

THE PREVALENCE OF ENTEROTOXIGENIC *E. COLI* ISOLATED FROM THE STOOLS OF CHILDREN AGED 0-10 YEARS WITH DIARRHEA IN MID-ANATOLIA REGION, TURKEY

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ABSTRACT

The stool samples from 245 patients with diarrhea were tested for heat labile toxin (LT) and heat stable toxins (ST) by passive latex agglutination and enzyme immunoassay methods respectively. Twelve (4.9%) enterotoxigenic *E. Coli* ETEC strains were isolated. Five strains (2%) expressed ST, and 7 (2.8%) expressed LT.

Key words: Enterotoxigenic *E. coli*, Traveler's diarrhea, Infectious Diarrhea, labile toxin, stable toxin

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of acute diarrhea in childhood. Generally affected populations are newborns, young children, and susceptible tourists ETEC strains are particularly common in tropical regions. In developing countries, children suffer from diarrhea attacks 7-8 times a year and ETEC strains are responsible for one of each 3 attacks (5,7,13). In developed countries, on the other hand, food and water-borne epidemics have been reported. However, the main problem for such countries is the diarrhea acquired by tourists while they are traveling in developing countries(6). ETEC has an important place among the etiologies for traveler's diarrhea.

To diagnose the presence of ETEC, heat labile toxin and/or heat stable toxin producing *E. coli* must be detected in the stool. From various parts of the world, ETEC has been reported to be the main etiological agent of diarrhea among children of 0-5 years of age at 1.86 %- 20.7% (1, 10, 16, 15).

The main goal of this study was to investigate whether ETEC is an important factor in diarrhea in children and to know its prevalence in our region.

This study was conducted at the microbiology laboratory of Kırıkkale University Hospital. Kırıkkale is a city in Mid-Anatolia region with a population of 280000 and land area of 4.630 km. Life in the city is mostly urban. The samples were collected from children of 0-10 years of age who applied with complaints of diarrhea to Children's Diseases Hospital in the region and the Hospital of Kırıkkale University Medical School.

Two hundred and forty-five diarrhea and 30 healthy stool samples were collected. Total 275 stool samples were inoculated onto eosin methylene blue agar plate (EMP). They were incubated at 37 °C overnight. Only one strain identified as *E. coli* from the stool sample of each patient was included in the evaluations. All the strains included in the study were

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stored at -80 °C. The strains were defrosted at 96-test groups, and tested for the production of heat labile and/or heat stable toxins.

Two-three colonies from pure cultures were put into tryptic soy broth and incubated at 37 °C for overnight. Next day, they were centrifuged at 900 g for 30 minutes. The supernatant was investigated for the presence of ST by Coli ST EIA test kit (Denka Seiken Co. Ltd. Tokyo-Japan). The sensitivity of this test, according to the values given by the manufacturing company, is 10-20 ng/ml and highly specific for *E. coli* ST+. LT was determined using VET-RPLA passive latex agglutination test kit (Oxoid, Oxoid Limited, Basingstoke Hampshire RG24 8PW, UK). Its sensitivity is 2 ng/ml. *E. coli* strains were put into Mundell's culture medium and incubated at 37 °C overnight; the cultured Mundell's medium was re-incubated at 37 °C for 4 hours after adding 10.000 u/ml polymyxin B as it is suggested in the kit procedure. The cultures were centrifuged at 4 °C, 900 g for 20 minutes, and supernatant was worked. Results were obtained by visual agglutination on black background.

The antibiotic resistance of 14 ETEC strains and 53 non ETEC strains, 28 of which were isolated from the diarrhea

stools and 25 of which were isolated from healthy stools was studied by disk diffusion method.

The results were evaluated with chi square test in SPSS 10,0 programme.

