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Epidemiology, Phenotyping and Antimicrobial Susceptibility Profile of Enterohaemrrhagic *Escherichia coli* Strains Isolated from Cases of Diarrhea

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RAAE-D and AN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors RAE-D, AAE and AN managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Work: this work aimed to study the Prevalence, Epidemiology, Phenotyping, and Antimicrobial susceptibility profiles of Enterohaemrrhagic *Escherichia coli* (EHEC) strains among cases of infantile and childhood diarrhea in Egypt.
Materials and Methods: This study was carried on 200 pediatric cases of acute diarrhea. *E. coli* was isolated from stool specimens and identified by conventional cultural methods which is confirmed by biochemical reactions. EHEC strains were identified by latex agglutination test. Antimicrobial susceptibility profile of the isolated EHEC strains was done by disc diffusion method.
Results: out of 200 cases of diarrhea *E. coli* could be isolated from 48 cases (24%). Of these 48 *E. coli* strains; 5 strains were identified as EHEC2.5% (5/200); one strain was identified as O157 and the other 4 strains were identified as non O157 (O26, O91, O103, O111, O128, O145). As regard

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the antimicrobial susceptibility profiles of the isolates EHEC highest sensitivity was recorded to Amikacin (100%) as the five strains of EHEC were sensitive to amikacin, followed by ceftriaxone (60%) as only 3 strains of EHEC recorded sensitivity to ceftriaxone. All the 5 strains of EHEC were resistant to sulphamethoxazole/trimethoprim and imepenem andcefepim, amoxicillin/clavulanic acids (00.00% sensitivity).

Conclusion: There was high incidence of EHEC among cases of infantile and childhood diarrhea. EHEC serotype O157:H7 is an important pathogen responsible for cases of bloody diarrhea. Phenotyping of *E. coli* must be added to the routine laboratory work of infantile diarrhea which helps in proper choice of antimicrobial chemotherapy that will improve the prognosis and sequel of diarrhea.

Keywords: Diarrhea; Enterohaemorrhagic; Escherichia coli; antimicrobial susceptibility.

1. INTRODUCTION

Diarrhea is a major cause of childhood morbidity and mortality in socio-economically developing countries like Egypt. Globally, more than one billion episodes of diarrhea occur every year among children under five years of age causing approximately 2.5 million deaths [1,2]. The WHO Child Health Epidemiology Reference Group estimates that 16% of deaths in African children younger than five years are directly attributable to diarrhea diseases [3].

Enterohemorrahagic *E. coli* (EHEC) are strains capable of producing Shiga toxin and typically cause bloody diarrhea [1-3]. Hemolytic-uremic syndrome (HUS) complicates 6 to 9 percent of EHEC infections overall, and about 15 percent of EHEC infections in children under age 10 [4]. Since the initial recognition of severe bloody diarrhea due to *E. coli* O157:H7 in the United States in 1982, outbreaks and sporadic infections have been attributed to EHEC worldwide [5]. In May 2011, a new Shiga toxin-producing EHEC strain, O104:H4, was identified as the cause of an outbreak in Germany and other countries in Europe [6]. Hospitalization is required in 23 to 47 percent of symptomatic patients with acute diarrhea due to EHEC, with a median hospital stay of 6 to 14 days [6,7]. The mortality rate is generally 1 to 2 percent, although it may be substantially higher among the elderly and among patients with HUS [8,9]. Uncomplicated EHEC infections generally resolve in approximately one week.

This work aimed to study the prevalence, phenotypes, and antimicrobial susceptibility profiles of the EHEC strains which are implicated in cases of infantile and childhood diarrhea in attempt to improve the prognosis of these cases with proper choice of antimicrobial therapy from disease onset.

2. MATERIALS AND METHODS

After Research Ethical Committee approval in Tanta Faculty of Medicine; and written informed consent from parents of all participants, this prospective randomized control study was carried out on 200 pediatric cases of acute diarrhea. Cases were selected from Diarrhea and Nutrition Unit of Pediatrics Department in Tanta University Hospital over a period of 6 months from August 2014 to February 2015.

