

ESCHERICHIA COLI O157: H7 INVESTIGATION IN CHILDREN WITH DIARRHEA: AN IRAQI CROSS-SECTIONAL STUDY

Nadia Aziz Nasir ¹, Saad Hasan Mohammed Ali ², Huda Q. Muhammad Abu AL-Ess ², Wijdan Akram Hussein ³, Muhi Kadhem Wannas Al-Janabi ⁴, Khalil Ismaiel A. Mohammed ², Jinan Mehdi Mosa ²

1. Community Medicine Department, College of Medicine, University of Baghdad
2. Clinical Communicable Diseases Research Unit, College of Medicine, University of Baghdad,
3. Community Medicine Department, University of Baghdad, Al-Kindi College of Medicine
4. Pediatrics Department, College of Medicine, University of Baghdad

ARTICLE INFO

Article history:

Received 22 March 2020

Revised 05 June 2020

Accepted 03 July 2020

Keywords:

Sensitive diarrhea, Escherichia coli O157:H7 strains, infection.

ABSTRACT

E. coli O157:H7 is a strain that produces a toxin known as Shiga, it is considered one of the most potent toxins, and it can cause severe infection. This study aimed to clinically investigate the infection caused by E. coli O157: H7 strain in children. The research was conducted in Iraqi laboratories and hospitals, where fecal samples were taken from children suffering from diarrhea and were investigated via laboratory protocols. The detection of Escherichia coli O157: H7 antigen in infected children was 44.2% in males and 55.8% in females, the average age was (17.2 ± 9.6 months) while the uninfected average age was (30.6 ± 19.8 months), a significant relation between E. coli O157: H7 infection and bloody diarrhea was noted. Infection with E. coli O157:H7 appears mostly in infants and may cause gastroenteritis irritation and a bloody stool.

© EuroMediterranean Biomedical Journal 2020

1. Introduction

In young children (especially under 5 year old), diarrheal infection is one of the familiar and main causes of illness and death (1).

In genetic diarrhea, the two E. coli O157:H7 pathogen types cannot be distinguished through biochemical testing (2).

Therefore, it is very important to determine and identify them pathogenically via developed PCR methods (3). E. coli O157:H7 infection is a major concern of public health all over the world, in spite of the total case numbers of E. coli O157:H7, infections are lower than for other enteric pathogens such as Salmonella or Campylobacter spp., but, the infections caused by E. coli O157:H7 show much higher hospitalization and death rates (4).

Based on clinical and epidemiological research, the strains of E. coli have been classified into six main categories; Enterotoxigenic E. coli (ETEC), Shiga toxin-producing E. coli (STEC), Enteroinvasive E. coli (EIEC), Diffusely Adherent E. coli (DAEC), Enterocytotoxic E. coli (EAEC), and Cytotoxic distending toxin-producing E. coli (CDT-EC) (5).

The most important strain is STEC where infection can be transmitted via consumption of contaminated food and water (6). E. coli O157:H7 was first recognized in 1982 as the main cause of severe bloody diarrhea and since that time, a number of large food and water-borne intestinal diseases have been reported in humans, specifically in children (7). In humans, STEC can cause gastrointestinal illness, ranging from hemorrhagic colitis diarrheas to the life-threatening hemolytic uremic syndrome (8).

This research work was conducted to identify the infection rate of E. coli O157:H7 in Iraqi children under 5 years, and to assess the laboratorial and clinical criteria of their diarrhea in three pediatric hospitals in Baghdad-Iraq.

2. Material and Methods

Samples were collected from three hospitals in Baghdad; the study investigated 94 samples from children under 5 years suffering from diarrhea.

