Frequency of *Escherichia coli* O157:H7 in children with diarrhoea in Zahedan, Islamic Republic of Iran

A.H.M. Fard,[1] M. Bokaeian[1] and M.E. Qureishi[1]

تكرار العدوى بالإشريكيات القولونية O157:H7 في الأطفال المصابين بالإسهال في زاهدان في جمهورية إيران الإسلامية

أمير حسين محققي فرد، محمد بكائيان، مظهر اقبال قريشي

الخلاصة: تؤدي الإشريكيات القولونية O157:H7 إلى الإسهال والزحار والتهاب القولون النزفي ومتلازمة الانحلال الدموي اليوريميائي، ومع ذلك فلا تزرع هذه الجراثيم في المختبرات بشكل روتيني. وقد أجرى الباحثون اختبارات على 322 عينة من أطفال تم تحويلهم لإصابتهم بالإسهال، إلى عيادة في زاهدان في جمهورية إيران الإسلامية، وقد أمكن استفراد 21 مستفرَدة من الإشريكيات القولونية السلبية السوربيتول، في حين أظهر تعيين النمط المصلي 4 ذراري إيجابية لـ O157 من بينها ذريتان تعرَّف الباحثون عليها بأنها الإشريكيات القولونية O157:H7، وكانت الحالتان لطفلين عمرهما 1.5 و4 سنوات، في حين كشفت اختبارات الحساسية للمضادات الحيوية عن ذراري حسَّاسة للأميكاسين والجينتاميسين والنتروفورانتوئين والتوبراميسين ومقاومة للكلورامفينكول والألوكسين والألوثين والكوتريموكسازول والسيفالوثين والكوتريموكسازول.

ABSTRACT ***Escherichia coli*** O157:H7 has been implicated in diarrhoea, dysentery, haemorrhagic colitis and uraemic haemolytic syndrome but is not routinely cultured in laboratories. We tested 322 samples from children referred with diarrhoea to a clinic in Zahedan, Islamic Republic of Iran. There were 21 sorbitol-negative ***E. coli*** isolated; serotyping revealed 4 strains positive for O157, out of which only 2 strains were identified as ***E. coli*** O157:H7. Both cases were boys, aged 1.5 and 4 years respectively. Antibiotic sensitivity testing found the strains were sensitive for amikacin, gentamicin, nitrofurantoin and tobramycin and resistant to chloramphenicol, cefalexin, cefalothin and co-trimoxazole.

Fréquence *d'Escherichia coli* O157:H7 chez les enfants souffrant de diarrhée à Zahedan en République islamique d'Iran

RÉSUMÉ La bactérie ***Escherichia coli*** O157:H7 est responsable de diarrhée, de dysenterie, de colite hémorragique et de syndrome hémolytique et urémique, mais elle n'est pas systématiquement mise en culture dans les laboratoires. Nous avons testé 322 échantillons provenant d'enfants souffrant de diarrhée et adressés à un centre de consultations de Zahedan (République islamique d'Iran). Nous avons ainsi isolé 21 ***E. coli*** sorbitol-négatives ; le typage sérologique a révélé 4 souches positives pour le sérogroupe O157, dont 2 seulement ont été identifiées comme étant ***E. coli*** O157:H7. Les deux cas étaient des garçons âgés respectivement de 1,5 et 4 ans. Le test de sensibilité aux antibiotiques a permis de déterminer que les souches étaient sensibles à l'amikacine, à la gentamicine, à la nitrofurantoïne et à la tobramycine, et résistantes au chloramphénicol, à la céfalexine, à la céfalotine et au co-trimoxazole.

[1]Department of Microbiology, School of Paramedicine, Zahedan University of Medical Sciences, Zahedan, Islamic Republic of Iran (Correspondence to A.H.M. Fard: ahmohagh@yahoo.com).
Received: 26/04/06; accepted: 08/06/06

Introduction

Escherichia coli O157:H7 is a part of the normal flora of the human and animal digestive system whose different subtypes can cause infections in humans [*1*]. One of its infections is created by Shiga toxin-producing *E. coli* (STEC), formerly known as verotoxin-producing *E. coli* (VTEC) [*2*], which comprise several serotypes. In recent years one particular serotype has been considered pathogenic and named *E. coli* O157:H7. Since recognition of the serotype, its isolation from stool samples has sharply increased, such that it has been ranked the 3rd most common bacterial pathogen of the human gut after *Salmonella* and *Campylobacter* spp. [*3,4*]. Isolation of this bacterium from cases of diarrhoea varies, although victims are mostly children under 5 years and occurrences are frequently in spring and summer seasons [*1,3–6*].

