

Original article**Frequency, antimicrobial susceptibility and plasmid profiles of *Escherichia coli* pathotypes obtained from children with acute diarrhea****Enayat Kalantar, PhD^{1*}, Fariborz Soheili, MSc², Heiman Salimi, MSc³, Mohammad Mehdi Soltan Dallal, PhD⁴**¹*Kurdistan Liver and Digestive Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran*²*Molecular Biology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*³*Department of Medical Microbiology, Beasat Hospital, Kurdistan University of Medical Sciences, Sanandaj, Iran*⁴*Department of Medical Microbiology, School of Health, Tehran University of Medical Sciences, Tehran, Iran***How to cite this article:**Kalantar E, Soheili F, Salimi H, Soltan Dallal MM. Frequency, antimicrobial susceptibility and plasmid profiles of *Escherichia coli* pathotypes obtained from children with acute diarrhea. Jundishapur J Microbiol. 2011; 4(1): 23-8.**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.***Address for correspondence:**

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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 this 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, amoxicillin, co-trimoxazole, 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 manufacture'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 1: Distribution of diarrheal episodes in different age group

Age (month)	Children with diarrhea (%)
1-12	26 (26.3)
13-24	37 (37.4)
25-36	14 (14.1)
37-48	8 (8.1)
<49	14 (14.1)
Total	99 (100)

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 1.7kb to 4.5kb. 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

Antibiotic	%
Nalidixic acid	36.4
Ciprofloxacin	30.3
Ceftriaxone	30.3
Nitrofurantoin	20.2
Ampicillin	79.8
Chloramphenicol	88.9
Amoxycillin	75.8
Co-trimoxazole	70.7
Cefixime	75.8
Tetracycline	89.9
Cephalotin	60.6

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.

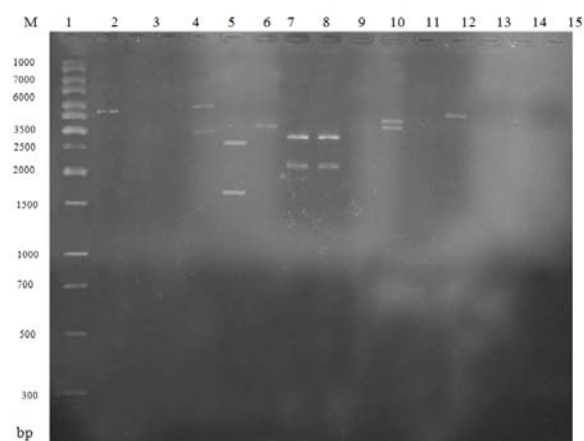


Fig. 1: Agarose gel electrophoresis showing plasmid profile of *E. coli* strains

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 Uma *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 enteropathogens 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 diarrheogenic *E. coli* and finally this is the first report of *E. coli* pathotypes causing diarrhea among children in Kurdistan Provinces. Therefore, it is recommended applying appropriate use of antibiotics and updating guidelines for appropriate use of antibiotics in Sanandaj.

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