* Corresponding author: Nadia Aziz Nasir, dr.alkarkhi@gmail.com

DOI: 10.3269/1970-5492.2020.15.28

All rights reserved. ISSN: 2279-7165 - Available on-line at www.embj.org

The three hospitals were: AL-Kadhymia Pediatric Hospital, Children Welfare Teaching Hospital and AL-Elweya Pediatric Hospital. The samples were gathered during the fall of 2015. This cross sectional prospective hospital-based study was carried out on 94 children under 5 years who presented with acute diarrhea at the outpatient clinics at Children Welfare Teaching Hospital, AL-Kadhymia Pediatric Hospital and AL-Elweya Pediatric Hospital in Baghdad in the period from Sep. to Dec. 2015. Their age ranged from one month to five years. Children with protozoal or parasitic infestations were excluded from the study. To detect E. coli O157:H7, around 100 µL (0.1 gm) was taken from each stool sample, then the diluted mixture was dispensed in to the (S)- labeled circular window on the test card and 10 minutes elapsed waiting for the appearance of red and green-colored bands. This qualitative immunochromatographic assay was performed to determine E. coli O157:H7 antigen in fecal samples and to identify the monoclonal antibodies and its reaction with E. coli O157:H7 antigens present on the pre-coated test band region. The detection followed the microscopical and microscopical laboratory examinations of the stool samples, using immunochromatographic assay (ICA) (CerTest Company, Spain) and was applied according to the instructions of the manufacturing company. According to the capillary action rule, the samples were allowed to react with a colored conjugate via a membrane through which a red colored line became visible (9). The T test, Fischer exact and Chi square tests were applied for the analysis of the obtained results using the SPSS program (version-20). The ethical approval from College of Medicine of University of Baghdad (Iraq) was obtained.

3. Results

Samples were collected from children aged less than 60 months (from 1 to 60 months). The relationship between gender and age is shown in table 1. Children with lower age (1-12 or 13-36 months are significantly more infected by E. Coli O175 strains (p-value <0.001). A significant association between bloody diarrhea and E. coli O157:H7 with major symptoms clearly appears in table 2.

Table 3 indicates the relationship between the E. coli O157:H7 antigen findings and stool laboratory findings. In particular, a stool color green is more frequent in E. Coli infection, similarly to the findings of pus and RBC cells in the stool samples.

Characteristics	E.coli O175 n (%)	Non-E. coli O175 n (%)	Total n (%)	p-value	
Gender	Male	19 (44.2)	25 (49)	44 (46.8)	0.6
	Female	24 (55.8)	26 (51)	50 (53.2)	
Age in Months	1-12	14 (53.8)	12 (46.2)	26 (27.7)	<0.001
	13-36	29 (65.9)	15 (34.1)	44 (46.8)	
	> 36	0 (0.0)	24 (100)	24 (25.5)	

Table 1. Socio- demographic characteristics of E. coli O175 infection in children

Clinical Features	E.coli O175 n (%)	Non-E.coli O175 n (%)	Total	p-value	
Fever	Yes	43 (100)	49 (96)	92 (79.9)	0.3
	No	0	2 (4)	2 (2.1)	
Nausea	Yes	34 (79.1)	39 (76.5)	73 (77.7)	0.9
	No	9 (20.9)	12 (23.5)	21 (22.3)	
Abdominal pain	Yes	27 (62.8)	34 (66.7)	61 (64.9)	0.5
	No	16 (37.2)	17 (33.3)	33 (35.1)	
Bloody diarrhea	Yes	43 (100)	22 (43.1)	65 (69.1)	<0.001
	No	0	29 (56.9)	29 (30.9)	
Gross pus stool	Yes	8 (18.6)	7 (13.7)	15 (15.9)	0.5
	No	35 (81.4)	44 (86.3)	79 (84.1)	
Gross mucous stool	Yes	35 (81.4)	43 (84.3)	78 (83)	0.7
	No	8 (18.6)	8 (15.7)	16 (17)	

Table 2. Association between clinical features and E. coli O175 infection in children

Laboratory stool findings	E.coli O175 n (%)	Non-E.coli O175 n (%)	Total	p-value	
Color	Brown	18 (46.2)	23 (45.1)	41 (43.6)	<0.001
	Green	20 (51.3)	15 (29.4)	35 (37.2)	
	Yellow	5 (2.6)	13 (25.5)	18 (19.2)	
Consistency	Loose	10 (23.3)	13 (25.5)	23 (24.5)	0.8
	Watery	33 (76.7)	38 (74.5)	71 (75.5)	
Fat Drops	Yes	14 (32.6)	25 (49.0)	29 (41.5)	0.1
	No	31 (72.1)	26 (51.0)	55 (58.5)	
Pus cells	Yes	43 (100)	28 (54.9)	71 (75.5)	<0.001
	No	0 (0)	23 (45.1)	23 (24.5)	
RBC	Yes	43 (100)	24 (47.1)	67 (71.3)	<0.001
	No	0 (0)	27 (52.9)	27 (28.7)	
Candida	Yes	2 (4.7)	0	2(2.1)	0.1
	No	41(95.3)	51 (100)	92 (97.9)	

