

Diarrhoeagenic *E. coli* pathotypes in children with and without diarrhoea in an Iranian referral paediatrics centre

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تكرار الأنماط المسببة للمرض وللإسهال من الإشريكية القولونية لدى الأطفال المصابين بالإسهال وغير المصابين به في أحد المراكز الإيرانية للإحالة في طب الأطفال

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الخلاصة: يمكن اعتبار الإشريكية القولونية المسببة للإسهال أهم العوامل المسببة للمرض وللإسهال في جمهورية إيران الإسلامية، ولاسيما لدى الأطفال. وتهدف هذه الدراسة إلى التعرف على تكرار مستفرقات الإشريكية القولونية المسببة للإسهال التي جُمعت من خمسين طفلاً مصابين بإسهال حاد، وخمسين طفلاً من الأطفال الشواهد، وذلك في مركز للإحالة في طب الأطفال في إيران على مدى سنة واحدة. واستخدم الباحثون الاختبار المتعدد للتفاعل السلسلي للبوليمراز للتعرف على الإشريكية القولونية المسببة للإسهال، فاكشفوها لدى 90% من مجموعة الحالات، ولدى 20% من مجموعة الشواهد. وكانت الإشريكية المولدة للذيفانات المعوية هي النمط المسبب للمرض الأكثر تكراراً الذي تم التعرف عليه لدى كلتا المجموعتين (26% لدى مجموعة الحالات و10% لدى مجموعة الشواهد)، واحتلت الإشريكية القولونية المنتجة للذيفان شبيغا المرتبة الثانية بين الأنماط المستفردة المسببة للأمراض (17%)، تلاها الإشريكية القولونية المعوية المكثسة (12%). ولم يكتشف الباحثون أية إشريكية قولونية من ذراري غزوية للأعضاء أو ممرضة للأعضاء. وقد وُجدَ في أكثر من 80% من المستفرقات جين *fimH*. ويرى الباحثون أن النسبة المرتفعة للإشريكية القولونية المسببة للإسهال وتنوع أنماط الإشريكية القولونية يؤكد الحاجة إلى الترتُّد المعزَّز للعوامل التي تسبب التهابات المعدة والأمعاء لدى الأطفال في إيران.

ABSTRACT Diarrhoeagenic *Escherichia coli* can be considered as the most important etiologic agents of diarrhoea in the Islamic Republic of Iran, particularly in children. This study determined the frequency of diarrhoeagenic *E. coli* isolates collected from children with acute diarrhoea ($n = 50$) and a control group ($n = 50$) at an Iranian referral paediatric centre during a 1-year period. Using multiplex PCR, diarrhoeagenic *E. coli* was identified in 90% of the case group and 20% of controls. Enterotoxigenic *E. coli* was the most frequently identified pathotype in both groups (26% in cases; 10% in controls). Shiga toxin-producing *E. coli* was the second most isolated pathotype (17%), followed by enteroaggregative *E. coli* (12%). No enteroinvasive *E. coli* and enteropathogenic *E. coli* strains were recovered. More than 80% of isolates harboured the *fimH* gene. This high proportion of diarrhoeagenic *E. coli* and diversity of *E. coli* types highlights the need for enhanced surveillance of gastroenteritis agents in children in this country.

Pathotypes *Escherichia coli* diarrhéogènes chez des enfants souffrant ou non de diarrhées dans un centre pédiatrique d'orientation-recours iranien

RÉSUMÉ Les souches d'*Escherichia coli* diarrhéogènes peuvent être considérées comme les agents étiologiques les plus importants à l'origine de diarrhées en République islamique d'Iran, notamment chez l'enfant. La présente étude a déterminé la fréquence d'*E. coli* diarrhéogènes à partir d'isolats recueillis chez des enfants souffrant de diarrhées aiguës ($n = 50$) et dans un groupe témoin ($n = 50$) au sein d'un centre pédiatrique d'orientation-recours iranien pendant un an. À l'aide de la PCR multiplexe, *E. coli* diarrhéogène a été identifié chez 90 % des patients et 20 % des témoins. *E. coli* entérotoxigène était le pathotype le plus fréquemment identifié dans les deux groupes (26 % des cas ; 10 % des témoins). *E. coli* producteur de shiga-toxine était le deuxième pathotype le plus fréquemment isolé (17 %), suivi par *E. coli* entéroagglutinant (12 %). Aucune souche d'*E. coli* entéroenvahissant ni d'*E. coli* enteropathogène n'a été découverte. Plus de 80 % des isolats hébergeaient le gène *fimH*. La proportion élevée de souches *E. coli* diarrhéogènes et la diversité des types d'*E. coli* soulignent la nécessité d'une surveillance accrue des agents de gastro-entérites chez les enfants de ce pays.

