

## Original Article

# Isolation and identification of Enteropathogenic Escherichia coli (EPEC) in paediatric diarrhoeal patients by detection of bfpA gene by PCR.

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### Abstract

This study has been undertaken to investigate the isolation and identification of EPEC strains from paediatric diarrhoeal patients. Total 300 samples were studied. Two hundred and seventy two samples from patients with diarrhoea and 28 samples from control children were collected from two tertiary care hospital. Esch. coli was isolated and identified from all the 300 samples including patient and control using standard microbiological techniques. EPEC strains were identified on the basis of presence of bundle forming pilus (bfpA) gene. Out of 272 samples from diarrhoeal patient only Esch. coli was isolated from 240 (88.2%) samples. Shigella spp. with Esch. coli were isolated from 27 (10%) specimens and Salmonella spp. with Esch. coli were isolated from 5 (1.8%) samples. Among 272 samples 20 (7.35%) isolates were identified as EPEC on the basis of presence of bfpA gene detected by polymerase chain reaction. EPEC strains were identified from those 240 samples, from which Esch. coli had been isolated only. No EPEC strain was identified from control children. Rapid and reliable detection of EPEC is required for successful microbiological surveillance and for treatment of EPEC mediated diarrhoeal disease. bfpA gene detection by polymerase chain reaction can be a appropriate method where facilities for polymerase chain reaction are available.

**Keywords:** EPEC, bfpA gene, PCR.

### Introduction:

Enteropathogenic Escherichia coli (EPEC), is a major cause of infantile diarrhoea among children in developing countries<sup>1</sup>. 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<sup>2</sup>. 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<sup>3</sup>. The bfpA gene sequenced from different EPEC strains shows a high degree of homology and appear to be specific for EPEC<sup>1</sup>. 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, 15.5% in 1995, 16% in 2000<sup>4,5,6</sup>. In developing countries, no attempt is usually made for characterizing Esch. coli strains during routine microbiology reporting. 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<sup>7</sup>. A few study on EPEC has been carried out in Bangladesh. Identificataion of bfpA gene of EPEC by PCR. can identify EPEC strains. Because of the costly and laboratory intensive diagnostic procedures, the diagnosis of EPEC infection remain obscure in many parts of the world. This study was done to evaluate the application of a PCR based test to differentiate Esch. coli isolates and determine their distribution among children with or without diarrhea.

### Materials And Methods:

Specimen was either stool or rectal swab (R/S). Samples were collected from Sir Salimullah Medical college and Mitford

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Hospital (SSMC & MH) and from Dhaka Shishu Hospital (DSH). From SSMC & MH 194 samples and from DSH 106 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<sup>5</sup>.

**Controls:** Twenty eight samples were collected from age matched controls children who had no complaints of diarrhoea during the previous month but had admitted to hospital for other clinical entities and from the children who did not received antibiotic therapy in the preceding 30 days<sup>6</sup>.

#### 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<sup>4</sup>.

#### 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<sup>8</sup>. 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°C for 24 hrs<sup>9</sup>.

#### Detection of bfpA genes of EPEC by PCR assays<sup>10</sup>:

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. 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°C for 4 min; then 30 cycles of 20 s at 94°C, 20s at 55°C and 10s at 70°C and a final, prolong extention at 72°C for 7 min. The amplified DNA products were resolved by 1% agarose gel electrophoresis and visualized by UV transillumination after ethidium bromide staining. Reference strains E2348/69 was used as positive control which was kindly donated by icddr, Dhaka, Bangladesh & ATCC E. coli (25922) strain was used as negative control for bfpA gene detection by PCR.

#### Results

Esch. coli. was isolated from all the samples. Out of 174 samples from SSMC & MH, only isolate was Esch. coli from 151(86.78%) specimens. Shigella spp. along with Esch. coli were isolated in 20(11.50%) samples and Salmonella spp. with Esch. coli were in 03(1.72%) specimens. Out of 98 samples from DSH, Esch. coli only was isolated in 89(90.82%) samples, Shigella spp. with Esch. coli were in 07(7.14%) specimens and Salmonella spp. with Esch. coli were in 02(2.04%) specimens. 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).

All Esch. coli isolates (272) were subjected to PCR for detection of bfpA gene. 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-II). The amplified product was visualized at 367 bp by agarose gel electrophoresis (Figure-1).