Table 3. Laboratory stool findings and E. coli O175 infection in children

4. Discussion

In this study immunochromatographic assay (ICA) was applied to detect the E. coli O157:H7 antigen. The results indicate that E. coli O157:H7 antigen was detected in 45.7% of the examined fecal samples. These results were much higher than in other Iraqi studies, such as those reported by Al- Awwadi et al. (2012) (10), Mohammed et al. (2012) (11). While other studies conducted by Elaine et al (2012) (12), and Vally et al. (2013) (13) recorded very low rates of infection with E. coli O157:H7 in diarrheal children in Australia and the USA, respectively.

However, by using multiplex PCR, Arif et al. (2010) (1) detected target genes of diarrheagenic E. coli in 38% diarrheal stool specimens from children in Sulaimani and Karkuk, Iraq.

Table 3 indicates the increase in E. coli O157:H7 infection with advancing age towards 36 months and declining after 36 months of age, this finding is in agreement with studies made by Tozzi and his coworkers in 2003 (14), and Ali in 2004 (15).

Differently from other viral agents of diarrhea in children, such as Rotavirus, bacterial agents responsible for diarrhea in children aged from 0 to 5 years were not preventable with an effective vaccination (16, 17).

Table 2 shows that Children with fecal specimens positive to E. coli O157:H7 had more nausea than those without E. coli O157:H7 antigen (79.1% versus 76.5 %). Children with acute diarrhea and positive fecal specimens for E. coli O157:H7 antigen had fever while 96% of those without E. coli O157:H7 antigen was febrile, and a statistical association between fever and E. coli O157:H7 infection was not scored. 62.8 % of children with abdominal pain and fecal specimens were positive for the E. coli O157:H7 antigen while 66.7% were negative for the E. coli O157:H7 antigen, therefore, no significant association was detected between abdominal pain and E. coli O157:H7 infection. However, there was a significant association between bloody diarrhea and E. coli O157:H7 infections as all (100%) of the children with acute diarrhea and positive fecal specimens for E. coli O157:H7 antigen had bloody diarrhea compared to 43.1% of those without the E. coli O157:H7 antigen.

As seen in table 3, the stool color, the presence of pus and red blood cells in stools were significantly associated with E. coli O157:H7 infection, while stool consistency, fat drops, and candida in stool were not significantly associated with E. coli O157:H7 infection.

The association between certain host-specific factors and progression risk of enteric *E. coli* O157:H7 infection has been noted and up to 15% of gastrointestinal cases progressed to hemolytic uremic syndrome (HUS) (18). The onset of HUS most frequently followed an episode of gastroenteritis that was often accompanied by bloody diarrhea (19).

Moreover, similarly to other gastrointestinal infection from any microbial agents, also *E. coli* O157:H7 infections could be associated with intussusception in the 30 days later than primary infection (20).

In addition, the infected patients with elevated white blood cell counts, fever, or bloody stools were also those to have a higher risk of progression to HUS than their counterpart patients without these findings (21).

In conclusion, this study has focused on the significance of symptoms that accompanied *E. coli* O157:H7 infection diarrhea in Iraqi children aged (1-60 months), and provides very helpful clinical and laboratory information.

