Prevalence of *Escherichia coli* Pathotypes Among Children With Diarrhea in Babol, Northern Iran

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Published Online: March 26, 2016

Keywords: Diarrheagenic *Escherichia coli*, Prevalence, PCR

**Abstract**

**Background:** Diarrheagenic *Escherichia coli* (DEC) are major causes of diarrhea in the world particularly among infants and young children.

**Objectives:** The aim of this study was to determine the prevalence of DEC strains in stool samples from children under 5 years old.

**Patients and Methods:** Stool specimens were collected from 200 children under 5 years visiting hospital due to gastroenteritis. *E. coli* pathotypes were detected by using conventional culture techniques and polymerase chain reaction (PCR).

**Results:** Sixty-eight (34%) out of 200 specimens were positive for DEC. Different pathotypes would show the following profiles: 43 (21.5%) for enteropathogenic *E. coli* (EPEC); 18 (9%) for enterotoxigenic *E. coli* (ETEC) including 10 (55.5%) st positive, 6 (33.3%) lt positive and 2 (11.1%) st and lt both positive; 6 (3%) for enteroaggregative *E. coli* (EAEC) and 1 (0.5%) for enteroinvasive *E. coli* (EIEC). Enterohemorrhagic *E. coli* (EHEC) was not isolated from any of the *E. coli* strains tested.

**Conclusions:** This study shows that DEC is a common cause of childhood diarrhea in Babol. EPEC and ETEC were the most frequent pathotypes in the population under study.

**Background**

*Escherichia coli* is one of the predominant facultative anaerobes in the human gastrointestinal tract. Many strains of *E. coli* are harmless and even provide many health benefits to the host. However, there are small groups of *E. coli* that have evolved and developed pathogenic strategies that can cause a broad spectrum of disease, including severe diarrheal disease.¹ Diarrheagenic *Escherichia coli* (DEC) are conveniently classified into 6 major pathotypes according to their virulence genes and including enterotoxigenic *E. coli* (ETEC), Shiga toxin producing *E. coli* (STEC), also referred to as verotoxigenic *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC).² EIEC is also the only *E. coli* pathotype to invade and multiply within host epithelial cells, and can cause invasive inflammatory colitis and dysentery, but most symptomatic infections are characterized by watery diarrhea indistinguishable from that produced by other diarrheagenic *E. coli* pathotypes.³ EHEC cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).⁴ ETEC is an important cause of diarrheal disease in infants (6-18 months), young children, and the elderly in the developing world. It is also known to be the major cause of “traveler’s diarrhea” acquired by tourists visiting developing nations.⁵ The main distinguishing feature of EPEC is the ability to induce a characteristic histopathology called the attaching and effacing (A/E) lesion.⁶ EAEC is increasingly recognized as an emerging enteric pathogen and cause of persistent diarrhea (greater than 2 weeks duration) in children and adults in both developing and developed countries.⁷ The pathogenicity of DAEC strains is inadequately understood but it has been associated with diarrhea in young children under 12 months which is typically mild without blood in the feces.⁸ In many studies, a different prevalence of DEC strains has been reported. In studies conducted in Iran, Bangladesh and Jordan a high prevalence of DEC strains was detected.⁹,¹⁰ The identification of diarrheagenic *E. coli* using biochemical and serological tests is unreliable. Therefore, several polymerase chain reaction (PCR) tests have been developed to amplify the target regions present in virulence genes and identify strains of DEC.¹¹

**Objectives**

The objective of the present study was to determine the prevalence of DEC among children with diarrhea in...
Babol, Northern Iran. Since DAEC is not well defined as a distinct pathotype, its detection was not included in this study.

**Patients and Methods**

**Isolation and Identification of Escherichia coli**

Stool samples, from June to October 2014 were collected from hospitalized children under 5 years old in the Babol. A total of 200 stool samples from diarrheal patients were processed by directly inoculating the fecal matter onto MacConkey Agar and culturing overnight at 37°C. The suspected pink colonies were then cultured on eosin methylene blue (EMB) agar plates to see the metallic sheen color of the *E. coli*. Stool samples were a metallic green sheen colony from each plate with a typical *E. coli* morphology was selected and examined by biochemical tests, including indole, methyl red, Voges–Proskauer, citrate and urease tests. The isolates that were positive to indole and methyl red tests but negative to Voges–Proskauer, citrate and urease tests were identified as *E. coli*.

**Reference Strains**

The following DEC reference strains were used as positive controls: ETEC ATCC 35401 (elt, est), EHEC ATCC 43889 (hlyA), EPEC ATCC 43887 (eae), EIEC ATCC 43893 (ial) and EAEC ATCC 29552 (pCVD432).

**DNA Templates for PCR Reactions**

All isolated *E. coli* strains were grown on Luria-Bertani (LB) broth at 37°C for 24 hours. Genomic DNA was extracted according to the method described by Gómez-Duarte et al. Bacteria were first harvested from 1.5 mL of an overnight LB broth culture, suspended in 200 μL of sterile water, and boiled at 100°C for 10 minutes. Following centrifugation of the lysate, the supernatant containing a crude DNA extract was used as a DNA template on a multiplex PCR for identification of *E. coli* pathotypes.