2.1 Inclusion Criteria

All infants or children suffering from acute diarrhea.

2.2 Exclusion Criteria

Cases which received antibiotic treatment for at least 5 days before the study and cases that had any other underlying systemic diseases or infections.

2.3 Microbiological Study

E. coli was isolated from stool specimens using conventional cultural methods which were confirmed by biochemical reactions including sugar fermentation, action on triple sugar, and IMVC formula [10].

2.3.1 Phenotyping of *E. coli* detection of EHEC strain O157: H7 and non O157 serotypes (O26, O91, O103, O111, O128 and O145) by (Oxoid Dryspot *E. coli* Seroscreen Kit)

This test uses antibody sensitized blue latex particles which agglutinate in the presence of specific *E. coli* cell wall antigens to form visible clumps [11].

2.3.2 Antibiotic sensitivity test

Antibiotic susceptibility testing of *E. coli* isolates was performed using the standardized disc agar diffusion method (Oxoid-England) [12].

2.3.4 The antibiotic discs that were used for this test include

Cefebime (30 µg), Amikacin (30 µg), Co-trimoxazole (25 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Amoxillin-clavulanic (10 µg), Cefotriaxone, Cefoperazone (10 µg).

2.4 Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.20.

3. RESULTS

The present work was carried out on two hundred children suffering from acute diarrhea in pediatric nutrition unit in Tanta University Hospital over a period of 6 months. The age of the cases ranged from 2 months to 5 years, made up of 120 males and 80 females. Table (1) Shows the Clinical Characteristics of the Studied Cases.

The results showed that *E. coli* were isolated from 48 cases (24%), of these 48 *E. coli* caused diarrhea 5 cases were EHEC (2.5%) (5/200) positive .As regard the results of phenotyping by latex agglutination test; of the 5 EHEC strains one isolate was EHEC O157 (1/5) and the other 4 isolates were EHEC non O157 (O26, O91, O103, O111, O128, O145). It is to be noted that none of the EHEC infected cases showed clinical manifestation of HUS or TMA. Table (2) presents the prevalence of EHEC in the study population.

As regard the antibiotic sensitivity of the Enterohemorrahagic *Escherichia coli* the result showed the highest sensitivity was to Amikacin as all the five EHEC isolates were sensitive to Amikacin (100%) followed by Cefotriaxone then to Cefoperazone (60%). On the other hand 100% of the EHEC were resistant to Cefipime, Sulphamethoxazole / Trimethoprim, Amoxicillin-Clavulanic, Ciprofloxacin and Imepeneme Table (3). The results of the study showed that out of the 5 EHEC positive cases 4 were residents of rural areas, 3 cases had the infection in summer and 2 cases in winter. Table (4) shows the history and manifestation of the EHEC positive case.

4. DISCUSSION

Diarrhea is one of the most common causes of morbidity and mortality in children worldwide. Diarrheal illness is the second leading cause of child mortality; among children younger than five years, it causes 1.5 to 2 million deaths annually [13]. In developing countries, infants experience a median of six episodes annually; children experience a median of three episodes annually [14]. In Egypt, diarrhea is an important cause of childhood illness, and usually manifests with symptoms of vomiting, diarrhea, and under nutrition or poor growth, causing prolonged morbidity and may end fatally. Bacterial gastroenteritis is a very common disorder. It has many causes which can range from mild to severe [15].

The present work was carried out on two hundred children suffering from acute diarrhea; their ages ranged between 2 months to 5 years, consisting of 120 males and 80 females. All were attending Diarrhea and Malnutrition Unit in Pediatrics Department, Tanta University Hospital during the period from August 2014 to February 2015.