E. coli O157:H7 causes a severe disease in humans that starts with profuse diarrhoea, and if not diagnosed or treated properly can lead to serious complications such as haemorrhagic colitis, haemolytic uraemic syndrome and thrombocytopenic purpura [*7–9*]. Nevertheless, diagnosis of the causative agent of diarrhoeas cannot depend only on the clinical features of the patients but requires proper diagnosis of the infectious agent in the laboratory [*4*]. Unfortunately, the diagnosis of *E. coli* O157:H7, in spite of its role in diarrhoea and its potentially severe outcome, is not considered a routine laboratory test especially in the Islamic Republic of Iran, and, except for very few reports, there are no comprehensive and documented data on the incidence and prevalence of diarrhoea caused by this particular strain [*10–12*].

The aim of this study was to establish the prevalence of *E. coli* O157:H7 in diarrhoeic children in Zahedan. For this purpose, children with diarrhoea referred to Ali-Asghar paediatric hospital in Zahedan, Sistan and Baluchistan province, south-east Islamic Republic of Iran, were screened.

Methods

In this cross-sectional study children admitted to Ali-Asghar paediatric hospital with diarrhoea during 2005 were screened for the presence of *E. coli* O157:H7. The children were selected sequentially on referral to the hospital until the desired number of cases, i.e. 322, was achieved. All children presenting with diarrhoea regardless of the causative agent (bacterial and non-bacterial) were included in the study. The selected children were first visited by a paediatrician and, if bacterial diarrhoea was suspected, the child's age and sex were noted and a stool sample was collected for subsequent culture.

Culture was done on sorbitol-MacConkey agar plate (Merck). The cultivation method was linear and the plates were incubated at 37 °C for 48 hours under aerobic conditions. Immediately after incubation, suspected colonies that remained colourless due to absence of sorbitol fermentation but which had the other criteria of enterobacteriaceae colonies were tested by other biochemical tests. These included: triple-sugar-iron agar, urease activity, Simmons citrate and sulfide-indole-motility agar. The isolated *E. coli* colonies were then serotyped using specific antisera (anti-O157 and anti-H7, Denka Seiken, Japan).

The sensitivity pattern of *E. coli* O157:H7 strains to different antibiotics was determined by the Kirby–Bauer standard protocol. The antibiotics tested were: amikacin (30 mg), gentamicin (10 mg), nitrofurantoin (300 mg), tobramycin (10 mg), chloramphenicol (30 mg), cefalexin (30 mg), cefalothin (30 mg) and co-trimoxazole (1.25 and 23.75 mg).

Results

A total of 322 stool samples were examined and from these 21 colonies of sorbitol-negative *E. coli* strains (6.5%) were isolated. Serotyping revealed 4 strains positive for O157, out of which only 2 strains showed positive reaction with anti-H7 and were identified as *E. coli* O157:H7. Both cases were in boys, aged 1.5 and 4 years respectively, and were isolated in May and July 2005. Neither of the boys had bloody diarrhoea, and no pus cells or blood were observed in the stool samples.

The isolated *E. coli* O157:H7 strains were sensitive to amikacin, gentamicin, nitrofurantoin and tobramycin and resistant to chloramphenicol, cefalexin, cefalothin and co-trimoxazole. Table 1 shows the susceptibility of the 2 *E. coli* O157:H7 strains to the different antibiotics.

Discussion

STEC yields different serotypes, from which *E. coli* O157:H7 is the most common [*13*]. Infections in humans have different manifestations such as non-bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome, asymptomatic discharge; even cases of death have been reported [*2,13*].

In our study, non-sorbitol fermentation (NSF) was used as a phenotypic criterion for diagnosis of the specimens. Out of 322 diarrhoea samples tested, 6.5% were sorbitol-negative. This corresponds with Aslani et al. who reported isolation of 4.9% of NSF strains in Ilam province of the Islamic Republic of Iran [*12*]. In a similar study the same workers reported 0.7% of recovery using NSF from Mazandaran and Golestan provinces [*11*].

