# Frequency and Antimicrobial Susceptibility of Diarrheagenic *Escherichia coli* Obtained from Patients with Acute Diarrhea in a Tertiary Care Hospital, Bangladesh.

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## Abstract

*Escherichia coli* (*E. coli*) is an important group of pathogens associated with diarrhea among children. Despite the fact that diarrhaegenic *Escherichia coli* (DEC) has been identified as a major etiologic agent of childhood diarrhea, only a few studies have been performed in Bangladesh to identify these organisms. The objective of this study was to determine the frequency and antimicrobial susceptibility of DEC obtained from patients with acute diarrhea. To detect DEC in patients with acute diarrhea, a total of 300 stool specimens were tested by multiplex polymerase chain reaction (PCR). The antimicrobial susceptibility of DEC were tested by Kirby-Bauer disc diffusion technique as per recommendation of CLSI (Clinical and Laboratory Standards Institute), 2010. Out of 300 stool specimens collected from patients with acute diarrhea, the DEC was detected in 18% (54/300) cases. The dominating strain was Enterotoxigenic *E. coli* ( ETEC) (13%, 39/300), followed by Enteroaggregative *E. coli* (EAEC) (5%, 15/300) and no Enterohemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* ( EIEC) and Enteropathogenic *E. coli* ( EPEC) could be detected. Detected ETEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, 94% sensitive to Nalidixic acid, 89% sensitive to Gentamycin, 83% sensitive to Ciprofloxacin, 79% sensitive to Cephalexin, 39% sensitive to Amoxycillin, 46% sensitive to Tetracyclin and 31% sensitive to Cotrimoxazole. Detected EAEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, Nalidixic acid, 90% sensitive to Gentamycin and Ciprofloxacin, 85% sensitive to Cephalexin, 41% sensitive to Amoxycillin, 49% sensitive to Tetracycline and 31% sensitive to Cotrimoxazole. Both ETEC and EAEC isolates exhibited decreased susceptibility for Amoxycillin, Tetracycline and Cotrimoxazole. Our results revealed that ETEC and EAEC, had significant association with acute diarrhea and should be considered as potential pathogens. Guidelines for appropriate use of antibiotics in tertiary care hospitals need updating.

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## Introduction

Acute diarrhea is a major cause of morbidity and mortality worldwide. The acute diarrhea remains a major public health challenge, especially in developing countries where it is a leading cause of death. Every year nearly 1.4 billion episodes of acute diarrhea occur in children of less than 5 years of age in developing countries[1]. It has been estimated that the mean number of episodes of diarrhea per year in children of under 5 years of age from a developing region is 3.2[1]. In addition diarrheal illness account for an estimated 12600 deaths each day in children of under 5 years of age in Asia, Africa and Latin America[2]. Kosek and associates (2003) reviewed studies from 1990 to 2000 and concluded that diarrhea accounts for 21% of all deaths at under five years of age causing 2.5 million death per year in developing countries[1].

A diversity of recognized microorganisms such as bacteria, viruses and parasites can be associated with severe acute diarrhea in children[3]. Numerous studies performed in different countries have reported diarrheagenic *E. coli* (DEC) as being the most frequent and important among bacterial pathogens associated with acute diarrhea in developing countries. However, the frequencies of these pathogens vary with geographic region and depend on the socioeconomic/sanitary conditions[4].

In Bangladesh, the acute diarrhea remains one of the most important health problems. One third of the total child death burden is due to diarrhea. Every year, a rural child suffers on average from 4.6 episodes of diarrhea, from which about 230,000 children die[5]. The DEC has been reported to be responsible for 34% of diarrheal diseases in Bangladesh[6].

There are now at least six types of diarrheagenic strains of *E. coli* on the basis of distinct epidemiology and clinical feature, special virulence determinants and association with certain serotypes. There are Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely adhering *E. coli* (DAEC). Of these, EPEC, ETEC, EIEC, EHEC and EAEC are clearly associated with different types of enteritis, while the DAEC is potential pathogens but its association with diarrhea has not been clearly assessed and further studies are required to confirm its etiological role in diarrheal diseases[3,7].

Due to lack of facilities, the DEC cannot be detected in the routine diagnostic microbiology laboratory in developing countries, which is important in understanding the disease spectrum, tracing the sources of infection and the burden of the disease. Such identification would also assist the clinician to dispense appropriate management[8].

