J. Dhaka National Med. Coll. Hos. 2013; 19 (02): 32-35

Original article

Enteropathogenic *Escherichia coli* (EPEC) associated with diarrhea in children under 5 years of age.

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Abstract:

This study has been undertaken to investigate the isolation of EPEC strains associated with diarrhea in children under 5 years of age.Two hundred and seventy two samples from patients with diarrhoea were collected from two tertiary care hospital. *Esch. coli* was isolated and identified from all the 272 samples from the patients using standard microbiological techniques. EPEC strains were identified on the basis of presence of bundle forming pilus (*bfpA*) gene. Among 272 samples 20 (7.35%) isolates were identified as EPEC on the basis of presence of *bfpA* gene detected by polymerase chain reaction. Maximum *bfpA* gene carrying EPEC strains (10%) were identified from 0-12 months age group. Rapid and reliable detection of EPEC in children under 5 years of age is required for successful microbiological surveillance and for treatment of EPEC mediated diarrhoeal disease.

Keywords: EPEC, *bfpA* gene, PCR.

Introduction:

Enteropathogenic *Escherichia coli* (EPEC), is a major cause of infantile diarrhoea among children in developing countries.[1] EPEC strains can colonise the intestine and cause attaching and effacing (A/E) lesions characterized by localized destruction of brush border microvilli, intimate attachment of the bacterium to the cell membrane and formation of an underlying pedestal-like structure of polymerized actin in the host cell.[2] The A/E lesion begins with the presence of adhesin, named bundle-forming pilus (BFP). The major structural subunit of BFP is bundlin, a highly polymorphic protein encoded by *bfpA* gene.[3] Detection of *bfpA* gene is more useful for diagnosis of EPEC diarrhea as the *bfpA* gene has a defined role. In Bangladesh rate of isolation of EPEC was 23% in 1985,[4] 15.5% in 1995,[5] 16% in 2000.[6] Diagnosis of EPEC infection needs serogrouping. But serogroupings of *Esch. coli* used for differentiating diarrhoeagenic *Esch. coli* are costly and time-consuming. Compared with the conventional assays such as serotyping, detection of EPEC by polymerase chain reaction (PCR) method is proved to be simpler, more rapid and less expensive.Therefore this method may be applied for the diagnosis of EPEC diarrhea.[7] EPEC was identified from fecal specimens as a unique pathogen or associated with other pathogens in acute and chronic diarrhea in children. This study was done to evaluate the application of a PCR based test to differentiate *Esch.* coli isolates and determine their distribution among children under 5 years of age.

Materials and Methods:

Specimen was either stool or rectal swab (R/S). Samples were collected from Sir Salimullah Medical college and Mitford Hospital (SSMC & MH) and from Dhaka Shishu Hospital (DSH). From SSMC & MH 174 samples and from DSH 98 specimens were taken.

Patients: Stool specimens were collected from 272 patients, under 5 years of age, presenting with acute diarrhea and who had not taken antibiotic during the last 30 days.[5]

Microscopic Examination(M/E) of Stool

All stool specimens were examined under microscope within 1 hour of collection for any ova, cysts, pus cells, RBCs and macrophages.[4]

Culture of specimen:

All the specimens were inoculated onto MacConkey's agar media as early as possible. The plates were incubated aerobically at 37°c for 24-48 hours. After overnight incubation, all the organisms were identified by their colony morphology, staining characters, motility and appropriate biochemical tests.[8] *Esch. coli* was confirmed by biochemical assays and 3-5 colonies from each sample grown on MacConkey agar media were subcultured into Trypticase soy agar (TSA) media separately dividing the media into compartments and incubated at 37^{0}C for 24 hrs.[9]

Detection of *bfpA* genes of EPEC by PCR assays:[10]

A total of 272 *Esch. coli* isolates from patients and 28 *Esch. coli* from controls were tested for bfpA gene of EPEC by PCR by using universal primer. A sweep of 3-5 colonies from TSA were suspended in 1 ml distilled water. DNA extraction was done by boiling lysis method. A final volume of 25μl of reaction mixture was prepared. Amplification was carried out under the following conditions: initial denaturation at 96^{0}C for 4 min; then 30 cycles of 20 s 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 strains E2348/69 was used as positive control which was kindly donated by icddr,b Dhaka, Bangladesh & ATCC E. coli (25922) strain was used as negative control for *bfpA* gene detection by PCR.

