Original article

Frequency, antimicrobial susceptibility and plasmid profiles of Escherichia coli pathotypes obtained from children with acute diarrhea

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How to cite this article:

Received: May 2010  Accepted: July 2010

Abstract

Introduction and objective: Escherichia coli is an important group of pathogens associated with diarrhea among children. The objective of this study was to determine the frequency, antimicrobial susceptibility and plasmid profiles of E. coli pathotypes obtained from children with acute diarrhea.

Materials and methods: In a prospective study during 2008, 466 rectal swabs of inpatient children were examined for the presence of E. coli strains.

Results: Of the total number of specimens examined, 99 (21.2%) were positive for E. coli. The highest number of isolates, 37(37.4%), was recovered from the 13-24 month age group. Of the 466 children, 191(41%) were girls and 275(59%) were boys. Based on the serological tests, 59(59.6%) of the E. coli strains were identified as enteropathogenic E. coli (EPEC) and 22 (22.2%) were identified as enteroinvasive E. coli (EIEC). The in vitro antibiotic susceptibility pattern of E. coli strains showed that 89.9 %, 88.9%, 79.8%, and 75% of isolates were found to be resistant to tetracycline, chloramphenicol, ampicillin and cefixime respectively. In plasmid profiling, out of the 99 E. coli pathotypes, 35 (35.4%) were found to possess plasmids, which ranged in sizes from 1.7kb to 4.5kb. These plasmids were seen in 33% of EPEC. Only two pathotypes possessed single size plasmid in EIEC.

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Conclusion: Our results revealed that EPEC, EIEC, enterohemorrhagic *E. coli* (EHEC), and enterotoxigenic *E. coli* (ETEC), had significant association with acute diarrhea among children in Sanandaj and should be considered as potential pathogens. Guidelines for appropriate use of antibiotics in Sanandaj need updating.

**Keywords:** Antimicrobial susceptibility; Plasmid profile; *Escherichia coli* pathotypes; Children; Acute diarrhea

**Introduction**
*Escherichia coli* is one of the most common causes of morbidity and mortality in children with diarrhea all over the world particularly in developing countries [1-2]. Diarrheagenic *E. coli* can be categorized into six groups: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffuse adhering *E. coli* (DAEC) [3]. This classification is based on agglutination using monovalent and polyvalent antisera. However, comparison of plasmid profiles is a useful method for assessing the possible relatedness of individual clinical isolates of a particular bacterial species in epidemiological studies [4].

Among all pathotypes of *E. coli*, enterotoxigenic *E. coli* is the most frequently isolated enteropathogen, accounting for approximately 210 million diarrhea episodes and approximately 380000 deaths annually. Among children aged <5 years in the developing country, the annual burden of diarrhea is estimated to be 1.5 billion episodes, accounting for three million deaths [5]. Moreover, the WHO has emphasized the need to understand the disease burden and epidemiology of diarrheal infections in developing countries [6].

In Iran, it has been estimated that diarrhea is responsible for 18 million cases of illness [7-8], 12 million medical visits, one million hospital admissions [9-10], and 516 deaths in children younger than five years of age [10]. The widespread occurrence of drug resistant *E. coli* in Iran has necessitated the regular monitoring of *E. coli* pathotypes. Study of the prevalence of diarrheagenic *E. coli* (DEC) categories and their importance in childhood diarrhea has not been carried out in Sanandaj yet. Therefore, to define the relation of various categories of *E. coli* with diarrhea in Sanandaj, we carried out this study to determine the frequency, antimicrobial susceptibility and plasmid profiles of *E. coli* pathotypes obtained from children with acute diarrhea.

**Materials and methods**

**Sample collection and susceptibility tests**
A total of 466 rectal swabs were examined for the presence of *E. coli* strains in children between the ages of one month to five years, in their study. The specimens were processed at the Beassat Hospital, Sanandaj, which is a reference center for children in Kurdistan province. All *E. coli* pathotypes were isolated and identified using standard methods [11].

Susceptibility of isolates to antibiotics namely; nalidixic acid, ciprofloxacin, ceftriaxone, nitrofurantoin, ampicillin, chloramphenicol, amoxycillin, cotrimoxazole, cefixime, tetracycline, and cephalotin was tested using the disk diffusion method on Mueller Hinton agar (Merck, Germany) based on recommendations of CLSI (formerly the National Committee for Clinical Laboratory
Standards) [12]. The isolates were determined with commercially variable monoclonal antisera (Bahar Afshan Co. Iran) against all *E. coli* serotypes and serological tests were performed by the slide agglutination method.

**Plasmid analysis**

Plasmid DNA was extracted from cultured cells using the high pure plasmid isolation Kit (Roche, Germany), according to the manufacturer's instructions. The DNA was electrophoresed on 0.8% agarose gel stained with ethidium bromide and visualized by UV-transillumination [13].

**Results**

A total of 466 rectal swabs were examined for the presence of *E. coli* strains in children between the ages of one month to five years. Of the total number of specimens examined, 99 (21.2%) were positive for *E. coli*. The highest number of isolates, 37 (37.4%), was recovered from the 13-24 month age group, followed by the 1-12 month age group which produced 26 (26.3%) isolates (Table 1).