Therefore, we carried out this study to determine the frequency and antimicrobial susceptibility of DEC obtained from patients with acute diarrhea.

## Methods

This cross-sectional study was carried out during the period from July' 2011 to December' 2011 in the department of Microbiology, Mymensingh Medical College and included all patients with acute diarrhea irrespective of age and sex, admitted in Mymensingh Medical College Hospital. A total of 300 stool specimens were examined by standard laboratory methods for identification of *E. coli*. Different DEC strains were detected by Multiplex PCR following standard methods**.** DNA of *E. coli was* extracted from few freshly isolated colonies grown on MAC plates mixed in 100 µl of sterile deionized water, by boiling at $100^0$C for 10 minutes and centrifuged supernatant was used as DNA template [8].

Multiplex PCR for categorization of *E. coli* into EAEC, ETEC, EPEC, STEC and EIEC was done using primers for identification of *aggR*, *CVD432* and *aspU* genes for EAEC, *elt* or *est* gene for ETEC, *eae* gene for EPEC, *eae* or *stx* genes for STEC and *ipaH* gene for EIEC (Table: I)[8,9]. The PCR amplification was carried out with a 50 µl reaction mixture

[buffer, dNTP, Primers, Taq DNA polymerase, nuclease free water and DNA tamplate] using the following thermal and cycling conditions: initial denaturation at 95$^0$C for 5 min, followed by 30 cycles, each containing denaturation at 95$^o$C for 1 min, annealing at 56$^o$C for 1 min, and extension at 72$^o$C for 1 min; and final extension at 72$^o$C for 10 min. The amplified products were then separated by horizontal electrophoresis on a 1.0% agarose gel, stained with ethidium bromide and visualized under UV Trans- illuminator[8,9].

Antimicrobial susceptibility testing by disc diffusion method:

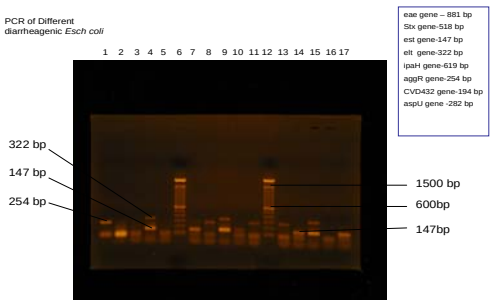
All the isolates were put into antibiotic susceptibility test by Kirby-Bauer disc diffusion technique as per recommendation of CLSI (Clinical and Laboratory Standards Institute), 2010. Panel of antibiotics were used. All tests were performed on Muller-Hinton agar. The surface was lightly and uniformly inoculated by cotton swab. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards. The swab stick was then took out and squeezed on the wall of the test tube to discard extra suspension. Inoculated plates were incubated at 37 $^0$C for 24 hours. On the next day, plates were read by taking measurement of zone of inhibition. Results were recorded and graded as Resistant (R) and Sensitive (S) according to the reference zone of inhibition of particular antibiotic (CLSI, 2010). Known control strain ATCC, No. 25922 and ATCC No. 25923 were used for quality control[10,11].

## Antimicrobial agents used

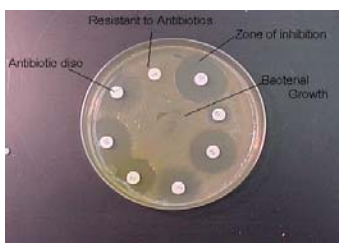
A total of 10 antimicrobial agents were used for determining antibiogram of isolated organisms according to Gram negative panel recommended by CLSI, 2010. Antibiotics were: Amoxycillin, Cephalexin, Ciprofloxacin, Gentamicin, Amikacin, Nalidixic acid, Ceftriazone, Cotrimoxazole, Nitrifurantioin and Tetracyclin[10,11].

Table I: PCR primers were used for detecting different diarrheagenic *Escherichia coli* in the present study.

