First report of *Escherichia coli* O157 among Iraqi children

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أول إبلاغ عن الإيشريكية القولونية O157 بين أطفال عراقيين

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الخلاصة: تم تحديد معدل انتشار الإيشريكية القولونية المنزفة للأمعاء ولاسيَّما النمط O157 وغيرها من الأنماط الممرضة للأمعاء لدى 200 طفل مصابين بإسهال مدمى ولدى 100 من الشواهد في نفس العمر، وذلك في مستشفيين من مستشفيات بغداد. وقد عُثر على عوامل جرثومية لدى 39.5٪ منهم وعلى عوامل طفيلية لدى 28.5٪ منهم، في حين لم يكشف أي عامل مسبِّب للمرض لدى 32٪ منهم. وقد وُجد النمط O157 من الإيشريكيات القولونية لدى 11.5٪ كما وُجد أكثر من عامل ممرض واحد لدى 15.5٪ من الحالات. إن أكثر العوامل المسبِّبة للمرض شيوعاً كانت الإيشريكيات القولونية الممرضة للأمعاء وتشكل 5٪ من الحالات والإيشريكيات القولونية غير النمط O157 أو غير الممرضة للأمعاء وتشكل 15٪. والمتحولات الأميبية الحالّة للنسج في 25٪ والجيارديا اللمبلية في 3.5٪. وقد كانت جميع المستفرَدات من الإيشريكية القولونيه حساسه للسيفاليكسين والسيبروفلوكساسين والجنتاميسين وحمض النالديكسيك ولكنها مقاومة للإريثروميسين وسلفات البولي ميكسين والفانكوميسين. وقد لوحظ شيوع المقاومة لستٍ أو أكثر من مضادات المكروبات (50٪ من المستفرَدات).

ABSTRACT We determined the prevalence of enterohaemorrhagic *Escherichia coli*, especially *E. coli* O157, and other enteropathogens among 200 children with bloody diarrhoea and 100 age-matched controls at two Baghdad hospitals. Bacterial and parasitic agents were found in 39.5% and 28.5% of cases, respectively; no pathogen was detected in 32%. *E. coli* O157 was identified in 11.5% and more than one pathogen was found in 15.5% of cases. The most common pathogens were enteropathogenic *E. coli* (EPEC) (5%); *E. coli* other than *E. coli* O157 or EPEC (15%); *Entamoeba histolytica* (25%) and *Giardia lamblia* (3.5%). All isolates of *E. coli* O157:H7 were sensitive to cephalexin, ciprofloxacin, gentamicin and nalidixic acid and resistant to erythromycin, polymyxin B and vancomycin. Resistance to 6 or more antimicrobial agents was common (50% of isolates).

Première notification d'*Escherichia coli* O157 chez des enfants iraquiens

RESUME Nous avons déterminé la prévalence d'*Escherichia coli* entérohémorragique, en particulier *E. coli* O157, et d'autres agents entéropathogènes chez 200 enfants souffrant de diarrhée sanglante et 100 témoins appariés sur l'âge dans deux hôpitaux de Bagdad. Des agents bactériens et parasitaires ont été trouvés dans 39,5 % et 28,5 % des cas respectivement ; aucun pathogène n'a été détecté dans 32,0 % des cas. *E. coli* O157 a été identifié dans 11,5 % des cas et plus d'un agent pathogène a été trouvé dans 15,5 % des cas. Les agents pathogènes les plus courants étaient *E. coli* entéropathogène (5 %), *E. coli* autre que *E. coli* O157 ou *E. coli* entéropathogène (15 %), *Entamoeba histolytica* (25 %) et *Giardia lamblia* (3.5 %). Tous les isolats de *E. coli* O157:H7 étaient sensibles à la céfalexine, la ciprofloxacine, la gentamicine et l'acide nalidixique et résistants à l'érythromycine, au sulfate de polymyxine B et à la vancomycine. Une résistance à 6 agents antimicrobiens ou davantage était courante (50 % des isolats).

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Introduction

Escherichia coli O157 has recently been recognized as a cause of haemorrhagic colitis [*1,2*], a diarrhoeal illness characterized by severe crampy abdominal pain, initially watery diarrhoea followed by grossly bloody diarrhoea, and little or no fever. Since the etiological role of this rare serotype of *E. coli* was first established by the study of two outbreaks in the United States of America in 1982 [*3*], infections due to this organism have been reported with increasing frequency. A significant risk of two life-threatening complications haemorrhagic colitis and the haemolytic uraemic syndrome—makes enterohaemorrhagic *E. coli* (EHEC) infection a public health problem of serious concern [*4,5*]. The most common serotypes are *E. coli* O157:H7 and *E. coli* O26:H11. These are unique from the enteropathogenic *E. coli* (EPEC) organisms that are the main causes of infantile diarrhoea.