Using standard methods for *E. coli* isolation followed by latex agglutination using specific antisera to identify EHEC strains [O157:H7 and non O157] , the present study recorded that out of 200 cases of childhood diarrhea, 48 cases showed *E. coli* infection (48/200) (24%); of these 5 or 25% cases werepositive as EHEC phenotypes. Out of the five cases isolated ideentified phenotypically as EHEC, one case was caused by EHEC O157 phenotype and the other 4 cases were due to EHEC non O157 (O26, O91, O103, O111, O128, O145) phenotypes. This means that the percentage of EHEC is somewhat. EHEC O157 phenotype represented 20% (1/5) while non O157 phenotype were 80% (4/5) of isolated EHEC strains in this study. It is to be noted that none of our EHEC infected cases showed clinical manifestation of HUS or TMA.

This agrees with the study of Allerbereg et al. [16] in Austria on 280 diarrheal cases. The ages of the cases in their study were ranged from 6 months to 6 years .Their results of the former study showed that there were 7 cases EHEC (2.5%), 3 cases EHEC O157 and 4 cases EHEC non O157.

Table 1. Clinical characteristics of the studied cases in relation to the phenotype of *E. coli*

	EHEC cases	Non EHEC cases	t.test	Chi-square	
				X2	P-value
Age in months (mean±SD)	6.20±4.38	13.20±4.85	2.326		0.241
Sex N (%)					
Male	1 (20.00%)	119 (61.00%)		3.256	0.046*
Female	4 (80.00%)	76 (39.00%)			
Weight (%of median) (mean±SD)	85.4±10.2	99.2±11.1	6.325		0.007*
Geography N (%)					
Urban	1 (20.00%)	63 (32.3%)		9.326	0.023*
rural	4 (80.00%)	132 (67.7%)			
Season N (%)					
Winter	2 (40.00%)	97 (49.5%)		1.241	0.258
spring		51 (26.2%)			
Summer	3 (60.00%)	44 (22.6%)			
Autumn		3 (1.5%)			
Feeding pattern N (%)					
Breast feeding	(40.00%)	156 (80%)		5.326	0.019*
Non breast feeding	3 (60.00%)	39 (20%)			
Character of diarrhea N (%)					
Watery	4 (80.00%)	129 (66.2%)		2.963	0.147
With mucus		54 (27.7%)			
With blood	1 (20.00%)	12 (6.2%)			
Vomiting N (%)					
Present	5	161 (82.6%)		1.050	0.241
Absent	(100.00%)	34 (17.4%)			
Fever N (%)					
Present	5(100.00%)	96 (49.2%)		1.621	0.174
Absent		99 (50.8%)			
Dehydration N (%)					
No	1 (20.00%)	45 (23.1%)		2.625	0.142
Some	2 (40.00%)	124 (63.6%)			
Severe	2 (40.00%)	26 (13.3%)			

**Significant*

Which again coincides with Vilchez et al. [17] study that done on 671 children from León, Nicaragua, During the period March 2005 to September 2006, a total of 526 faecal samples from children aged 0–60 months (381 with and 145 without diarrhoea) were studied by PCR. EHEC was only detected in children with diarrhoea (2.1%) but none from children without.

Table 2. The prevalence of EHEC of the studied population

	+ve		-ve		Total	
	N	%	N	%	N	%
EHEC	5	2.5	195	97.5	200	100

In Al- Gallas et al. [18] a total of 271 stool specimens were collected from children (diarrheagenic, n = 115 and control, n = 54) from Tunis. EHEC strains were 10.4% and for children in the control group, EHEC strains were 11.1% by PCR.

Urbina et al. [19] study (1998-2000) was done on 253 young children and infants with acute diarrhea, most of whom were less than 3 years old. Enteric pathogenic *Escherichia coli* were (6.0%). EHEC were detected in 7 cases (2.8%).