The following antimicrobial disks were used: ampicillin (10 µg)(AMP), amoxicillin clavulanic acid (30 µg)(AMC), gentamycine (10 µg)(GN), ciprofloxacin (5 µg)(CIP), trimethoprim-sulfamethoxazole (25 µg) (SXT) (Oxoid, Oxoid Limited Basingstoke, Hampshire, England). *E. coli* ATCC 25922 was used as a reference strain for quality control. The results were interpreted according to criteria of the National Committee for Clinical Laboratory Standards (11)

Of 245 diarrhea stool samples, 12 (4.9 %) ETEC strains were isolated. Of these, 5 (2 %) were ST and 7 (2.8%) were LT. Among the *E. coli* strains isolated from 30 healthy stools, 2 (6.7%) ST were isolated. Statistical significance difference between healthy stools and diarrhea stool samples was not considered ($P > 0.05$).

The antibiogram results of *E. coli* strains isolated from the stool samples of children of 0-10 years of age have been provided in Table 1.

Table 1. Antibiotic resistance of ETEC and *E. coli* isolated from stool samples of diarrheic and non-diarrheic children (0-10 years-old).

strain	CIP %(n)	AMC %(n)	AMP %(n)	SXT %(n)	GN %(n)	Multiple resistant %(n)
ETEC (14)	21(3)	29(4)	36(5)	21(5)	7(1)	29(4)
Diarrheic strains (28)	11(3)	64(18)	75(21)	39(11)	11(3)	68(19)
Healthy strains (25)	0(0)	56(14)	60(15)	20(5)	8(2)	56(14)
Total (67)	9(6)	54(36)	61(41)	28(19)	9(6)	55(37)

n: the number of resistant strains, %:resistance rate, CIP: ciprofloxacin, AMP: ampicillin, AMC: amoxicillin clavulanic acid, SXT: trimethoprim-sulfamethoxazole, GN: gentamycine

The resistance rates of ETEC strains to antibiotics were 21% for CIP, 29% for AMC, 36% for AMP, 21% for SXT, and 7% for GN. In the isolates from diarrhea stools, these rates were 11%, 64%, 75%, 39%, and 11% for CIP, AMC, AMP,

SXT, and GN, respectively. In the isolates from the samples of healthy children, the rates of resistance to CIP, AMC, AMP, SXT, and GN were 0%, 56%, 60%, 20%, and 8% respectively (Table1). No ESBL growth was observed in any of the strains

including all the ETEC.

Statistical significance difference between resistance prevalences to antibiotics of ETEC and non ETEC strains was not considered ($P > 0.05$ for all analyses).

Although not statistically significant, except with CIP, the resistance rates of ETEC to all the other antibiotics were lower than those of non ETEC strains. ETEC were most susceptible to gentamycine (susceptibility rate: 93%). The resistance rates to AMP (36%) and AMC (29%) were significantly high. The strains resistant to more than 2 antibiotics were considered to have multiple resistance. Multiple resistance rates were also high. Among all the strains studied with antibiogram, the strains that were resistant to GN were also resistant to AMP, AMC, and SXT, while they were susceptible to CIP. Nevertheless, considering all the strains together, ETEC strains were the most CIP resistant group of strains.

ETEC is an important etiological agent of diarrhea in developing countries. Reports from Bangladesh, Mexico, Peru, Egypt, Argentina, India, Nicaragua, and Tunisia indicate an ETEC prevalence rate of 18-38 % in symptomatic children. These rates are much lower in reports from Vietnam and Brazil (4%, 3.7 %) (14,2,8,3). In a study conducted in our country in 2005, Ozerol *et al* determined a 10% prevalence rate for ETEC in children (0-5 years) with diarrhea (12). In Kırıkale, we determined a prevalence rate of 4.9% in symptomatic children of 0-10 years of age. This rate is similar to the rates determined in Vietnam and Brazil (8,3). However, it is lower than the rate reported by Ozerol *et al*. In the study by Ozerol *et al*, an age group of 0-18 years was studied and 69 samples from 0-5 years old group and 39 samples from 6-18 years (total= 108 samples) were evaluated. In that study, 7 ETEC were isolated. The prevalence of ETEC was 10% for 0-5 year-old group, while no ETEC were isolated from 5-18 year-old group. If the rate for 0-10 year-old group had been calculated, the ETEC prevalence rate would probably have been under 10% (12). Evaluation of the results of the study by Ozerol *et al* and our study shows that the prevalence rate of ETEC in our country is not high.

