Original Article

Antimicrobial Susceptibility Pattern of Enteropathogenic *Escherichia coli* (EPEC) in Paediatric Diarrhoeal Patients

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Abstract

Enteropathogenic Escherichia coli (EPEC) mediated infantile diarrhoea among children is an important cause of morbidity and mortality in developing countries. The antimicrobial susceptibility pattern of EPEC strains isolated from children under 5 years of age was studied. Stool samples from 272 patients with diarrhoea were collected from two tertiary care hospitals. Out of 272 stool samples, 20 (7.35%) isolates were identified as EPEC on the basis of presence of bfpA gene detected by polymerase chain reaction and antibiotic susceptibility testing was performed on these EPEC strains by Kirby-Bauer disc diffusion method. The antimicrobial susceptibility test revealed that the EPEC isolates were highly resistant to ampicillin (100%), nalidixic acid (95%) and tetracycline (95%) and were sensitive to ceftazidime (95%), cefotaxime (90%), ceftriaxone (95%), imipenem (100%) and levofloxacin (85%). Isolation of EPEC is of great importance since they are responsible for acute diarrhoeal diseases in large number of children under the age of five years. The high antimicrobial resistance observed in our study indicates indiscriminate or improper use of antimicrobials, besides the risks of self-medication.

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Introduction

Diarrhoea caused by multidrug-resistant bacteria has been recognized as an important public health problem among children in developing countries and is a research priority of the diarrhoeal disease control program of the World Health Organization.[1] Enteropathogenic *Escherichia coli* (EPEC), is a major cause of infantile diarrhoea and subsequent morbidity and mortality among children in developing countries.[2] Children with acute diarrhoea are treated by oral rehydration fluid plus administration of an antibiotic. EPEC shows high level of resistance to ampicillin, tetracycline, co-trimoxazole while the most effective drugs are ceftazidime, ceftriaxone, imipenem, cefotaxime, levofloxacin.[3,4] Prior knowledge on local antimicrobial susceptibility patterns of infective agent is important in selecting the appropriate empirical therapy as the culture and antibiotic susceptibility test result is generally not available before 72 hours. Therefore, since many patients with enteritis are treated empirically with antibiotics, it is important to know the antimicrobial resistance patterns of prevalent EPEC causing diarrhoea in children. The objective of the study was to determine the antibiotic susceptibility pattern of EPEC causing diarrhoea among children under the age of five years. EPEC was detected by the presence of bfpA gene in Escherichia coli (*Esch. coli*) using polymerase chain reaction (PCR).[4,5]

Materials and Methods

Stool or rectal swab (R/S) samples were collected from Sir Salimullah Medical college and Mitford

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Hospital (SSMC & MH) and Dhaka Shishu Hospital (DSH) from 272 patients under 5 years of age, presenting with acute diarrhoea and who did not take any antibiotic during the last 30 days.[6] All the samples were collected within the period from January to December 2011. Standard microbiological techniques were followed for culture and isolation of *Escherichia coli* (*Esch. coli*).

Detection of bfpA genes of EPEC by PCR assay

Three to five colonies of *Esch. coli* from primary plate were taken for the detection of bfpA gene by PCR assay using universal primer.[7] DNA extraction was done by boiling lysis method. A final volume of 25μl of reaction mixture was prepared. All reaction mixture components (12.5μl of super mix, 5.0μl of template DNA, 2.5μl of each primer, 2.5μl of deionised water) were dispensed into PCR tube. The reaction mixture was labeled and stored at -20° C until used. Amplification was carried out under the following conditions: initial denaturation at 96^{0}C for 4 min; then 30 cycles of 20s at 94^{0}C, 20s at 55^{0}C and 10s at 70^{0}C and a final, prolong extention at 72^{0}C for 7 min. The amplified DNA products were resolved by 1% agarose gel electrophoresis and visualized by UV transllumination after ethidium bromide staining. Reference EPEC strain E2348/69 (kindly donated by icddr,b Dhaka, Bangladesh) was used as positive control and ATCC *Esch. coli* (25922) strain was used as negative control for bfpA gene detection by PCR.