Table 3. Antibiotic sensitivity test of the isolated strains of EHEC in acute cases

Antibiotic cases	Amikacin	Amoxicillin-clavulanic	Cefoperazone	Cefotrioxone	Ciprofloxacin	Cefibim	Sulphamethole/Trimethoprim	Imepenem
Case-1	+++	R	R	R	R	R	R	R
Case-2	+++	R	++	+++	R	R	R	R
Case-3	+++	R	R	++	R	R	R	R
Case-4	+++	R	++	R	R	R	R	R
Case-5	+++	R	+	++	R	R	R	R

Table 4. The history and manifestation of the EHEC positive cases

	Age	Sex	Weight % of median	Clinical picture	Feeding pattern	Resident	Season	*E. coli*
Case 1	3 months	female	84%	Vomiting, fever and watery diarrhea tinged with blood (4 times) for 2 days. no dehydration	Exclusive bottle feeding	rural	winter	Non o157
Case 2	9 months	female	98%	Vomiting, fever and watery diarrhea (7 times) for 3 days. Some dehydration	Breast feeding	urban	winter	Non o157
Case 3	18 months	female	83%	Vomiting, fever and watery diarrhea (6 times) for 4 days. Some dehydration	Breast feeding	rural	summer	O157
Case 4	7 months	female	78%	Vomiting, fever and watery diarrhea (10 times) for 3 days. Severe dehydration	Bottle feeding	rural	summer	Non o157
Case 5	6 months	male	79%	Vomiting, fever and watery diarrhea (5 times) for 2 days. Severe dehydration.	Bottle feeding	rural	summer	Non o157

As regard the antibiotic sensitivity of the Enterohemorrhagic *Escherichia coli*, the results of this study showed that the highest sensitivity was to Amikacin as the five EHEC were sensitive to Amikacin (100%) followed by Cefotrioxone then to Cefoperazone (60%). On the other hand 100% of the EHEC wre resistant to Cefipime, Sulphamethoxole/Trimethoprim, Amoxicillin-Clavulanic, Ciprofloxacin and Imepenem.

This is in agreement with Swierczewski et al. [20], which showed that EHEC isolates were multidrug resistant to ampicillin, tetracycline and trimethoprim / sulfamethoxazole. These antibiotics are commonly prescribed and readily available at local chemists.

This result agreed also with the study of Mariana AR et al. [21] which was done on 437 children up to 6 years old with acute diarrhea in that study, most of the isolates were sensitive to all of the antimicrobials tested as determined by the agar disc diffusion method. They were all sensitive to Cefotaxime. The resistance observed by these authors was to ampicillin, tetracycline, streptomycin, sulfisoxazole, chloramphenicol, gentamicin and trimethoprim/sulfamethoxazole.

Nitschke et al. [22] and Bielaszewska et al. [23] studies (2012) in Germany that done on reported 2987 cases of Shiga-toxin mediated gastroenteritis. In contrast to earlier reports, we could not observe any case of deterioration attributable to antibiotic treatment. A recent publication on the use of Azithromycin in EHEC O104:H4 infection found no increase in frequency of HUS or worsening of EHEC related symptoms. Treatment with Azithromycin was correlated with a shorter time of EHEC colonisation. In vitro data indicate different effects on Shiga-toxin production depending on the antibiotic agent used: Ciprofloxacin induces Shiga-toxin production while Meropenem, Azithromycin, Tigecyline, and Rifaximin do not influence Shiga-toxin production [24,25].

Contrary to our results, Most studies found no difference or favored a negative impact of antibiotics on risk for diarrhea HUS outcome [26,27]. One retrospective cohort analysis of a large outbreak that occurred in Sakai City, Japan, demonstrated benefit for using the antibiotic fosfomycin within the first 2–3 days from beginning of EHEC infection symptoms. It was associated with a significantly decreased risk of the hemolytic–uremic syndrome [28].

Smith et al. [29] in showed that the use of bactericidal antibiotics, particularly β-lactams, to treat O157 infection was associated with the subsequent development of HUS.

5. CONCLUSION

A relatively high incidence of EHEC among cases of infantile and childhood diarrhea was observed in this study. EHEC serotype O157:H7 is an important pathogen responsible for cases of bloody diarrhea. Phenotyping of *E. coli* should be added to the routine laboratory investigations of infantile diarrhea which helps in proper choice of antimicrobial chemotherapy that will improve the prognosis and sequel of diarrhea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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