Although several national reports in Iraq have focused on the etiology of diarrhoea in children [*6–8*], there has been no comprehensive study on the incidence or other epidemiological characteristics of diarrhoea-associated EHEC, particularly *E. coli* O157. This study reports on the clinical, epidemiological and laboratory features of bloody diarrhoea associated-*E. coli* O157 among Iraqi children.

Methods

From October 1999 to July 2000, 200 children presenting with bloody diarrhoea at the Central Children's Hospital and the Al-Kadimyia Teaching Hospital in Baghdad were investigated. Histories were established by the use of questionnaires eliciting data from parents on the child's age, sex, residence, presence of associated symptoms of fever, vomiting, and abdominal pain, and duration of diarrhoea. For the control group, we enrolled 100 age-matched healthy subjects excluding children who had experienced diarrhoea within the previous week.

The children's stool samples were collected into sterile containers and specimens were cultured directly onto sorbitol-MacConkey agar and Salmonella–Shigella agar (SS agar). Colonies growing on the plate media were identified with standard biochemical tests [*9*]. Biotypes were determined using a commercial identification system (API 20 E, bioMerieux, Marcy-Etoile, France).

For the detection of *E. coli* O157, bacteria identified by biochemical analysis as *E. coli* underwent further testing [*10*], including fermentation of cellobiose and rhamnose and production of enterohaemolysin. Furthermore, a latex agglutination test was performed using a commercial latex kit (Wellcolex, Dartford, Kent, United Kingdom).

Wet smears of faecal specimens of patients were microscopically examined for leukocytes, erythrocytes, helminth ova and cysts, and trophozoites of *Entamoeba histolytica* and *Giardia lamblia*, using saline and iodine stained preparation [*11*].

Antimicrobial susceptibility test

Each *E. coli* O157:H7 isolate was tested for its antimicrobial susceptibility using the agar diffusion test [*12*]. The antimicrobial agents included: 30 µg ampicillin/cloxacillin, 30 mg cephalexin, 30 µg gentamicin, 30 µg ciprofloxacin, 30 µg cefotaxime sodium, 5 µg erythromycin, 10 µg fusidic acid, 30 µg nalidixic acid, 300 units poly-

myxin B sulfate, 30 µg rifampicin, 25 µg co-trimoxazole, 300 µg tetracycline and 30 µg vancomycin.

Results

All 100 controls were investigated for *E. coli* O157 and none were positive. No further investigations were performed for the controls. *E. coli* O157 was isolated in 23 (11.5%) of the 200 cases, of which 13 (6.5%) were *E. coli* O157:H– and 10 (5.0%) were *E. coli* O157:H7.

The rate of detection of enteric agents was higher among inpatients (39.2%) than among outpatients (28.8%). Bacteria were the most frequently identified pathogens (39.5%). Parasites were identified in 28.5%. Mixed infections were found in 15.5% of cases (Table 1).

Of the identified enteropathogens, *E. histolytica* and *E. coli* non-O157 were found in 25% and 15% of cases, respectively. Other agents identified were EPEC (5.0%), *G. lamblia* (3.5%) and *Salmonella* spp. (3.0%). Less commonly encountered pathogens were *Shigella* spp. (1.0%) and *E. hermanii* (1.0%). The main causative agents of bloody diarrhoea in our study were *E. coli* O157 and *E. histolytica*.

During this study, vomiting, fever and other clinical characteristics were investigated. Low-grade fever was complained of by 17.3% of the cases with *E. coli* O157, while 91.3% had abdominal pain and 47.8% experienced vomiting (Table 2). Leukocytes and mucus were not detected by general stool examination. Most *E. coli* O157 infection occurred during summer months (47.8%) compared with the winter months (8.7%). There were 58% of cases living in urban areas and 42% living in rural areas (Table 2).

Among *E. coli* O157 patients, the risk of developing diarrhoea was greater in the first year of life. The highest rates were for children aged 0–12 months (56.5%). The mean age was 22.3 months. The infection rate among males (69.6%) was higher than among females (30.4%). The male to female ratio was 2.3:1 (Table 2).

All *E. coli* O157:H7 isolates were resistant to more than 1 antimicrobial agent. Multiple resistance to 6 or more agents was common, occurring in 50% of isolates. All isolates were resistant to erythromycin, polymyxin B sulfate and vancomycin and sensitive to rifampicin, cephalexin, gentamicin, nalidixic acid and ciprofloxacin. Three isolates (30%) were resistant to tetracycline and 2 (20%) to cefotaxime sodium (Table 3).