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Introduction

Diarrhoeal disease is still a major health problem, especially in developing countries, where it is considered one of the leading causes of morbidity and mortality especially in children aged less than 5 years [1]. Among the bacterial causes of diarrhoea, diarrhoeagenic *Escherichia coli* is the most important etiologic agent of children's diarrhoea in the Islamic Republic of Iran [2,3].

Identification of *E. coli* pathotypes is limited in many developing countries because conventional microbiological testing is unable to distinguish between normal flora and pathogenic strains [4]. Molecular identification and classification of diarrhoeagenic *E. coli* is based on the presence of different chromosomal or plasmid-encoded virulence genes, which are absent in the commensal *E. coli* [5]. Five categories of *E. coli* have been associated with diarrhoea in several epidemiological studies: enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and Shiga toxin-producing *E. coli* (STEC) [5]. Infections caused by pathogenic *E. coli* are often initiated by binding of the bacteria to the host cell surface via specific bacterial adhesins. Binding of fimbrial adhesins enabling bacteria to adhere to host cells. Type 1 fimbriae were the adhesins first described in *E. coli* [6].

The features of acute diarrhoea vary from place to place depending on local meteorology, geography and socioeconomic variables. Knowledge of the major etiologic agents of this disease is important for epidemiological surveillance and correct treatment. The goal of our study was to evaluate the frequency of *E. coli* pathotypes and type 1 fimbriae in children attending a referral hospital in Tehran, Islamic Republic of Iran.

Methods

Study design and setting

In this study, a total of 100 random *E. coli* isolates of stool samples were collected and processed during a 1-year period (2010–11) from patients attending the Children's Medical Centre. In addition to being a referral tertiary care centre this is one of the major teaching hospitals of Tehran University of Medical Sciences. It admits patients from all regions of Islamic Republic of Iran, representing a wide spectrum of socioeconomic levels.

Sample

A random sample of 50 children aged > 1 month to 12 years old with acute diarrhoea referred to the Children's Medical Centre over a 1-year period were selected as case patients and enrolled into the study. Diarrhoea was defined, according World Health Organization guidelines as the occurrence of 3 or more, loose, liquid or watery stools within 24 hours [7]. To ensure optimum recovery of *E. coli* all specimens were obtained within 24 h of the onset of illness and before any antimicrobial treatment had begun. During the same period, 50 children with no evident signs and symptoms of gastroenteritis were recruited from the general population as controls. These patients were apparently healthy, with the same age range and sex, and included healthy volunteers, individuals presenting to clinics for routine health maintenance visits and individuals presenting to the emergency department.

Data collection

After informed consent was obtained from each child's parent, stool samples were collected and transported immediately to the microbiology laboratory for analysis within 2 hours of collection.

Bacteriological procedures

All bacterial isolates were microbiologically identified in the microbiology

laboratory of the hospital using standard identification methods [8].

DNA extraction

E. coli clinical isolates were processed for isolation of genomic DNA as previously described [9].

Detection of *fimH* gene and *E. coli* pathotypes

Detection of specific virulence genes by polymerase chain reaction (PCR) is frequently used because this method gives rapid, reliable results with a high sensitivity and a high specificity [5,10]. Having confirmed the specificity of each primer set by single PCR, we combined primer sets and tested the control strains in several PCR cycling protocols. The targets selected for each category were *fimH* for type 1 fimbriae, *aggR* for EAEC, *eae* for EPEC, *stx1* and *stx2* for STEC, *lt* and *st* for ETEC, and *invE* for EIEC. For each of the target genes, different pairs of primers were selected from the literature [11,12].