Out of 28 age matched control, 20 (71.4%) stool samples were collected from SSMC & MH, and 08 (28.6%) from DSH. Esch. coli were isolated from all samples but bfpA gene was not identified from any one of them (Table-III)

**Table-I:** Organism isolated from patients with diarrhea.

Name of Hospital	Only Esch. coli and Shigella	Mixed growth of Esch. coli and Salmonella	Mixed growth of Esch. coli and	Total
SSMC & MH (n=174)	151	20	03	147
DSH (n=98)	89	07	02	98
Total (n=272)	240(88.2)	27(10)	05(1.8)	272(100)

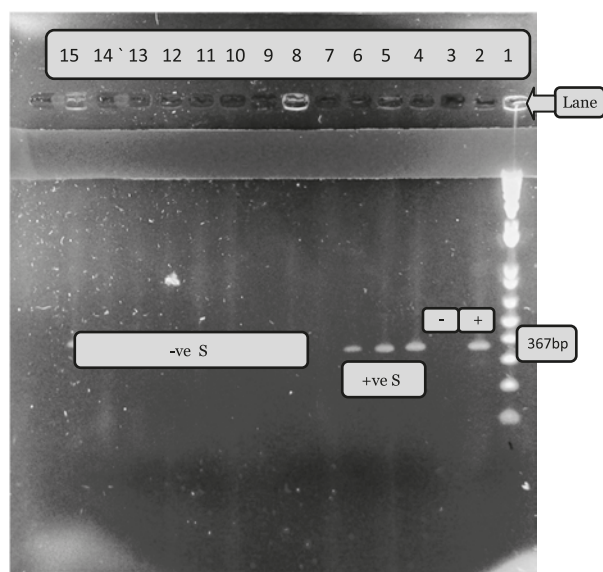
Note: Figure within parentheses indicate percentage.

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

No of Sample (n=272)	Culture	No of organism	bfpA gene	
			Positive	Negative
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

**Table-III:** Sources, frequency, and test results of control samples.

Name of Hospital	Number	Total	Culture	Presence or absence of bfpA gene
SSMC& MH	20	28	Esch.coli	Absent
DSH	08		Esch.coli	Absent

**Figure 1:** Agarose gel electrophoresis of PCR-amplified DNA fragments of bfpA genes in EPEC.

Lane:1, DNA molecular size marker (1 kb);  
 Lane:2, positive control;  
 Lane:3, negative control;  
 Lane:4-6, positive sample;  
 Lane:7-15, negative sample.

**Discussion:**

Identification of Enteropathogenic Escherichia coli (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<sup>11</sup>. But the diagnosis and characterization of EPEC is important, as it is one of the important causes of infantile diarrhea which needs antimicrobial treatment<sup>12</sup>.

Total 272 samples either stool or rectal swab collected from the patients with diarrhoea were investigated. Twenty eight stool samples were collected from age matched children as control. 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 as expected. From diarrhoeal stools solely Esch. coli were detected in 240 (88.2%) samples. Shigella spp. associated with Esch. coli were isolated from 27 (10%) samples and Salmonella spp. along with Esch. coli were isolated from 05 (1.8%) specimens. Similar types of isolations had been reported by Albert et al., (1995) from Bangladesh. Svenungsson et al., (2000) reported different percentages of Shigella (4%) and Salmonella (7%) in association with Esch. Coli<sup>10</sup>.

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)<sup>13</sup>. These 20 strains were identified as EPEC strains. Around 3.2% EPEC diarrhea was reported from Thailand (2004)<sup>14</sup>, 6.6% from Vietnam (2005)<sup>15</sup>, 15.8% from India (2008)<sup>16</sup>.

No bfpA gene was detected from the Esch. coli which were association with Shigella and Salmonella spp. Similar results had been reported by Gunzburg, et al., (1995). The bfpA gene was not identified from Esch. coli isolated from control stools. Similar results have been reported by Gunzburg, et al., (1995)<sup>17</sup>.

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. Because a single organism can contain different 'O' antigens and it can cross react with other organisms<sup>18</sup>.

The determination of virulence factors are needed to identify the EPEC strains<sup>19</sup>. 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<sup>9</sup>. Therefore gene detection by PCR can be a effective method for diagnosis of EPEC infection.

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