While LT expression was more common in the strains isolated in Mexico, Peru, Argentina, and India, ST expression was more common in the strains isolated in Bangladesh, Egypt, and Nicaragua. In these countries, the rate for simultaneous expression of LT and ST varied between 7% and 49 % (14). In the study from our country by Ozerol *et al*, the rate of ST expression in the ETEC strains isolated from children aged 0-5 years with diarrhea was 28%, while for LT expression, the rate was 71% for the same group of children (12). In our study, 41.8% of the ETEC strains isolated from children with diarrhea expressed ST, whereas 58.3% expressed LT. The ETEC strains isolated in our region express LT more frequently as the strains isolated in Mexico, Peru, Argentina, and India. Furthermore, to date, no ETEC strains that express both toxins have been detected in our region. The higher prevalence of ETEC strains that express LT than those expressing ST is an important finding because the LT expressing ETEC strains are considered to be less pathogenic than those expressing ST. The colonization factors (CF) in LT expressing ETEC are fewer than in ST expressing ETEC and frequently, they are accompanied by mild infections. The ETEC strains that express ST or LT/ST simultaneously are often isolated from severe diarrhea infections (14). In our region, the low prevalence of ETEC and among the ETEC isolated, the high prevalence rate of less pathogenic, LT expressing ETEC strains is a positive finding with regard to child health and tourism.

Although no LT expressing ETEC was isolated from the 30-sample control group, in 2 (6.6%) stools, ST expressing ETEC was isolated, which is a significant finding. The coexistence of ST expressing ETEC with colonization factors is more prevalent. Therefore, ST expressing ETEC are more likely to colonize and dwell in the bowels for a long time. In conclusion, although these children are not ill at the moment, they may pose a risk for contagion. In fact, these colonizations might be responsible for repeated ETEC attacks over a year-period in developing countries.

In diarrhea caused by ETEC, fluid replacement therapy is

usually effective. However, in persistent cases, antibiotic treatment may be required. Although in the years when ETEC strains were first isolated, they were found to be susceptible to antibiotics, in time they have acquired resistance to the most commonly used antibiotics. CIP was a safe drug for prophylaxis against travel related diarrhea (4, 14). The resistance rates of ETEC strains to drugs have increased in the last decade. In a study from Kuwait, the resistance rates of ETEC strains were found to be 66,7% for AMP, 11,1% for GN, 11,1 for CIP. The resistance rates reported from Mozambique are 61% for SXT and 46% for AMP, 8% for AMC. In an earlier study conducted in Malatya city of Turkey, the resistance rates were reported to be 54.4% for AMP and 36% for SXT (1, 9, 12). In our study, drugs of common use were tested. The results indicated that the resistance rates of ETEC strains to antibiotics were lower than the resistance rates of non-ETEC *E. coli* strains. On the other hand, except with GN, the resistance rate to the other four antibiotics evaluated (CIP, AMC, AMP, SXT) and multiple antibiotic resistance rate were found to be over 20%. This suggests the onset of problems in ETEC associated diarrhea requiring treatment. Although the low resistance to GN is an advantage, the strains that are resistant to GN are also resistant to the other antibiotics constitute another difficulty in the treatment.

In this study, a low ETEC prevalence was found. The majority of the isolated strains (58.3%) were less pathogenic, LT expressing ETEC strains. These are positive findings with regard to child health and tourism. Nevertheless, high rates of resistance to antibiotics may lead to problems in the cases requiring antibiotic treatment.

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