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

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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 (*stx1, stx2, 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 ceftazolin and no resistance against imipenem and Amikacin, respectively while, the isolated strain of aEPEC was sensitive to all the studied antibiotics except ceftazolin. 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 enterobacteriaceae family are Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobic organisms which found as normal inhabitant 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...
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 hemolytic 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 (stx1 and stx2) 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 toxin 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 stx1, stx2, 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 37°C. 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 (stx1, stx2, eae and bfp A)

##### 2.2.1. DNA extraction and PCR

Genomic DNA template for PCR were obtained from overnight 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.

| Gene   | Primer sequence (5’-3’ ) | Conditions used                                                                 | Gene product | Reference |
|--------|--------------------------|--------------------------------------------------------------------------------|--------------|-----------|
| Stx-1  | ATAAATCGCCATTCGTTGACCTAC 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]     |
|        | GGCACGTGCTCAGAAMSCTGGCTCC TGGACATTCTGACATTCTG | 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]     |
| eae A  | GTGCGGAGTACTGCGGAGACT CCCCATTCTTTTTTTCACCTGCG | Denaturation for 3 minutes at 95°C, 35 cycles (95°C for 20 seconds, 72°C for 90 seconds and 72°C for 5 minutes and a final extension step at 72°C for 5 minutes | 890 bp       | [18]     |
| bfp A  | AATGGTGGCATTGCCTTGCTTGC GCACCTTTATCCACCTGGTA | Denaturation for 5 minutes at 94°C, 29 cycles (94°C for 1 minute, 60°C for 1 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

Sero-typing 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 & clavulanicacid (AMC, 30 µg), Cefazolin (CZ, 30 µg), Cefuroxime (CXM, 30 µg), Ceftriaxone (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 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 determination 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* (stx1 and/or stx2) genes and were positive or negative for *eae*A were identified as STEC and strains that were negative for *stx* (stx1 and/or stx2) 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 stx2 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*A 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).

#### 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 | %* |
| *Stx1*          | 8    | 16       | 17.97 | 0 | 0 |
| *Stx2*          | 2    | 6        | 6.74 | 0 | 0 |
| *Stx1* and *Stx2* | 4    | 4        | 4.49 | 0 | 0 |
| *eae*A          | 0    | 0        | 0 | 1 | 1.12 |
| *pfb*A          | 0    | 0        | 0 | 0 | 0 |
| **Total**       | 14   | 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 stx1 and stx2, eight strains were positive for stx1 only and two strains were positive for stx2 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 children 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 stx1, stx2 and both stx1 and stx2

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%), cefatrizone (85.71%), moderate resistance against amoxicillin/clavulanic acid (57.14%),

ccefipime (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 cefatrizone (75%) and low resistance against cefipime and amoxicillin/clavulanic acid (37.5%), ampicillin/ sulbactam (25%) and gentamcin (12.5%) were detected in eight of the O157:H7 STEC strains that were positive to stx1 gene, while these strains were found to be completely sensitive to amikacin, imipenem and ofloxacin (100%). Complete resistance against cefazolin, cefuroxime, cefatrizone and amoxicillin/clavulanic acid and partial resistance against ampicillin/ sulbactam, cefipime and ofloxacin (50%) were detected in the two O157:H7 STEC strains that were positive to stx2 gene, while these strains were found to be completely sensitive to amikacin, imipenem and gentamcin (100%). Four O157:H7 STEC strains that were positive to both stx1 and stx2 gene were completely resistant against cefazolin, cefuroxime and cefatrizone (100%), high resistance against ampicillin/ sulbactam, cefipime and amoxicillin/clavulanic acid (75%) and low resistance against gentamcin 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/clavulanic acid (53.84 %), cefuroxime (42.31%), cefatrizone (34.6%) and cefipime (23.1%).While, low resistance occurred against ampicillin/sulbactam (11.54%), gentamcin (7.7%) and ofloxacin (3.85%). No resistance was observed against amikacin and imipenem (0%).

| Characteristics | Shiga toxin producing Escherichia Coli (STEC)( n=40) | atypical Enteropathogenic Escherichia Coli (aEPEC)(n=1) | Total |
|-----------------|-----------------------------------------------|-----------------------------------------------|-------|
| Gender          |                                               |                                               |       |
| Male            | 4                                             | 22                                            | 26    |
| Female          | 10                                            | 4                                             | 14    |
| Age(year)       |                                               |                                               |       |
| 0-1             | 3                                             | 6                                             | 9     |
| >1-2            | 4                                             | 7                                             | 11    |
| >2-5            | 7                                             | 13                                            | 21    |
| Appearance      |                                               |                                               |       |
| Watery diarrhea | 10                                            | 17                                            | 28    |
| Mucoid diarrhea | 4                                             | 9                                             | 13    |
| Seasons         |                                               |                                               |       |
| Spring          | 5                                             | 4                                             | 9     |
| Summer          | 8                                             | 20                                            | 29    |
| Autumn          | 1                                             | 2                                             | 3     |
| Winter          | 0                                             | 0                                             | 0     |

*The calculated percentage from the total number of the isolated E.coli strain
| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|-----------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 173       | S  | R   | S   | S   | R  | S   | S   | S   | S   | R   | S   |
| 178       | S  | R   | S   | S   | R  | S   | S   | R   | R   | S   | S   |
| 146       | S  | R   | S   | S   | R  | S   | S   | R   | S   | S   | S   |
| 119       | S  | R   | R   | S   | R  | R   | S   | R   | S   | R   | S   |
| 120       | S  | R   | S   | S   | R  | R   | S   | S   | S   | S   | S   |
| 160       | S  | S   | S   | S   | R  | S   | S   | S   | S   | S   | S   |
| 143       | S  | R   | S   | S   | R  | R   | S   | S   | S   | R   | S   |
| 53        | S  | R   | R   | S   | R  | R   | S   | R   | S   | R   | S   |