The multiplex PCR reactions were performed using 10× PCR buffer; 100 mM MgCl₂; 10 mM dNTP; 1.5 U Taq DNA polymerase and each of primers. The PCR programme was 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, for 30 cycles, and 72 °C for 3 min. PCR products were then electrophoresed on a 1.5% agarose gel.

Results

Background characteristic of patients

In this study, multiplex PCR was used to detect pathotypes of *E. coli* in 50 children with diarrhoea and 50 control children. The patients were analysed in 4 age groups < 6 months ($n = 11$), 6–11 months ($n = 20$), 12–23 months ($n = 50$) and ≥ 24 months ($n = 19$). There were 32 males and 68 females. Children were enrolled during all seasons: spring ($n = 21$), summer ($n = 27$), fall ($n = 22$), and winter ($n = 30$).

Diarrhoeagenic *E. coli* strains isolated

A total of 55 isolates of diarrhoeagenic *E. coli* were isolated from the 100 children, including 45/50 (90%) from patients with diarrhoea compared with 10/50 (20%) from the control group.

ETEC was the most common pathotype, found in 26/100 (26%) of the total children: 21/50 (42%) cases with diarrhoea and 5/50 (10%) controls. Among 26 ETEC strains, 8/26 were positive for the *lt* gene (7 cases; 1 controls) and 15/26 for the *st* gene (11 cases; 4 controls), while 3/26 strains possessed both genes (3 cases; 0 controls) (Table 1). STEC was found in 17/100 (17%) children (14 cases; 3 controls) (Table 1). *stx1* in combination with *stx2* was seen exclusively in the case group (10/50 cases). EAEC was isolated from 12/100 (12%) children. Among the EAEC isolates, the *aggR* gene was seen in 10/50 (20%) cases and 2/50 (4%) controls. We did not find any *eae* and *invE* positive strains. No EIEC or EPEC strains were recovered.

Two different types of diarrhoeagenic *E. coli* were isolated from 8/50 (16%) cases and none of the controls. The most frequent combination was

Table 1 Frequency of different diarrhoeagenic *Escherichia coli* strains isolated from the case group of children with diarrhoea and the control group

Pathotype	No. of strains isolated		
	Cases (n = 50)	Controls (n = 50)	Total (n = 100)
STEC			
Stx1-producing	1	1	2
Stx2-producing	3	2	5
Stx1 + stx2-producing	10	0	10
ETEC			
<i>lt</i> -producing	7	1	8
<i>st</i> -producing	11	4	15
<i>lt</i> + <i>st</i> -producing	3	0	3
EAEC	10	2	12
EIEC	0	0	0
EPEC	0	0	0
Total isolates	45	10	55

STEC = Shiga toxin-producing *E. coli*; ETEC = enterotoxigenic *E. coli*; EAEC enteroaggregative *E. coli*; EIEC = enteroinvasive *E. coli*; EPEC = enteropathogenic *E. coli*.
n = total number of children.

STEC with ETEC in 7/8 of these cases (Table 2).

In our study, type 1 fimbriae was expressed by the majority of ETEC isolates (19/26, 73%). In addition, this gene was detected in 14/17 (82%) STEC isolates (Table 2).

Variations by age and season

No difference in the rate of detection of diarrhoeagenic *E. coli* proportion was

found between the dry (summer) and rainy season (autumn) samples. Of the faecal strains obtained in winter, 24/30 (80%) were diarrhoeagenic *E. coli*: 18/30 (60%) cases and 6/30 (20%) controls.

We divided the samples according the age (Table 2). Despite detection of diarrhoeagenic *E. coli* in faecal specimens from children in all ages, there was

Table 2 Frequency of different diarrhoeagenic *Escherichia coli* strains isolated from the case group of children with diarrhoea and the control group, by age group