As stated earlier, although NSF has been considered the primary phenotypic criterion for identification of *E. coli* O157:H7 [*11,12*], other researchers have reported fermentation of sorbitol by this strain [*4*]. The latter finding necessitates employment of serological procedures, but it is worth mentioning that not all NSF strains necessarily belong to *E. coli* O157:H7 [*10*]. Hence, out of 322 examined samples identified positive as *E. coli* by NSF, only 0.6% of the total were identified as *E. coli* O157:

Table 1 **Susceptibility of *Escherichia coli* O157:H7 strains to different antibiotics**

Antibiotic	Disc potency (mg)	Zone of inhibition (mm) 1st strain		2nd strain	
Amikacin	30	23	S	25	S
Gentamicin	10	20	S	20	S
Nitrofurantoin	300	21	S	20	S
Tobramycin	10	23	S	21	S
Cefalexin	30	18	R	18	R
Cefalothin	30	15	R	17	R
Chloramphenicol	30	23	R	21	R
Co-trimoxazole	1.25, 23.75	30	R	28	R

S = sensitive, R = resistant.

H7. Similar studies in the Islamic Republic of Iran by other workers also revealed very low figures; for instance, in Ilam province, Golestan and Mazendaran provinces the figure was 0% [*10,12*], while in Isfahan it was 1.4% [*11*]. The proportion may have been higher in Isfahan because only people with diarrhoea were investigated, whereas in the 3 studies reporting 0%, healthy individuals were checked for the strain. Interestingly, Stephan et al. in Switzerland examined 5590 samples from healthy personnel working in the beef industry and isolated only 1 strain of *E. coli* O157:H7 [*14*]. Nevertheless, literature surveys of different workers indicate that geographical distribution of the strain varies from place to place; for instance, in Poland in a 5-year study of diarrhoeic children *E. coli* O157:H7 was not isolated at all [*15*]. Similarly, Baffone et al. could not isolate *E. coli* O157:H7 from any of 606 diarrhoea samples in Italy [*16*]. However, their samples were collected from elderly patients, while according to different studies the strain is more abundant among children than the elderly [*1,4*]. In another investigation by Allerberger et al. in Australia, who examined samples from 280 children suffering from diarrhoea, 3 cases (1%) proved positive [*4*]. Klein et al. studied 1851 samples from children with diarrhoea between 1998 and 2001 in Seattle, United States of America, and reported *E. coli* O157:H7 in 1.5% [*5*].

Our findings were concordant with the above studies, whereas other researchers have reported differently; for instance in Lagos, Nigeria, researchers recovered *E. coli* O157:H7 from 6% of diarrhoea sufferers [*17*]. In another investigation in Wisconsin during 1992–9 the occurrence of 1333 infections as a result of *E. coli* O157:H7 was documented [*18*]. During an outbreak of diarrhoea in northern Palestine in 1992 Adwan et al. examined 250 collected stool samples and reported 124 positive cases of *E. coli* O157 (about 50%) [*3*]. In the latter study, 2 techniques were adopted, culture and polymerase chain reaction assay (no anti-sera were used for confirmation). The reasons that can be suggested for this high frequency of recovery of the strain are the timing of sample collection since it was during an outbreak of diarrhoea and overcrowding, insanitary conditions and poverty in the area [*3*].

In the present study in Zahedan, isolation of *E. coli* O157:H7 occurred in the warm seasons of spring and summer. In other studies too the peak incidence was in the warmer months of the year [*4,19*].

Both our patients who had *E. coli* O157:H7 were male, aged 1.5 and 4 years respectively. Other researchers have pointed out the peak age in positive cases was 2.5 years and cases were more frequent in boys [*4,17*]. More detailed data are required about the age and sex distribution of those suffering from *E. coli* O157:H7 infection.

In our investigation both cases of *E. coli* O157:H7 were recovered from children with watery diarrhoea without blood and faecal leukocytes and this corresponds to the findings of Allerberger et al. [*4*], whereas Klein et al. isolated the strain from patients with dysentery [*5*]. It has been noted that this serotype does not necessarily cause dysentery and asymptomatic cases may have the strain [*4,13*]. As most of the cases of diarrhoea caused by this bacterium are watery and have similar features to other types of diarrhoea, there is a need for laboratory diagnosis.

In the search for the drug sensitivity pattern of *E. coli* O157:H7, we found a good response to amikacin, gentamicin, nitrofurantoin and tobramycin but resistance to chloramphenicol, cefalexin, cefalothin and

co-trimoxazole, although the number of strains isolated in this study was not enough to provide a full drug resistance pattern of *E. coli* O157:H7 in Zahedan. Adwan et al. found 55% of isolated strains resistant to gentamicin and 48% to amikacin, and 49% of the total strains showed resistance to at least 5 antibiotics [*3*]. Drug resistance has been attributed to misuse of broad-spectrum antibiotics. In contrast, in Nigeria, all 6 isolated strains of *E. coli* O157:H7 showed sensitivity to most of the antibiotics tested [*17*].

