Epidemiology, Phenotyping and Antimicrobial Susceptibility Profile of Enterohaemorrhagic 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.

ABSTRACT

Aim of the Work: this work aimed to study the Prevalence, Epidemiology, Phenotyping, and Antimicrobial susceptibility profiles of Enterohaemorrhagic 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
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 imipenem 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].

Enterohemorrhagic *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].

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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), Cotrimoxazole (25 μg), Ciprofloxacin (5 μg), Imipenem (10 μg), Amoxicillin-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 Enterohemorrhagic *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 were positive as EHEC phenotypes. Out of the five cases isolated identified 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 |
|--------------------------|-------------|----------------|--------|------------|
| 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)     | 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                   | 2 (40.00%)  | 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 (63.8%)    |        |            |
| 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 regards 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 Cefotaxime then to Cefoperazone (60%). On the other hand 100% of the EHEC were resistant to Cefpime, 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, Tigecycline, 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.

REFERENCES

1. Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef—United States, June-July 2002. MMWR Morb Mortal Wkly Rep. 2002;51:637.
2. Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. N Engl J Med. 2011;365:1771.
3. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolyticuræmic syndrome. Lancet. 2005;365:1073.
4. Crump JA, Sulka AC, Langer AJ, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med. 2002;347:555.
5. Centers for Disease Control and Prevention (CDC). *Escherichia coli* O157:H7 infection associated with drinking raw milk—Washington and Oregon, November-December 2005. MMWR Morb Mortal Wkly Rep. 2007;56:165.
6. Crump JA, Sulka AC, Langer AJ, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med. 2002;347:555.
7. Varma JK, Greene KD, Reller ME, et al. An outbreak of *Escherichia coli* O157
infection following exposure to a contaminated building. JAMA. 2003;290:2709.

8. Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin-producing Escherichia coli in Montana: bacterial genotypes and clinical profiles. J Infect Dis. 2003;188:719.

9. Werber D, Fruth A, Heissenhuber A, et al. Shiga toxin-producing Escherichia coli O157 more frequently cause bloody diarrhea than do non-O157 strains. J Infect Dis. 2004;189:1335.

10. Nataro JP, Kaper JB. Clin. Micro. Rev. 1998;11:142-201.

11. Chapman 1-PA. Evaluation of Commercial latex slide test for identifying Escherichia coli O157. 1989;42:1109-1110

12. Betteelheim KA. J. Med. Microbiol. 1998;47:1037-1038.

13. Waters JR, Sharp JC, Dev VJ. Infection caused by Escherichia coli O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987-1991. Clin Infect Dis. 1994;19:8

14. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolyticuremic syndrome. Lancet. 2005;365:1073.

15. Curtis V, Cairncross S, Yonli R. Domestic hygiene and diarrhea pinpointing the problem. Trop Med Int Health. 2000;5:22-32.

16. Allerberger F, Sölder B, Vogtgeseder W, Larcher C, et al. Infektionen mit enterohämorrhagischen Escherichia coli O157:H7 in Österreich. Pädiatrie und Pädiologie. 1995;30:49-53.

17. Vílchez S, Reyes D, Paniagua M, Bucardo F, Mollby R, Weintraub A. Prevalence of diarrhoeagenic Escherichia coli in children from León, Nicaragua. J Med Microbiol. 2009;58:630-637.

18. Nasek Al-Gallas, Olfa Bahri, Aida Bouratbeen, et al. Etiology of Acute Diarrhea in Children and Adults in Tunis, Tunisia, with emphasis on Diarrheagenic Escherichia coli: Prevalence, Phenotyping, and Molecular Epidemiology Am J Trop Med Hyg. 2007;77:571-582.

19. Urbina D, Arzuza O, Young G, Parra E, Castro R, Puello M. Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhea on the Colombian Northern Coast. Int Microbiol. 2003;6:27-32.

20. Brett E, Swierczewski, Elizabeth A. Odundo, Margaret C. Koech, Janet N. Ndonye, Ronald K. Kirera, Cliff P. Odhiambo, et al. Surveillance for enteric pathogens in a case-control study of acute diarrhea in Western Kenya. Trans R Soc Trop Med Hyg. 2012;15(3):549-551. (Accepted).

21. Rivero MA, Passucci JA, et al. Role and clinical course of verotoxigenic Escherichia coli infections in childhood acute diarrhoea in Argentina. J Clin Pathol. 2010;59:345-352.

22. Nitschke M, Sayk F, Hartel C, Roseland RT, Hauswaldt S, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enterohemorrhagogenic Escherichia coli O104:H4. JAMA. 2012;307:1046-1052.

23. 209-Bielaszewska M, Idelevich EA, Zhang WX, et al. Effects of antibiotics on Shiga toxin 2 production and bacteriophage induction by epidemic Escherichia coli O104:H4 strain. Antimicrob Agents Chemother. 2012;56:3277-3282.

24. Smith MJ, Melton-Celsa AR, F. Sinclair J, et al. Monoclonal antibody 11E10 which neutralizes Shiga toxin type 2 (Stx2), recognizes three regions on the Stx2 A subunit, blocks the enzymatic action of the toxin in vitro, and alters the overall cellular distribution of the toxin. Infect. Immun. 2009;77:2730-2740.

25. 211-Grisaru S, Morgunov MA, Samuel SM, et al: Acute renal replacement therapy in children with diarrhea-associated hemolytic uremic syndrome: a single center 16 years of experience. Int J Nephrol. 2011:930539.

26. Brussow H, Canchaya C, Hardt W. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Rev. 2004;68:560-602.

27. Sheoran AS, Chapman S, Singh P, Donohue-Rolfe A, Tzipori S. Stx2-specific human monoclonal antibodies protect mice against lethal infection with Escherichia coli expressing Stx2 variants. Infect Immum. 2003;71(6):3125-3130.
28. Ikeda K, Ida O, Kimoto K, Takatorige T, Nakanishi N, Tatara K. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying Escherichia coli O157:H7 infection. Clin Nephrol. 1999;52:357-362.

29. Smith KE, Wilker PR, Reiter PL, Hedican EB, et al. Antibiotic treatment of Escherichia coli O157 infection and the risk of hemolytic uremic syndrome, Minnesota. Pediatr Infect Dis J. 2012;31(1):37–41.