| Designation-Sequence(5 to 3)- | Target gene- | Amplicon size(bp) |
|---|---|---|
| SK1 CCC GAA TTC GGC ACA AGC ATA AGC<br>SK2 CCC GGA TCC GTC TCG CCA GTA TTC G | *eae* | 881 |
| VTcom-u GAG CGA AAT AAT TTA TAT GTG<br>VTcom-d TGA TGA TGG CAA TTC AGT AT | *stx* | 518 |
| AL65 TTA ATA GCA CCC GGT ACA AGC AGG<br>AL125 CCT GAC TCT TCA AAA GAG AAA ATT AC | *est* | 147 |
| LTL TCT CTA TGT GCA TAC GGA GC<br>LTR CCA TAC TGA TTG CCG CAA T | *elt* | 322 |
| ipaIII GTT CCT TGA CCG CCT TTC CGA TAC CGTC<br>ipaIV GCC GGT CAG CCA CCC TCT GAG AGT AC | *ipaH* | 619 |
| aggRks1 GTA TAC ACA AAA GAA GGA AGC<br>aggRkas2 ACA GAA TCG TCA GCA TCA GC | *aggR* | 254 |
| Eaggfp AGA CTC TGG CGA AAG ACT GTA TC<br>Eaggbp ATG GCT GTC TGT AAT AGA TGA GAAC | CVD432 | 194 |
| aspU-3 GCC TTT GCG GGT GGT AGC GG<br>aspU-2 AAC CCA TTC GGT TAG AGC AC | *aspU* | 282 |




Photograph of multiplex PCR showing *aggR* gene (254 bp) of EAEC in lane 1, *est* gene (147 bp) and *elt* gene (322 bp) of ETEC in lane 4, only *est* gene (147 bp) of ETEC in lane 14 and ladder marker (100bp) in lane 5 and 12.



Photograph of antibiotic susceptibility test of isolated ETEC by Kirby-Bauer disc diffusion technique on Muller-Hinton agar medium.

## Results

In the present study, majority of the cases (62%) belonged to <5 years of age, in which 18% (54/300) cases were in the age group <1 year and 44% (132/300) cases were in the age group 1-5 years. The rest 38% (114/300) cases were in the age group >5 years (Figure: I).

Of the 300 specimens examined, the DEC was detected in 18% (54/300) cases. The dominating strain was ETEC (13%, 39/300), followed by EAEC (5%, 15/300) and no EHEC, EIEC and EPEC could be detected (Table: II).

Detected ETEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, 94% sensitive to Nalidixic acid, 89% sensitive to Gentamycin, 83% sensitive to Ciprofloxacin, 79% sensitive to Cephalexin, 39% sensitive to Amoxycillin, 46% sensitive to Tetracyclin and 31% sensitive to Cotrimoxazole(Table:III). Detected EAEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, Nalidixic acid, 90% sensitive to Gentamycin and Ciprofloxacin, 85% sensitive to Cephalexin, 41% sensitive to Amoxycillin, 49% sensitive to Tetracycline and 31% sensitive to Cotrimoxazole(Table: IV). Both ETEC and EAEC isolates exhibited decreased susceptibility for Amoxycillin, Tetracycline and Cotrimoxazole.

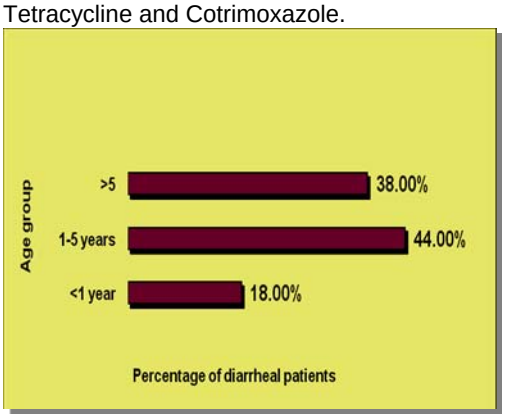



Figure I: Age distribution of the study population.

Table II: Distribution of different diarrheagenic *Escherichia coli* (DEC) Strains in the study population (n=300).

| Diarrheagenic *Escherichia coli* | Number of cases (%). |
|---|---|
| ETEC | 39 (13) |
| EAEC | 15 (5) |
| EPEC | 0 (0) |
| EIEC | 0 (0) |
| EHEC | 0 (0) |
| Total | 54 (18) |

Table III: Antibiotic sensitivity patterns of Enterotoxogenic *E. coli* (ETEC) isolated from the stool samples (n=39).

| Antibiotic | Number of ETEC strains sensitivity to different antibiotics (%). |
|---|---|
| Ceftriaxone | 39(100) |
| Amikacin | 39(100) |
| Nitrofurantioin | 39(100) |
| Nalidixic acid | 36(94) |
| Gentamycin | 34(89) |
| Ciprofloxacin | 32(83) |
| Cephalexin | 30(79) |
| Tetracycline | 18(46) |
| Amoxycillin | 15(39) |
| Cotrimoxazole | 12(31) |