Antimicrobial susceptibility test

Susceptibility of isolated EPEC strains to different antibiotics was determined by Kirby-Bauer disc-diffusion technique as specified by the National Committee for Clinical Laboratory Standards (NCCLS).[8] The antibiotic discs used in this study were Ampicillin (10 μg), ceftazidime (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), gentamycin (10 μg), imipenem (10 μg), levofloxacin (5 μg), nalidixic acid (30 μg), piperacillin tazobactum (110 μg), tetracycline (30 μg), cotrimoxazole (trimethoprim sulphamethoxazole) (25 μg).[5] All the antibiotic discs used for the study were obtained from Oxoid Ltd. Bashingstore Hampaire, UK.

Inoculum standardization

With a sterile wire loop 3-5 isolated colonies were transferred to a screw-capped tube containing 4 ml of sterile normal saline, turbidity of which was then adjusted to 0.5 McFarland turbidity standards.

Inoculation of test plate and disc placement

Within 15 minutes after standardization of inoculums, a sterile cotton swab was immersed into bacterial suspension. The swab was then streaked evenly on the surface of the plate in three different planes by rotating the plate to get a uniform distribution of inoculum. The inoculum was allowed to dry for 15 minutes at room temperature with lid closed. The antimicrobial discs were then placed on the inoculum surface by a sterile fine pointed forceps 10-15 mm away from the edge of the Petri dishes and 24 mm gap between the discs. The plates were incubated at 37° C for 24 hours.

Measurement of inhibition zone

After incubation, each plate was examined, and the diameter of complete inhibition zone was measured with the help of a scale placed on the undersurface of the Petri dish. Measurement of diameter in millimeter was made in two directions at right angle to each other through the centre of each disc and the average of the two readings was taken.

Interpretation of zone size

The zone of inhibition in growth produced by each antimicrobial agent on the test organisms were categorized into sensitive (S) and resistant (R) according to NCCLS.[8]

Results

All 272 stool samples yielded growth of *Esch. coli* in culture. All *Esch. coli* isolates (272) were subjected to PCR assay for the detection of bfpA gene. Table-1 shows that bfpA gene was detected in 20 *Esch. coli*

Table-1: *Isolation of bfpA gene positive EPEC from the patients with diarrhoea*

No of Sample	bfpA gene for EPEC	
	Positive N (%)	Negative N (%)
272	20 (7.4)	240 (92.6)

Note: *Three to five colonies of Esch. coli from each primary plate was taken and tested*

Table-2: *Antimicrobial susceptibility pattern of isolated EPEC strains*

Antimicrobial Agent	Susceptibility pattern	
	S No (%)	R No (%)
Ampicillin	0 (0)	20 (100)
Ceftazidime	19(95)	1(05)
Cefotaxime	18(90)	2(10)
Cefoxitin	16(80)	04(20)
Ceftriaxone	19(95)	01(05)
Chloramphenicol	11(55)	09(45)
Ciprofloxacin	12(60)	08(40)
Cotrimoxazole	16(80)	04(20)
Gentamycin	16(80)	04(20)
Imipenem	20(100)	00(00)
Levofloxacin	17(85)	03(15)
Nalidixic acid	1(05)	19(95)
Piperacillin tazobactum	12(60)	08(40)
Tetracycline	1(05)	19(95)

Note: *S- Sensitive, R-Resistant.*

strains out of 272 samples tested. These 20 strains in which bfpA gene was detected were identified as EPEC.

Table-II shows the antimicrobial susceptibility pattern of 20 EPEC strains isolated from patients. EPEC strains were highly susceptible to ceftazidime (95%), cefotaxime (90%), ceftriaxone (95%), imipenem (100%) and levofloxacin (85%). Isolated EPECs were highly resistant to ampicillin (100%), nalidixic acid (95%) and tetracycline (95%).

