbiol*, 9 (2006) 103-110.
[56] P.K. Ghosh, A. Ali, Isolation of atypical enteropathogenic Escherichia coli from children with and without diarrhoea in Delhi and the National Capital Region, India, *Journal of medical microbiology*, 59 (2010) 1156-1162.
[57] H. Momtaz, F.S. Dehkordi, M.J. Hosseini, M. Sarshar, M. Heidari, Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran, *Gut pathogens*, 5 (2013) 39.
[58] I.K. Behiry, E.A. Abada, E.A. Ahmed, R.S. Labeeb, Enteropathogenic Escherichia coli associated with diarrhea in children in Cairo, Egypt, *TheScientificWorldJournal*, 11 (2011) 2613-2619.
[59] M.J. Albert, A.S. Faruque, S.M. Faruque, R.B. Sack, D. Mahalanabis, Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh, *Journal of clinical microbiology*, 37 (1999) 3458-3464.
[60] P. Van de Perre, Transfer of antibody via mother's milk, *Vaccine*, 21 (2003) 3374-3376.
[61] S. Mukherjee, R.E. Mosci, C.M. Anderson, B.A. Snyder, J. Collins, J.T. Rudrik, S.D. Manning, Antimicrobial Drug–Resistant Shiga Toxin–Producing Escherichia coli Infections, Michigan, USA, *Emerging infectious diseases*, 23 (2017) 1609.
[62] Egbule OS, Owhe-Ureghe UB, O. EE, Occurrence of Multidrug Resistance among E. coli O157:H7 Isolated from Stool Samples obtained from Hospitalized Children., *J Prob Health* 4(2016).