Table IV: Antibiotic sensitivity patterns of Enteroaggregative *E. coli* (EAEC) isolated from the stool samples (n=15).

| Antibiotic | Number of EAEC strains sensitivity to different antibiotics (%). |
|---|---|
| Ceftriaxone | 15(100) |
| Amikacin | 15(100) |
| Nalidixic acid | 15(100) |
| Nitrofurantioin | 15(100) |
| Ciprofloxacin | 13(90) |
| Gentamycin | 13(90) |
| Cephalexin | 12(85) |
| Tetracyclin | 7(49) |
| Amoxycillin | 6(41) |
| Cotrimoxazole | 5(31) |

## Discussion

Acute diarrhea is one of the most common illness and cause of death in young children in Bangladesh. In the present study, majority of the cases (62%) belonged to <5 years of age, in which 18% cases were in the age group <1 year and 44% cases were in the age group 1-5 years. The rest 38% cases were in the age group >5 years. Albert and associates (1999) as well as Stoll and associates (1982) reported that diarrhea was more common in children of <5 years of age in Bangladesh[12,13], which supports the present study findings. In a recent study from Vietnam by Nguyen and associates (2005), it was found that diarrhea was more frequent in children of less than 5 years of age[2], which correlates with the present study findings.

In the present study, the DEC was detected in 18% (54/300) cases (Table II). In 2002 and 1999, it was reported that DEC was responsible for 34% and 26% of diarrheal diseases in Bangladesh respectively[6, 12]. All the above study findings were higher than the present study. Various factors might be responsible for such a difference In the present study, the samples collected were neither directly inoculated in culture media nor transported by transport media, history of taking antibiotics before sample collection and seasonality might be important factors to reduce the identification of DEC in the study population[13]. In Vietnum, Nguyen and associates (2005) and in Mozambique, Rappelli and associated (2005) found DEC was responsible for 22.5% and 20% of diarrheal diseases respectively[2,7], which correlate well with the present study findings. In the present study, among the diarrheagenic *E. coli* (DEC), the ETEC was detected in 72% (39/54) cases and the EAEC was detected in 28% (13/54) cases. No EHEC, EPEC and EIEC could be detected. The ETEC was found to be the most prevalent DEC in the study population. In 2002, it was reported that ETEC was most prevalent DEC, responsible for diarrheal diseases in Bangladesh which supports the present study results[14]. The EAEC was the second most prevalent DEC in the present study population. EAEC has been found as a common diarrheal pathogen in children in many developing countries[5]. The EPEC could not be detected in the present study population. The most important feature of the diarrheal diseases due to EPEC infection is the remarkable age distribution. The EPEC infection is primarily a disease of infants younger than 1 year of age[16]. In the present study, only 18% cases were found in the age group below 1 year. Nessa and associates (2007) as well as Unicomb and associates (1996) could not detect EPEC from any age group in Bangladesh[8,17]. In the present study, the EIEC and EHEC could not be detected in the study population. In many previous studies carried out in Bangladesh, the EIEC and EHEC could not be detected in diarrheal patients[18,19,20,21]. The absence of these strains is not surprising since these pathotypes are not frequently detected in developing countries of Africa and Asia[7].

In the present study, detected ETEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, 94% sensitive to Nalidixic acid, 89% sensitive to Gentamycin, 83% sensitive to Ciprofloxacin, 79% sensitive to Cephalexin, 39% sensitive to Amoxycillin, 46% sensitive to Tetracyclin and 31% sensitive to Cotrimoxazole (Table: III). Detected EAEC were 100% sensitive to Ceftriaxone, Nitrofurantioin, Amikacin, Nalidixic acid, 90% sensitive to Gentamycin and Ciprofloxacin, 85% sensitive to Cephalexin, 41% sensitive to Amoxycillin, 49% sensitive to Tetracycline and 31% sensitive to Cotrimoxazole (Table: IV). Both ETEC and EAEC isolates exhibited decreased susceptibility for Amoxycillin, Tetracycline and Cotrimoxazole. This trend agrees with the findings in other study in Germany[22]. kalantar and associates (2011) found similar type of results that supports the present study findings[23].

## Conclusion

We therefore, recommend the routine isolation and identification of *E.coli* strains form the patients with acute diarrhea and application of appropriate use of antibiotics and updating guidelines for appropriate use of antibiotics in tertiary care hospitals .

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