Discussion

Identification of EPEC is difficult for most clinical laboratories, due to lack of distinct phenotypic differences with non pathogenic *Esch. coli* strains which are present in stool as normal flora.[9] But the diagnosis of EPEC is important, as it is one of the important causes of infantile diarrhoea which needs antimicrobial treatment as has been reported by many studies in developing countries.[1,10]

A total of 272 samples either stool or rectal swab collected from the patients with diarrhoea were investigated. The EPEC strains identified on the basis of presence of bfpA gene detection by PCR. In this study *Esch. coli* were isolated from all specimens. All *Esch. coli* isolates were investigated by PCR to detect the presence of bfpA gene. The bfpA genes were identified in 20 (7.35%) *Esch. coli* isolates. Similar result was reported by Iman *et al.*, (2011).[11] Around 3.2% EPEC diarrhoea was reported from Thailand in 2004,[12] 6.6% from Vietnam in 2005,[13] 15.8% from India in 2008.[14] Lower isolation rate of EPEC in our study could be due the fact that only bfp A gene was detected by PCR and other genes like eaeA gene was not considered. Also, lower isolation rates might be due to inclusion of breast fed children. Breast milk (colostrum) from mothers living in endemic areas have been reported to contain high levels of immunoglobulin A (IgA) antibodies against the EPEC virulence factors. Other reasons could be increased awareness about food and hand hygiene, resulting from intensive education programs carried out by the media after H5N1 (Avian flu) and H1N1 (Swine flu) outbreaks in 2006 and 2008 respectively.[11] Another cause of lower isolation rate of EPEC in the present study was probablly for not detecting EPEC by serotyping as polyvalent and specific monovalent antisera for EPEC sero-groups were not available.

Acute or chronic enteritis due to EPEC is an emerging problem in many parts of the world. It has been estimated that 9.2 million deaths in the developing world have been caused by infectious diseases, and diarrhoeal diseases are the fourth most prevalent cause.[15] Most mild diarrhoea cases are successfully managed with oral rehydration therapy. Antimicrobial treatments are added only for more severe or persistent diarrhoeal cases. Ampicillin and co-trimoxazole have been recommended by the World Health Organization. Local information about antimicrobial resistance should be used in clinical management, and treatment guidelines should be updated.[16] In the present study, all EPEC strains were isolated from diarrhoeal patients.

The susceptibility test results showed that, most of the EPEC strains were multidrug resistance. EPEC strains were highly susceptible to ceftazidime (95%), cefotaxime (90%), ceftriaxone (95%), imipenem (100%), levofloxacin (85%). The most effective beta-lactam antibiotics were ceftazidime, ceftriaxone, imipenem and piperacillin-tazobactam. Such results may indicate that the isolated strains of EPEC were not extended-spectrum beta-lactamase producers, since resistance to third generation cephalosporin was not observed.[17] The results imply that the strains were

likely to have originated from the community, which supports the observation of low levels of resistance to such drugs.[18,19] Sensitivity was moderately high for gentamicin (80%) and piperacillin tazobactum (60%). Moreover, such antimicrobials are generally used in hospitals, and bacteria resistant to theses agents originating from the community are not expected.[20] Several studies in different parts of the world showed similar sensitivity pattern of EPEC.[5,21] Low levels of resistance against levofloxacin were observed in this study. The literature has reported varying rates of resistance against both levofloxacin and ciprofloxacin, which can be explained by the use of these drugs in some countries as a treatment for enteric infections.[22,23] However, studies to assess the role of these antimicrobial agents in the treatment of EPEC infections in children are needed. It has been shown that the treatment of diarrhoea caused by EPEC with antibiotics, specifically co-trimoxazole, decreases the duration and intensity of the diarrhoea.[24]

Overall, our results reinforce the importance of EPEC in the etiology of acute diarrhoea in children aged less than 5 years and its rapid detection by PCR. The high level of antimicrobial resistance observed in our study raises a broader discussion about the indiscriminate use or misuse of antibiotics and the risks of empirical antibiotic therapy in children of very young age.

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