Table 1. **Enteropathogens detected in faecal samples from the 200 children with bloody diarrhoea**

Enteric agent	Total (n = 200) %
Bacteria	39.5
EPEC	5.0
E. coli O157	11.5
E. coli O157:H7	5.0
E. coli O157:H–	6.5
E. coli non-O157	15.0
Shigella spp.	1.0
Salmonella spp.	3.0
Escherichia hermanii	1.0
Enterobacter spp.	1.5
Citrobacter spp.	0.5
Klebsiella spp.	0.5
Pseudomonas aeruginosa	0.5
Bacteria only	30.0
Parasites	28.5
Entamoeba histolytica	25.0
Giardia lamblia	3.5
Parasites only	22.5
Mixed infection	15.5
No pathogen identified	32.0

Table 2 **Demographic and clinical details of the 23 children testing positive for *Escherichia coli* O157 (10 with *E. coli* O157:H7 and 13 with *E. coli* O157:H–)**

Pathogen	Age of the child	Sex	Residence	Vomiting	Abdominal pain	Fever	Duration of diarrhoea (days)	Associated pathogens
E. coli O157:H7								
	10 months	M	Urban	–	+	–	10	*Giardia lamblia*
	2 months	M	Urban	–	+	–	9	–
	1.5 months	M	Rural	+	+	Low-grade	5	EPEC
	10 months	F	Urban	+	+	–	3	*Entamoeba histolytica*
	1 month	M	Rural	–	+	–	7	*Entamoeba histolytica*
	2.5 months	M	Rural	+	+	–	5	*Entamoeba histolytica*
	2.5 months	F	Rural	–	+	–	6	–
	2.3 years	M	Urban	+	+	–	8	*G. lamblia*
	2.6 years	F	Urban	+	–	–	7	–
	3.5 months	M	Urban	–	+	Low-grade	6	EPEC
E. coli O157:H–								
	1.6 years	M	Urban	–	+	–	10	*G. lamblia*
	2.6 years	M	Rural	–	+	–	9	–
	2.2 years	M	Urban	–	+	–	5	EPEC
	1.8 years	F	Urban	+	+	–	3	–
	1.8 years	M	Rural	–	+	–	7	*Entamoeba histolytica*
	2.2 years	M	Rural	+	+	–	5	*Entamoeba histolytica*
	2 months	F	Urban	–	+	–	6	–
	2 months	M	Rural	+	+	–	8	–
	1.9 years	F	Urban	+	–	–	7	–
	1 month	M	Urban	–	+	Low-grade	5	–
	11 months	M	Rural	+	+	–	9	EPEC
	2 months	M	Urban	+	+	Low-grade	7	*Entamoeba histolytica*
	2.3 years	F	Rural	–	+	–	6	–

M = male; F = female; present (+); absent (–); EPEC = enteropathogenic E. coli.

Table 3 **Frequency of drug resistance among *Escherichia coli* O157:H7 strains isolated from children with bloody diarrhoea**

Antimicrobial agent	Resistance of EHEC O157:H7 isolate[a]									
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}
Ampicillin/cloxacillin	+	+	+	+	−	+	+	+	+	+
Cephalexin	−	−	−	−	−	−	−	−	−	−
Gentamicin	−	−	−	−	−	−	−	−	−	−
Ciprofloxacin	−	−	−	−	−	−	−	−	−	−
Cefotaxime sodium	−	±	+	−	±	−	+	−	−	−
Erythromycin	+	+	+	+	+	+	+	+	+	+
Fusidic acid	±	+	−	+	±	+	−	+	+	−
Nalidixic acid	−	−	−	−	−	−	−	−	−	−
Polymyxin B sulfate	+	+	+	+	+	+	+	+	+	+
Rifampicin	±	±	−	±	±	−	±	−	−	−
Trimoxazole	+	+	+	+	−	+	+	+	−	+
Tetracycline	−	−	+	+	−	+	−	−	−	−
Vancomycin	+	+		+	+	+	+	+	+	+

[a]*Resistance (+); intermediate resistance (±); sensitivity (−).*
EHEC = enterohaemorrhagic E. coli.

Discussion

This hospital based investigation of enteric infections identified the incidence of *E. coli* O157 associated with bloody diarrhoea among children in the Baghdad area. *E. coli* O157 was not detected among the controls, which suggested that in a paediatric population *E. coli* O157 is rarely associated with asymptomatic carriers. Similar findings have also been reported in Brazil indicating that enterohaemorrhagic *E. coli* (EHEC) in developing countries is most frequently isolated from symptomatic patients [*13*]. Throughout this study, it was remarkable that the majority of children were hospitalized—possibly because of severe dehydration.