Results :

Table-I: Correlation of the culture with *bfpA* gene carrying EPEC by PCR.

No of Sample	Culture	No of organism	*bfpA* gene	
			Positive	Negative
272	Only *Esch. coli*	240	**20** (7.35%)	220
	Mixed growth of *Esch. coli* and *Shigella*	27	00(00%)	27
	Mixed growth of *Esch. coli* and *Salmonella*	05	00(00%)	05

All *Esch. coli* isolates (272) were All *Esch. coli* isolates (272) were subjected to PCR for detection of *bfpA* gene. In total, from 240(88.2%) samples, the only isolate was *Esch. coli. Shigella* spp. with *Esch. coli* were isolated from 27 (10%) samples and *Salmonella* spp. with *Esch. coli* were isolated from 05 (1.8%) samples. *Esch. coli* was the most frequently isolated bacteria (88.2%) (Table-I). *bfpA* gene was detected from 20 *Esch. coli* strains from the 240 samples where only *Esch. coli* had been isolated. These 20 strains from which *bfpA* gene had been detected were identified as EPEC. *bfpA* gene was not detected from any strains of *Esch. coli* where associated *Salmonella* or *Shigella* spp. were isolated (Table-I).

Table-II: *bfpA* gene positive EPEC among different age group of study population.

Age group Months	No of sample	*bfpA* gene positive
0-6	77	08(10.39)
7-12	95	10(10.53)
13-24	58	02(3.45)
25-60	42	00 (00)
Total	272(100)	**20(7.35)**

Note: Figure within parentheses indicate percentage.

Table-II depicts that, out of 20 detected EPEC strains highest (10) isolates were from 7-12 months age group (10.53%) followed by 8 strains which were isolated from 0-6 months age group (10.39%) and 2 strains those were isolated from 13-24 months age group (3.45%).

Discussion :

Identification of Enteropathogenic *Escherichia coli* (EPEC) in different age group 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.[11] But the diagnosis and characterization of EPEC in under 5 years of age is important, as it is one of the important causes of infantile diarrhea.[12]

Only *Esch. coli* were isolated from 240 samples. All *Esch. coli* isolates were investigated to find out 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).[13] These 20 strains were identified as EPEC strains. Around 3.2% EPEC diarrhea was reported from Thailand at 2004,[14] 6.6% from Vietnum at 2005,[15] 15.8% from India at 2008.[16]

No *bfpA* gene was detected from the diarrhoeal patients caused by *Esch. coli* which were association with

Shigella and *Salmonella* spp. Similar results had been reported by Gunzburg, *et al.*, (1995).[17]

Maximum *bfpA* gene carrying EPEC strains (10.53%) were identified from 7-12 months age group whereas 19.8% EPEC strains had been reported by Albert *et al.*, (1995), in the similar age group (Table-II). Their occurance decreases with an increase in age (Gonzalez *et al.*, 1997). [18]

Higher and lower isolation rates in different age might be due to inclusion and exclusion of breast fed children. The reasons of lower isolation rates of EPEC may be due to 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 and increased awareness about food hygiene and hand hygiene, resulting from intensive education programs carried out by the the media after H5N1 (Avian flu) and H1N1 (Swine flu) outbreaks in 2006 and 2008 (Iman *et al* ., 2011) respectively. [13]

Traditionally, diarrhoeagenic *Esch. coli* strains from stools are identified with the help of microscopic examination, serotyping and conventional culture methods. Polyvalent and monovalent antisera for serogroupings of diarrhoeagenic *Esch. coli* are very expensive in comparison to the costs needed for gene detection by PCR method. Moreover, these antisera are not easily available and serogrouping by 'O' antigens are not sufficient to identify EPEC strains. The determination of virulence factors are needed to identify the EPEC strains in different age.[19] The main virulence characteristics of EPEC strains depend on *bfpA* gene. Though the diagnosis of EPEC infections based on tissue culture assay is the best method, but the facility of this investigations are not easily available. On the other hand diagnosis of EPEC strains by gene detection method gives more accurate results and it is less time consuming than other methods.[9] Therefore gene detection by PCR can be a effective method for diagnosis of EPEC diarrhoea in children under 5 years of age.

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