

**Research Article**

## **ANTIBIOTIC RESISTANCE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM DIARRHEAL CHILDREN IN MILAD HOSPITAL DURING 2012-13**

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### **ABSTRACT**

In this study drug susceptibility of enteropathogenic *Escherichia coli* (EPEC) was detected in diarrheal patients. The increase in antibiotic resistance in pathogenic bacteria, especially in children, is considered as one of the world's health problems. The aim of this study was to evaluate the antibiotic resistance in EPEC which isolated from children with diarrhea admitted to the Milad hospital in Tehran. This study was conducted from March 2011 through March 2013. fecal or rectal swab specimens from children (<5 years) were collected and then processed, using standard methods, to detect *Salmonella* spp., *Shigella* spp, and EPEC. The antibiograms of the enteropathogens were determined using the disk diffusion method. The *E. coli* positive cultures were set for serological test, by slide agglutination test, according to BioRad procedures, using EPEC polyvalent O antisera for EPEC, (I, II, III, and IV) separately. A total of 7321 samples were processed: 433 enteric pathogen were isolated from samples. From 433 positive samples, 146 came out positive for EPEC. Based on the results of antimicrobial testing resistance to: ampicillin, cefixime, Co-trimoxazole, Nalidixic acid, ciprofloxacin, ceftriaxone, and chloramphenicol were 82.19%, 79.45%, 64.38%, 59.5%, 36.98%, 34.93%, 13.6% respectively. The highest number of EPEC isolated belonged to polyvalent serogroup was 3 serogroup (85%), 4: 3%, 2: 5% and 1: 7%. Highest sensitivity was to chloramphenicol in this study. But in clinical practice, it has serious side effects. Therefore it suggests that application of antibiogram test is necessary before antibiotic prescription for successful treatment and prevention of diarrhea caused by multi-drug resistance agents.

**Keywords:** Diarrhea, EPEC, Antibiogram, Susceptibility, Disk Diffusion

### **INTRODUCTION**

Infantile diarrhea is one of the important illnesses with high morbidity and mortality in children. It is caused by a wide range of microbial agent's including viruses, bacteria and parasites. Among the bacterial pathogens, diarrheagenic *E. coli* is the most (Asadi *et al.*, 2010). Enteropathogenic *Escherichia coli* (EPEC) strains are among the most important pathogens infecting children worldwide, because they are common and are often associated with prolonged illness with its attendant risk of malnutrition. These pathogens induce a distinctive histopathology known as attaching effacing (A/E) lesion, which is characterized by the intimate contact between bacteria and the epithelial surface of the enterocyte. The protein intimin (*eaeA*), necessary for the A/E lesion, has been used for the molecular identification of EPEC. The current average prevalence of EPEC in pediatric diarrheal episodes in developing countries, using molecular methods, is 5%–10% (Francesca *et al.*, 2011). Infective diarrhea is a common childhood disease, the etiology of which changes according to geography, climatic conditions, nutritional status, and other circumstances. Previous studies showed that for urban centers rotavirus and *Salmonella* sp. are the important causal agents public health measures are poor, enterotoxigenic *Escherichia coli* (ETEC), *Shigella* sp., and other bacterial agents assume an important role (Yam *et al.*, 1987).

Enteropathogenic *Escherichia coli* (EPEC) is an important cause of infantile diarrhea worldwide and particularly in developing countries. EPEC strains adhere intimately to the brush border of the intestinal epithelium and initiate a complex signaling cascade by virtue of a chromosomal pathogenicity island, the locus for enterocyte effacement (Tamara *et al.*, 2010).

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Antimicrobial resistance is increasing worldwide. Resistance in intestinal organisms is of interest it can compromise treatment of infections caused by pathogenic strains but also because the gut is a complex, diverse and heavily populated niche and resistant organisms there can transmit resistance genes horizontally. Many investigators have documented a high prevalence of antimicrobial resistance among EPEC strains in different parts of the world but few of these studies have been performed on recent isolates. Resistance appeared at the

Beginning of the antibiotic era and epidemiological data suggests that its prevalence is associated with the 1970s and 1980s and diversity of antimicrobial use the genetic basis for this resistance and the evolutionary consequences are rarely studied (Isabel *et al.*, 2010).