In general, bacteria were the most frequently identified pathogens. This was similar to reports from South Africa that bacterial pathogens were important etiological agents of diarrhoea in developing countries [*14*]. Although recognized pathogens were found in the majority (68%) of single or mixed infections in our study, almost one-third (32%) remained of unknown etiology. The failure to find some agents may be attributed to prior antibiotic use or because some pathogens were excluded from our line of investigation. The present study focused on the detection of *E. coli* O157 rather than on other microorganisms that might possibly have accounted for a portion of the undiagnosed cases.

The other causative agents of bloody diarrhoea in our study were *E. histolytica, E. coli* non-O157 and EPEC. These findings correspond with those reported by Mahdi et al. [*8*]. In the present study, the incidence of *E. histolytica* (25%) was

higher than other studies have previously reported in Iraq [*7,15*]. This was possibly indicative of an increase in poor hygiene, contamination of water supplies and overcrowding since the beginning of the USA-led embargo on Iraq.

E. coli O157 was found in 23 (11.5%) cases. It was recovered in significant numbers from stools collected within 7 days of illness onset. Although this microorganism has emerged as a cause for public health concern throughout the world, it had not previously been the subject of enquiry in Iraq. Poor hygienic measures in Iraq could be associated with an increase in the incidence of *E. coli* O157. Our present findings were similar to reports from the USA, which found that *E. coli* O157 was the most commonly isolated enteric bacterial pathogen from bloody diarrhoea [*16*].

In the present study, *E. coli* O157:H– was more prevalent (6.5%) than O157:H7 (5.0%). This finding did not support the Pai study, which reported greater prevalence of *E. coli* O157:H7 [*16*]. The high prevalence of O157:H– in our study might be explained by the hypothesis that the observed *E. coli* O157:H– were actually *E. coli* O157:H7 strains that had lost their flagella [*17*].

Bloody diarrhoea, abdominal pain, vomiting, lack of leukocytes and stool mucus, and low-grade fever were characteristic features of the *E. coli* O157 cases in our study. These findings are in keeping with the clinical features of EHEC [*16,18*].

The mean duration of diarrhoea associated with *E. coli* O157 in our study was 7 days. Similar findings have been reported from the USA [*16*]. Prolonged duration of diarrhoea was observed more frequently in cases of EHEC. This might be due to the overuse of antimicrobial agents leading to a disturbance of microbial flora, and hence, the production of IgA as intestinal flora assisted the host in resisting infectious diseases of the gastrointestinal tract through microbial interference activities [*1,19*].

The risk of developing diarrhoea was greater in the first 2 years of life among *E. coli* O157 cases—probably due to the poor defensive mechanisms against infection in this age group. The mean age of *E. coli* O157 cases in our study was similar to that reported in the USA [*20*]. Age has been considered to be a risk factor for developing serious complications such as haemolytic uraemic syndrome.

The cases of *E. coli* O157 infection in the present study occurred predominantly during hot summer months. This might have been because shedding of EHEC by animal reservoirs increases significantly during the summer [*21*] and high temperatures encourage multiplication and growth of bacteria. Dehydration is also more common during relatively hot weather and might have lead to more rapid deterioration of affected children [*22*].

There were more cases with *E. coli* O157 infection from urban areas in our study than from rural areas, which might reflect the higher urban–rural ratio of total cases in the study. Furthermore, rural children with diarrhoea might have been treated in their villages without referral to one of the city hospitals. Mehdi et al. reported similar findings [*8*]. Although most human infection with *E. coli* O157 has occurred in urban areas, people living in rural areas might be at greater risk of infection, presumably because of their greater exposure to livestock [*18*].

All *E. coli* O157:H7 isolates were resistant to more than one of the antimicrobial agents tested. The resistance rate in the present study was higher than reported from Chile [*23*]. This might be due to antibiotic overuse, leading to the development of resistant strains. The use of antibiotics

prior to the onset of symptoms was considered to be a risk factor for developing complications such as haemolytic uraemic syndrome. The mechanism by which antibiotic overuse increases the risk of infection or the risk of developing complications might involve the enhancement of toxin production by the bacteria or an alteration in the normal competing bowl flora leading to an overgrowth of *E. coli* O157. Trimethoprim-sulfamethoxazole has been reported to enhance toxin production by *E. coli* O157 strains *in vitro* [*24,25*].

The findings of the present study strongly suggest that *E. coli* O157 is an important pathogen in our paediatric population. Identification of these bacteria should be routinely performed in laboratory testing. This evidence of its high prevalence emphasizes the need to strengthen prevention and control strategies.

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