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Children with diarrhea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-12</td>
<td>26 (26.3)</td>
</tr>
<tr>
<td>13-24</td>
<td>37 (37.4)</td>
</tr>
<tr>
<td>25-36</td>
<td>14 (14.1)</td>
</tr>
<tr>
<td>37-48</td>
<td>8 (8.1)</td>
</tr>
<tr>
<td>&lt;49</td>
<td>14 (14.1)</td>
</tr>
<tr>
<td>Total</td>
<td>99 (100)</td>
</tr>
</tbody>
</table>

Of the 466 children, 191 (41%) were girls and 275 (59%) were boys. With a total incidence of 21.2% (99/466), 34 out of 191 girls (7.3%, 34/466) and 65 out of 275 boys (14.0%, 65/466) were diagnosed with acute diarrhea. Based on the serological tests, 59 (59.6%) of the *E. coli* strains were identified as EPEC, 22 (22.2%) as EIEC and the remaining 18 (18.2%) comprised of EHEC 11 (11.1%) and ETEC 7 (7.1%) pathotypes.

In Table 2, *in vitro* antibiotic susceptibility pattern of *E. coli* isolates is shown. 89.9%, 88.9% and 79.8% of isolates resistant to tetracycline, chloramphenicol, and ampicillin, respectively. Similarly, in the case of cefixime, 75.8% of isolates were resistant. In plasmid profiling, out of the 99 *E. coli* pathotypes, 35 (35.4%) were found to possess plasmids, which ranged in sizes from 1.7 kb to 4.5 kb. These plasmids were detected and seen in 33% of EPEC. Only two pathotypes possessed single size plasmid in EIEC (Fig. 1).

Table 2: Antibiotic resistant pattern of *E. coli* pathotypes

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>36.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30.3</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>20.2</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>79.8</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>88.9</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>75.8</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>70.7</td>
</tr>
<tr>
<td>Cefixime</td>
<td>75.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>89.9</td>
</tr>
<tr>
<td>Cephalotin</td>
<td>60.6</td>
</tr>
</tbody>
</table>

**Discussion**

Diarrhea caused by *E. coli* patotypes has been recognized as an important health problem among children in the developing countries and is a research priority of the diarrheal disease control program of the WHO [14]. The epidemiological significance of each *E. coli* category in childhood diarrhea varies with the geographical area. It has become clear that there are important regional differences in the prevalence of the different categories of DEC.
Our study, which covered a 12-month period, was the first direct field investigation in Sanandaj associated with acute diarrhea in children. The prevalence of acute diarrhea among children caused by *E. coli* strains in Beassat hospital was 21.2%. This percentage is lower compared with other studies carried out in Iran [15-16]. However, some studies from other parts of the world report similar findings in regards to prevalence of acute diarrhea [17-19]. In this study, EPEC was the most commonly isolated category. In addition, we identified 22 EIEC strains and observed a very low frequency of ETEC. This is consistent with the findings of previous studies from Tehran and other part of the world [20-22].

**Antimicrobial susceptibility testing**

Our analysis of the antibiotic resistance patterns showed that 89.9%, 88.9% and 79.8% of isolates were found to be resistance to tetracycline, chloramphenicol, and ampicillin, respectively. These results confirmed data reported by other authors, indicating that EPEC were frequently and increasingly demonstrating multiple resistances to antibiotics tested [21]. The reason for the high resistance to antibiotics observed in this study may be due to an increasing consumption rate of antibiotics, transmission of resistant isolates between people, consumption of food from animals receiving antibiotics and predominantly, the wide use of self-medication.

**Plasmid profiles**

Plasmid profiling of antibiotic resistant *E. coli* isolates revealed that the isolates contained various size plasmids. In 35.4% of *E. coli* strains isolated from children with acute diarrheal infections, plasmids of different molecular size were found (Fig. 1). In several cases, different isolates showed very similar plasmid profiles. However, isolates that showed multiple drug resistance were also found to harbour plasmids with sizes ranging from 1.7kb to 4.5kb which is similar to what was observed by Umu *et al.* [23] who reported 105 of the *E. coli* isolated from children with diarrhea.

Plasmid profile analysis has been widely used in epidemiological investigations [24-25]. Although some strains exhibited different antibiotic resistance patterns, some of their plasmids had similar migration patterns on agarose gel electrophoresis. Multiple resistances are conferred by R-plasmids of different sizes. The high prevalence of antibiotic resistance conferring plasmids observed in this study may be due to the increasing widespread use of antibiotics.

**Strength of the study**

This study, which covered a 12-month period, was the first direct field investigation in Sanandaj associated with acute diarrhea in children. This was also the first report of *E. coli* pathotypes causing diarrhea amongst children in the Kurdistan provinces.
The limitation of this study was the possible presence of entropathogens such as some diarrheagenic viruses in the area that we did not test. More genetic studies are required to identify the genetic mechanisms allowing some pathogenic E. coli to retain a high potential for recombination. Moreover, further studies are needed to investigate the ecological, socio-economical, and epidemiological basis of E. coli infections as an emerging pathotype in children in Kurdistan.

Conclusion
In most of the clinical laboratories in Iran, particularly Kurdistan, E. coli is not considered as an etiologic agent responsible for diarrhea in children. Our results revealed that it is possible there is a relation between EPEC, EIEC, EHEC, and ETEC and acute diarrhea among children in Sanandaj, therefore, they should be considered as potential pathogens.

We therefore, recommend the routine isolation and identification of E. coli strains from children with acute diarrhea in all the clinical laboratories in Sanandaj. In short there is no formal surveillance system for diarrheagenic E. coli and finally this is the first report of E. coli pathotypes causing diarrhea among children in Kurdistan Provinces. Therfore, it is recommended applying appropriate use of antibiotics and updating guidelines for appropriate use of antibiotics in Sanandaj.

Acknowledgement
The authors are grateful to the Vice-chancellor in Research affairs for the financial support (Grant no. 14-8937).

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