**Stx1 Positive**

| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 135        | S  | R   | R   | R   | R  | R   | R   | S   | R   | S   |
| 136        | S  | R   | S   | S   | R  | R   | S   | S   | R   | S   |

**Stx1 & Stx2 Positive**

| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 177        | R  | R   | S   | S   | R  | S   | S   | R   | R   | S   |
| 61         | S  | R   | R   | S   | R  | R   | S   | R   | S   | S   |
| 55         | S  | R   | S   | S   | R  | R   | R   | S   | R   | S   |
| 20         | S  | R   | R   | S   | R  | R   | S   | R   | S   | S   |

**Total resistance percentage**

|                  | %   | %   | %   | %   | %   | %   | %   |
|------------------|-----|-----|-----|-----|-----|-----|-----|
| Positive         | 7.14 | 92.85 | 42.85 | 7.14 | 100 | 57.14 | 50 |
| NON              | 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/clavulanic acid; FEB: Cefipime; CN: Gentamcin; CRO: Cefatriaxone; IPM: Imipenem.

S: Sensitive   R: Resistant

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| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 190        | S  | R   | S   | S   | S  | S   | S   | S   | S   | R   | S   |
| 184        | S  | S   | S   | S   | S  | S   | S   | S   | S   | S   | S   |
| 31         | S  | S   | S   | S   | R  | S   | S   | S   | S   | S   | S   |
| 118        | S  | R   | S   | S   | R  | S   | R   | S   | S   |
| 129        | S  | S   | S   | S   | R  | S   | R   | 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   | S   |
| 66         | S  | S   | S   | S   | R  | R   | S   | S   | S   | S   |
| 116        | S  | S   | S   | S   | R  | S   | S   | S   | S   | S   |

**Stx1 Positive**

| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 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   | S   | R   | S   | S   |

**Stx1 & Stx2 Positive**

| Sample No. | AK | CXM | SAM | OFX | CZ | AMC | FEB | CN | CRO | IPM |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|
| 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**

|                  | %   | %   | %   | %   | %   | %   | %   | %   | %   |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Positive         | 42.31 | 11.54 | 3.85 | 88.46 | 53.84 | 23.1 | 7.7 | 34.6 |
| NON              | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

*The calculated percentage from the total number of the isolated non-O157 STEC isolates.*

**Abbreviations:** AK: Amikacin; CXM: Cefuroxime; SAM: Ampicillin/sulbactam; OFX: Ofloxacin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; FEB: Cefipime; CN: Gentamcin; CRO: Cefatrizone; IPM: Imipenem. S: Sensitive   R: Resistant
Resistance against cefazolin (93.75 %), amoxicillin/clavulanic acid (62.5 %), cefuroxime (43.75 %), ceftriaxone (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 stx1 gene, while these strains were found to be completely sensitive to amikacin, imipenem (100 %).

High resistance against cefazolin (83.3 %), moderate resistance against cefuroxime, ceftriaxone and amoxicillin/clavulanic 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 stx2 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 stx1 and stx2 gene were highly resistant against cefazolin (75 %) followed by amoxicillin/clavulanic acid and cefuroxime (25 %), while these strains were found to be completely sensitive to amikacin, imipenem, ofloxacin, gentamicin ampicillin/sulbactam, cefipime, ceftriaxone (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 stx2, some of them possess stx1 and stx2 and a little possess only stx1 [30]. These virulence genes are the two most significant virulence factors for humans. Studies had reported that stx2 was a more important virulence factor than stx1, which was associated with human diseases [31].

Our results showed that among 40 STEC isolates, 24 strains harbored stx1 (26.96 %), 8 strains harbored stx2 (8.988 %), 8 strains harbored stx1 and stx2 (8.988 %) and no strain harbored eaeA (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, stx1and stx2 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 assistants [34] determined that majority of human STEC isolates lacked the virulence gene (eae). A study done by Audu [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 stx1, stx2, stx1and stx2 were 28.57 %, 11.7 % and 1.8 % respectively. Abbasi et al [27] found that among 15 STEC isolates, STEC were STEC which disagreed with our results.

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

| Sample No. | AK  | CXM | SAM | OFX | CZ  | AMC | FEB | CN  | CRO | IPM |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| eaeA 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/clavulanic acid; FEB: Cefipime; CN: Gentamicin; CRO: Ceftriaxone; IPM: Imipenem.

S: Sensitive  R: Resistant
strains contain stx2 was higher than stx1 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 stx1 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 stx2 frequency was found to be higher than the stx1 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.

Abassi 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 stx1&stx2. Bahmanabadi and his assistants [44] identified only 7 EPEC isolates from 101 E. coli strains. These EPEC strains carried eae but did not carry stx1, stx2and bfpA 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, Bianco 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 bfpA 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, ceftriaxone and cefipime, low resistance to ampicillin/subbactam (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, moderate resistance to cefuroxime, ceftriaxone and cefipime, low resistance to ampicillin/subbactam (11.54%), gentamicin (7.7%) and ofloxacin (3.58%) and no resistance to amikacin and imipenem.
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/clavulanic (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.

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