Pathotype	No. of strains isolated									
	Control group					Case group				
	0-5 yrs	6-11 yrs	12-23 yrs	≥ 24 yrs	Total	0-5 yrs	6-11 yrs	12-23 yrs	≥ 24 yrs	Total
STEC	0	0	0	0	0	1	0	0	1	2
ETEC	0	0	1	0	1	0	1	3	1	5
EAEC	0	0	1	0	1	0	0	3	0	3
STEC + EAEC	0	0	0	0	0	0	0	1	0	1
STEC + <i>fimH</i>	0	1	2	0	3	0	1	3	1	5
ETEC + <i>fimH</i>	1	1	2	0	4	0	2	4	3	9
EAEC + <i>fimH</i>	0	0	1	0	1	0	2	3	1	6
STEC + ETEC + <i>fimH</i>	0	0	0	0	0	1	0	3	2	6
STEC + ETEC	0	0	0	0	0	0	0	1	0	1
Total children	4	11	25	10	50	7	9	25	9	50

STEC = Shiga toxin-producing *E. coli*; ETEC = enterotoxigenic *E. coli*; EAEC enteroaggregative *E. coli*; EIEC = enteroinvasive *E. coli*; EPEC = enteropathogenic *E. coli*; *fimH* = type 1 fimbriae.

a higher frequency of recovery of strains among children aged 12–23 months in the case group [21/25 (84%)] than the control group [7/25 (28%).

Discussion

Knowledge of the status of the enteropathogenic bacteria responsible for diarrhoea in the Iranian population is essential for implementation of appropriate public health measures to control these diseases [3]. The present study was performed to identify the frequency of diarrhoeagenic *E. coli* as a potential etiologic agent of diarrhoeal disease in an Iranian referral paediatrics centre. The most frequent *E. coli* pathotype in our study was ETEC, which corresponded to 26% of the total children studied (81% of the case group and 19% of the control group). Among diarrhoeagenic *E. coli*, ETEC has been noted as the most common, particularly in the developing world [13]. Variation in the prevalence of ETEC toxin types may occur from year to year and among different geographic areas. In our study, strains carrying only the *st* gene were more

prevalent (15/26, 58%) than those carrying genes for *lt* (8/26, 31%) or both *st* and *lt* genes (3/26, 11%). This result agrees with the report of other studies in Egypt and Tunisia that the *st*-expressing ETEC was the most common form [14,15].

STEC was the second most common diarrhoeagenic *E. coli* pathotype, isolated from 17% of children. According to a previous report from the Islamic Republic of Iran, this organism has high frequency in the Iranian population [16]. Although EPEC was the third most abundant *E. coli* after STEC and EAEC in previous studies in our country, we did not find this pathotype in this study [11,12]. The lack of EIEC isolates in our study and the low rate of its recovery in other studies suggests that this pathotype may play a less important role in childhood diarrhoea in developing countries [17,18].

Isolation of combinations of 2 diarrhoeagenic *E. coli* types in our study (8% of children, 16% of cases) was higher than the recent report in León, Nicaragua. In our study, the combination of ETEC and STEC was the most frequent, while in Vilchez et al.'s study

coinfection with EAEC and EPEC was common and reported in 3.9% of cases [19].

A high frequency of diarrhoeagenic *E. coli* was found in the age group 12–23 months in the case group: 18/30 (60%). In addition, 7/25 (28%) of the control group in this age had diarrhoeagenic *E. coli* pathotypes. We speculate that children at this age are immunologically naive and may not possess a specific immune response to new pathogens. In addition, the behaviour of children in this age group might be another factor that exposes them to more risk factors compared with younger and older children.

Conclusion

Our results show a high rate of diarrhoeagenic *E. coli* among Iranian children with diarrhoea. The finding of diverse *E. coli* types, even within a small number of *E. coli* isolates, focuses attention on the importance of pathogenic *E. coli* and stresses the need for enhanced surveillance of gastroenteritis agents in children in the Islamic Republic of Iran.

Competing interests: None declared.

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Diarrhoeagenic diseases

Diarrhoeal diseases represent a major health problem in developing countries. The wide diversity of bacterial and viral infections that may cause diarrhoea [5] complicates accurate surveillance and diagnosis, especially in developing countries with little or no access to modern laboratory procedures

Numerous types of diarrhoeagenic *E. coli* strains have been identified worldwide, including enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), Shiga toxin-secreting (STEC), diarrhoea-associated haemolytic (DHEC), entero-aggregative (EAaggEC), and cytolethal distending toxin-secreting (CDTEC) *E. coli* strains. The prevalence of these strains and the burden of disease they cause are however unequal.

Further information on diarrhoeal diseases can be found at: http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index.html