References

- Nessa K, Ahmed D, Islam J, Kabir FL, Hossain MA. Usefulness of a multiplex PCR for detection of diarrheagenic *Escherichia coli* in a diagnostic microbiology laboratory setting. *Bangladesh Journal of Medical Microbiology*. 2007; 1(2): 38-42.
- Kalnauwakul S, Phengmak M, Kongmuang U, Nakaguchi Y, Nishibuchi M. Examination of diarrheal stools in Hat Yai city, South Thailand, for *Escherichia coli* O157 and other diarrheagenic *Escherichia coli* using Immunomagnetic Separation and PCR Method. *Southeast Asian Journal Tropical Medicine and Public Health*. 2007; 38(5): 871-880.
- Arif SK, Salih LIF. Identification of Different Categories of Diarrheagenic *Escherichia coli* in Stool Samples by Using Multiplex PCR Technique. *Asian Journal of Medical Sciences*. 2010 2(5): 237-243.
- Ji-Youn L, Jang W, and Carolyn J. A Brief Overview of *E. coli* O157:H7 and Its Plasmid O157. *J. of Microbiology and Biotechnology*. 2010; 20(1): 5-14.
- Torres AG, Zhou X, Kaper JB. Adherence of diarrheagenic *Escherichia coli* strains to epithelial cells. *Infection Immunology*. 2005; 73: 18-29.
- Al-Dawmy FAA, Yousif AA. Prevalence of *E. coli* O157:H7 in intestinal and Urinary tract infection in children. *International J. of Advance Research*. 2013; 1(8): 111-120.
- Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *The New England Journal of Medicine*. 2003; 308: 681-685.
- Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. *International Journal of Medical Microbiology*. 2005; 295: 405-18.
- Huian SM, Abdelhafeiz M, Ahmed R, Hind MA. Detection of Virulence Factors of *Escherichia coli* Strains Isolated from Children with Diarrhea. *EC Microbiology*. 2018 14(8): 475-486.
- Al-Awwadi N, Alshimary A, Al-kafaji H, Al-badry H, Wanys Z. The detection of shiga toxin producing *E. coli* (O157: H7) infection in children diarrhea in Nasseriya city. *Global Journal of Physical and Applied Science and Technology*. 2012, 3(1): 1-6.
- Mohammed H, Awatif H, Issa L. Detection of rfbO157 and fliCH7 Genes in *Escherichia coli* Isolated from Human and Sheep in Basrah Province. *Rafidain Journal of Science*. 2013; 23(1): 19-33.
- Elaine S, Barbara E.M, Robert M.H, Patricia M.G. Estimates of Illnesses, Hospitalizations and Deaths Caused by Major Bacterial Enteric Pathogens in Young Children in the United States. *Pediatric Infection Disease Journal*. 2013; 32(3): 217-221.
- Vally H, Hall G, Dyda A, Desmarchelier J. Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000-2010. *BMC Public Health*. 2012; 12(63): 1-12.
- Tozzi AE, Caprioli A, Minelli F, et al. Shiga Toxin-Producing *Escherichia coli* Infections Associated with Hemolytic Uremic Syndrome, Italy, 1988-2000. *Emergency Infection Diseases*. 2003, 9(1): 106-108.
- Ali, NH. 2004. *Escherichia coli* O157:H7 Infection and Hemolytic Uremic syndrome among Iraqi Diarrheal Children. *Bahrain Medical Bulletin*. 26(2): 1-7.
- Costantino C, Restivo V, Tramuto F, Casuccio A, Vitale F. Universal rotavirus vaccination program in Sicily: Reduction in health burden and cost despite low vaccination coverage. *Hum Vaccin Immunother*. 2018;14(9):2297-2302.
- Restivo V, Caracci F, Sannasardo CE, Scarpitta F, Vella C, Ventura G, Tramuto F, Costantino C. Rotavirus gastroenteritis hospitalization rates and correlation with rotavirus vaccination coverage in Sicily. *Acta Biomed*. 2018 Oct 8;89(3):437.
- Tawfeek H.I, Najim N.H, Al-Mashkhi S. (2002). Studies on diarrhoeal illness among hospitalized children under 5 years of age in Baghdad during 1990-1997. *Eastern Mediterranean Health Journal*. 2002, 8(1): 12-19.
- Gerber A, Karch H, Allerberger F, Verweyen H.M, Zimmerhackl LB. Clinical Course and the Role of Shiga Toxin-Producing *Escherichia coli* Infection in the Hemolytic-Uremic Syndrome in Pediatric Patients, 1997-2000, in Germany and Austria: A Prospective Study. *Journal of Infection Diseases*. 2002, 186: 493-500.
- Restivo V, Costantino C, Giorgianni G, Cuccia M, Tramuto F, Corsello G, Casuccio A, Vitale F. Case-control study on intestinal intussusception: implications for anti-rotavirus vaccination. *Expert Rev Vaccines*. 2018 Dec;17(12):1135-1141.
- Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J. Pediatrics*. 1990 (116): 544-551.