In the present study, we isolated and identified the enteropathogens *E. coli* causing diarrhea among children less than 10 years old in Tehran, Iran, during the different seasons, and study their patterns of resistance to different antimicrobial agents used for the treatment of patients with diarrhea. The research aims to answer two questions first, what causes the disease? Second, is it possible the find a conclusive cure?

## **MATERIALS AND METHODS**

### ***Microbiological Study***

Stool specimens were collected from the patients and transported to the microbiology department of the laboratory. The specimens were examined for consistency, color, and atypical components such as mucous, blood, and parasites, examined by light microscope for the presence of red blood cells, pus cells, parasitic ova, and protozoa, inoculated onto Blood, MacConkey, Salmonella Shigella (SS) agar media, and Selenite F broth, and incubated at 37°C for 24 h. The isolates were subjected to the following tests: Gram staining, citrate utilization, oxidase test, and triple sugar iron agar (TSI)

### ***Serological Examination And Confirmatory Tests for EPEC***

The *E. coli* positive cultures were set for serological\_test, by slide agglutination test, using EPEC polyvalent O anti sera for EPEC, (I, II, III, and IV) separately.

### ***Antimicrobial Susceptibility Testing***

Susceptibility to some antimicrobial agents for all EPEC isolates was determined by the standard disk diffusion method on Muller-Hinton incubated for 18-24 hours at 37 °C. ampicillin, cefixime, Co-trimoxazole, Nalidixic acid, ciprofloxacin, ceftriaxone, and chloramphenicol were used. After incubation, the diameter of each inhibition zone was measured with a pair of calipers, and recorded in mm. The results then interpreted according to CLSI documentation (MacFaddin, 2000)

*Escherichia coli* ATCC 25922 was used as negative control (obtained from American Type Culture Collection, USA). Selection of antibiotic discs was performed according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2010).

### ***Statistical Analysis***

Statistical analysis was done with excel version 2007, using P values <0.05 were considered significant.

## **RESULTS AND DISCUSSION**

A total of 146 consecutive non repeat samples from children under 10 years old with diarrhea were included in our study. Our control group revealed no EPEC or other enteric pathogens ( $P < 0.05$ ).

In the present study, the isolates belonging to EPEC serotypes were tested by diffusion disk, and were interpreted according to Clinical Laboratory Standards Institute guidelines (CLSI, 2010).

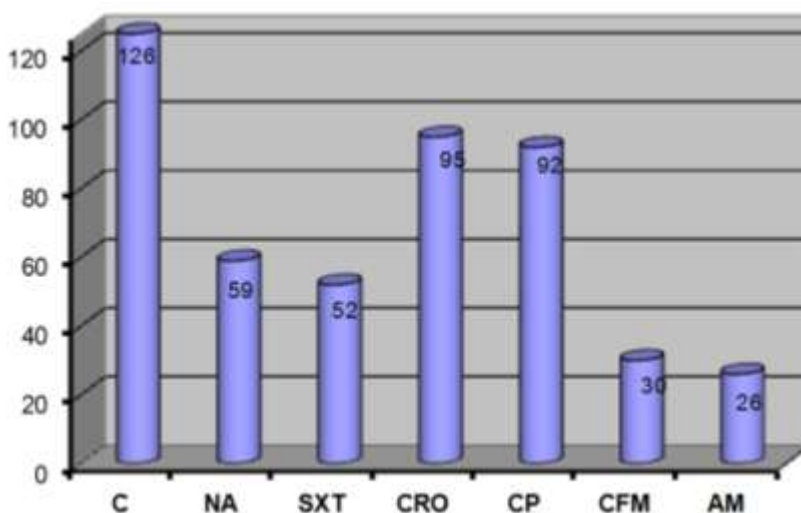
The highest number of EPEC isolated belonged to polyvalent serogroup was 3 serogroup(85%), 4: 3%, 2: 5% and 1: 7%.however, present results were not agreement with Al-hilali and Al-charrakh in Iraq (Al-hilali, 2010).