**Polymerase Chain Reaction Detection of Diarrheagenic Escherichia coli**

Two multiplex PCR assays were performed for the detection of five pathotypes of DEC. The oligonucleotide primers used in this study are listed in Table 1.

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Size of Product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 1. PCR Primers Used in This Study</strong></td>
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</tbody>
</table>

**Results**

From the 200 stool samples, DEC strains were identified in 80 of the samples, and DEC was detected in 68 (34%) of the diarrheic children under 5 years old within the period of the study. Of the 68 DEC strains detected by PCR, 43 (21.5%) were EPEC, 18 (9%) ETEC, 6 (3%) EAEC and 1 (0.5%) EIEC isolates. Among 18 ETEC isolates, *st* gene was detected in 10 (55.5%), *lt* gene in 6 (33.3%) and both *lt* and *st* genes were present in 2 (11.1%) isolates. The frequency of each DEC pathotype is shown in Table 2.

EHEC was not isolated from any of the *E. coli* strains tested. The most frequently isolated was EPEC. The amplified products for the *elt* gene and the *est* gene (ETEC) were of 322 bp and 170 bp, respectively. The PCR products were electrophoresed on a 2% agarose gel and visualized with UV transilluminator after EtBr staining.
for eae gene was of 229 bp. The amplified products for the ipaH gene and the iai gene (EIEC) were 933 bp and 320 bp, respectively. The PCR product for pCVD432 gene (EAEC) was 630 bp (Figures 1 and 2).

Discussion
Among the bacterial pathogens, E. coli plays an important role in causing diarrhea in children under 5 year old. In the present study, 68 (34%) isolates were identified as DEC strains by multiplex PCR. Hegde et al19 reported a prevalence of 26% DEC strains from 200 stool samples from children with diarrhea in India. In a study conducted by Gómez-Duarte et al28 in Colombia, a lower prevalence of 14.4% has been reported. The prevalence of DEC varies in the world from region to region and even between countries. Albert et al20 reported that in children with diarrhea, EPEC had the highest prevalence. Alikhani et al21 identified that EPEC was the most prevalent pathotype among DEC. In another study in Iran, Alikhani et al21 reported that the most frequently identified DEC was EPEC (47.5%). Usein et al21 also showed that 9% of the children with diarrhea carried EPEC isolates. In this study, EPEC was the predominant E. coli pathotype (21.5%). The reason for different rates of identification of the EPEC may be due to health status in various areas. In our study, the second most common pathotype of DEC was the ETEC 18 (9%). The prevalence of ETEC was lower in our study than in some previous studies. The rate of isolation of the ETEC reported by Wolk et al22 and Viboud et al23 was 20.7% and 18.3%, respectively. In studies conducted by Hegde et al20 and Nguyen et al,24 the rate of isolation of the ETEC was 3.5% and 2.2%, respectively. In this study, the detection rate for EAEC was 3.0%. Our finding is approximately similar to those reported by Aranda et al,25 Ifeanyi et al,26 and Aslani et al,27 in which they found that the rate of EAEC was 2%, 2% and 10.7%, respectively. The very low percentage (0.5%) prevalence of EIEC obtained in this study is in close agreement with the study reported by Hegde et al,19 who reported a prevalence of EIEC in children with diarrhoea as 1.5% in India. In the present study, we did not isolate any EHEC strains. The results of current investigation are consistent with the studies reported by Moyo et al28 and Nguyen et al.24 In conclusion, EPEC was the most commonly identified DEC strain in the region studied. Therefore, further studies are needed to investigate virulence properties and antimicrobial resistance of EPEC strains.

Acknowledgments
The authors thank Seyed Ali Sajjadi from Amirkola children’s hospital for the excellent technical assistance.

Authors’ Contribution
FM performed practical experiments and collected samples. MA performed the tests, wrote the manuscript, and performed interpretation of the results. YY contributed to the performing of the experiments and writing of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures
None.

Funding/Support
No funding of any kind has been received for this study.

References

Table 2. Diarrheagenic Escherichia coli Among Children With Diarrhea

<table>
<thead>
<tr>
<th>DEC</th>
<th>No. (%)</th>
</tr>
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<tbody>
<tr>
<td>EPEC</td>
<td>43 (21.5)</td>
</tr>
<tr>
<td>ETEC</td>
<td>18 (9)</td>
</tr>
<tr>
<td>EAEC</td>
<td>6 (3)</td>
</tr>
<tr>
<td>EIEC</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>DEC negative</td>
<td>132 (66)</td>
</tr>
<tr>
<td>Total</td>
<td>200 (100)</td>
</tr>
</tbody>
</table>

Abbreviations: DEC, diarrheagenic E. coli; EPEC, enteropathogenic E. coli; ETEC, enterotoxigenic E. coli; EAEC, enteropathogenic E. coli; EIEC, enteroinvasive E. coli.