There is some ambiguity about the appropriate antibiotic therapy for patients with *E. coli* O157:H7 infection, since some workers believe that treatment may prevent the occurrence of haemolytic uraemic syndrome, while others have ruled out this theory [*13*].

Recommendations

We propose further investigations of the prevalence of *E. coli* O157:H7, with inclusion of different patient groups, ages and an increase in the number of samples. Comprehensive assays should be applied to establish the antibiotic sensitivity pattern of *E. coli* O157:H7 strains in Zahedan. Healthy carriers of the organism, particularly those involved in the food and meat industry, should be thoroughly examined to discover the frequency of *E. coli* O157:H7 strains in Zahedan and methods of prevention in the community.

References

1. Bélanger SD et al. Rapid detection of Shiga toxin-producing bacteria in feces by multiplex PCR with molecular beacons on the smart cycler. ***Journal of clinical microbiology***, 2002, 40(4):1436–40.
2. Elliot EJ et al**.** Nationwide study of haemolytic uremic syndrome: clinical, microbiological and epidemiological features. ***Archives of disease in childhood***, 2001, 85:125–31.
3. Adwan K et al. Isolation and characterization of Shiga toxigenic ***Escherichia coli*** strains from northern Palestine. ***Journal of medical microbiology***, 2002, 51(4):332–5.
4. Allerberger F et al. Prevalence and clinical manifestations of Shiga toxin-producing ***Escherichia coli*** infections in Austrian children. ***European journal of clinical microbiology & infectious diseases***, 1996, 15(7):545–50.
5. Klein EJ et al. Shiga toxin producing ***Escherichia coli*** in children with diarrhea; a prospective point-of-care study. ***Journal of pediatrics***, 2002, 141(2):172–7.
6. Keskimäki M et al. Shiga toxin-producing ***Escherichia coli*** in Finland from 1990 through 1997: prevalence and characteristics of isolates. ***Journal of clinical microbiology***, 1998, 36(12):3641–6.
7. Besser R, Griffin EPM, Slutsker L. ***Escherichia coli*** O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. ***Annual review of medicine***, 1999, 50:355–67.
8. McLigeyo SD. Haemolytic uremic syndrome: a review. ***East African medical journal***, 1999, 76:148–53.
9. Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin-producing ***Escherichia coli*** infections. ***Clinical microbiology reviews***, 1998, 11:450–9.
10. Fazeli H et al. ***A comparative study of PCR and culture in identification of*** Escherichia coli ***O157:H7 in diarrheic patients referring to Al-Zahra Medical centre in Esfahan.*** Paper presentated at the 6th Congress on Microbiology, Tehran, Islamic Republic of Iran, 2003.

11. Aslani MM, Bouzari S. An epidemiological study on verotoxin-producing ***E. coli*** (VTEC) infection among population of Iran (Mazandaran and Golestan provinces). ***European journal of epidemiology***, 2003, 18(4):345–9.

12. Aslani MM et al. Verotoxin-producing ***Escherichia coli*** (VTEC) infection in randomly selected population of Ilam province (Iran). ***Scandinavian journal of infectious diseases***, 1998, 30(5):473–6.

13. Mead PS, Griffin PM. ***Escherichia coli*** O157: H7. ***Lancet***, 1998, 352:1207–12.

14. Stephan R, Ragettli S, Untermann F. Prevalence and characteristics of verotoxin-producing ***Escherichia coli*** (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. ***Journal of applied microbiology***, 2000, 88(2):335–41.

15. Sobieszczaska B et al. Prevalence of non-O157 ***Escherichia coli*** strains among shiga-like toxin-producing (SLTEC) isolates in the region of Lower Silesia, Poland. ***Scandinavian journal of infectious diseases***, 2004, 36(3):219–21.

16. Baffone W et al. Detection of ***Escherichia coli*** O157:H7 and other intestinal pathogens in patients with diarrhoeal disease. ***European journal of epidemiology***, 2001, 17(1):97–9.

17. Olorunshola ID, Smith SI, Coker AQ. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. ***Acta pathologica, microbiologica, et immunologica scandinavica***, 2000, 108(11):761–3.

18. Proctor ME, Davis JP. ***Escherichia coli*** O157:H7 infections in Wisconsin during 1992–99. ***Wisconsin medical journal***, 2000, 99(5):32–7.

19. Pradel N et al. Les infections a ***Escherichia coli*** producteurs de verotoxines: etude de la prevalence chez l'enfant dans la region Auvergne [Verotoxin-producing ***Escherichia coli*** infections: study of its prevalence in children in the Auvergne region]. ***Archives de pédiatrie***, 2000, 7(Suppl. 3):544s–50s.