433 positive samples, 146 came out positive for EPEC. Based on the results of antimicrobial testing resistance to: ampicillin, cefixime, Co-trimoxazole, Nalidixic acid, ciprofloxacin, ceftriaxone, and chloramphenicol were 82.19%, 79.45%, 64.38%, 59.5%, 36.98%, 34.93%, 13.6% respectively (Figure 1)

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All isolates showed 82.19% resistance to Ampicillin this study results were lower than Alaa Hani Al-Charrakh *et al* 2012. The rate of resistance to quinolons (Nalidixic acid) and fluoroquinolones (Ciprofloxacin) were detected in 59.5% and 36.98% respectively (Figure 1). These Anti bacterial susceptibility pattern of bacterial agents isolates in different parts of the country provides essential information regarding the selection of antibiotics for all patients living in different areas. Tabatabaei in 2008 were studied on stool infections, the result of their study almost agreed with our study (Tabatabaei, 2008).

However, it might be possible that this high level of 3rd generation cephalosporin and Ampicillin in present study was most probably due to acquisition of  $\beta$  lactamase, possibly during therapy. Chloramphenicol is an antibiotic with a broad antibacterial spectrum and industrially manufactured by synthetic method. Two types of risks have been identified in relation to medicinal use of chloramphenicol. Firstly, aplastic anemia, a form of anemia when the bone marrow ceases to produce sufficient red and white blood cells, is the most dangerous effect produced by medicinally used Chloramphenicol (Benestad, 1979). secondly, limited evidence exists for the genotoxic carcinogenicity of chloramphenicol in humans exposed to therapeutic doses (Doody *et al.*, 1996).



**Figure 1: Antibiotic sensitivity of 146 EPEC isolates to different antibiotics**

Worldwide, the most common pathogens that cause acute gastroenteritis are: Salmonella spp., Shigella spp., Campylobacter spp., E. coli O157:H7, Listeria monocytogenes, Vibrio cholerae, Yersinia enterocolitica, Rotavirus, Cryptosporidium spp., Entamoeba histolytica, and Giardia lamblia. These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks (Centre for Food Safety and Applied Nutrition, USA).

In Sao Paulo, Brazil, the etiologic profile of acute diarrhea in 154 children aging less than 5 years was studied. Intestinal pathogens were detected in 112 (72.8%) cases. The association of two or more intestinal pathogens occurred in 47 (30.5%) cases. The pathogens identified were, Rotavirus; 32 (20.8%), bacteria; 53 (34.4%), both; 25 (16.2%), and 2 (1.4%) with Giardia intestinalis (in one case associated with Rotavirus and in another one associated with bacteria). Altogether, there were 105 bacterial isolates; 90 were Escherichia coli (EPEC 27, Diffuse adhering E. coli (DAEC) 24, ETEC 21 and EAEC 18), 12 were Shigella spp., 2 were Salmonella spp., and one was Yersinia spp. Children with mixed infections (viral and bacterial) had increased incidence of severe vomiting, dehydration and hospitalization (Souza *et al.*, 2002).

Strains of E.coli which are capable of causing diarrhea, under certain conditions, Example, when the immune system is compromised, or due to environmental exposure, is referred to as diarrheagenic E. coli.

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Diarrheagenic *E. coli* strains possess specific fimbrial antigens that enhance their intestine-colonizing ability and allow adherence to the small mucosa bowel. Once having colonized, the strains use very different pathogenic strategies to cause changes in the arrangement of the bowel's mucosa (Donnenberg, 1999). Similarly, in Thailand isolated DEC showed high resistance to commonly used antibiotics such as ampicillin and nalidixic acid (Kalnauwakul *et al.*, 2007).

The high antibiotic resistance also indicates a negative impact on therapy with these classes of antibiotics. The periodic monitoring of antibiotics to detect any changing patterns would be necessary for effective treatments. A further study to evaluate the extent of antibiotic resistance transmission and the impact of such transmission on the effectiveness of antibacterial use in human medicine is imperative.

### Conclusion

Our data show that the prevalence of EPEC producing extended spectrum  $\beta$ -lactamases as a cause of infectious diarrhea in children younger than 5 years of age. In recent years, antibiotic resistance of diarrheagenic pathogens has reached alarming proportions worldwide. The misuse of antibiotics has been found to be the most important selecting force in the generation of bacterial resistance to antimicrobial drugs. This study of EPEC experimental infection provides a better understanding of the effects of antibiotics on bacterial infections and may provide a suitable model for the design and testing of antimicrobial products for treating of diarrhea. The development of newer antibiotics may offer a short term solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation.

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