Enteropathogenic Escherichia coli Diarrhea in Hospitalized Children in Bangladesh

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The role of enteropathogenic Escherichia coli (EPEC) was evaluated in a group of children with endemic diarrhea admitted to Dhaka Shishu Hospital in Dacca, Bangladesh. EPEC was detected in fecal samples of 23% of 104 cases and 8% of 74 concurrent control children. The most commonly isolated EPEC strains were serogroups O20a, O20c:K61; O20a, O20b:K84; O26:K60; and O18a, O18c:K77. Except for O26:K60, these groups had not been reported from Bangladesh. On testing for enterotoxin production, only two strains (serogroups O26:K60, O18a, and O18c:K77) were enterotoxigenic. None was enteroinvasive as tested in the guinea pig conjunctivitis model. Our study supports the concept that EPEC may be an important cause of endemic diarrhea in Bangladesh.

Enteropathogenic Escherichia coli (EPEC) has been implicated as a cause of diarrhea and gastroenteritis in children (26). Several serogroups have been reported to have caused outbreaks of diarrheal diseases in different locations. Edwards and Ewing (5) identified 9 such OK groups of E. coli, and the World Health Organization International Escherichia Centre listed 15 O serogroups of E. coli as enteropathogenic to man (27). Sakazaki and co-workers (21, 22) identified 34 OK groups of E. coli as possible enteropathogens.

Because of the failure by many workers (6, 20) to demonstrate the production of enterotoxin by the classical EPEC strains, the pathogenic role of EPEC became controversial. Thus, for quite a long time (from the late 1960s to the late 1970s), few studies on EPEC diarrhea were done (31), and many laboratories throughout the world stopped serotyping E. coli isolated from diarrheal cases. However, recent experiments with animal models and human subjects have shown that EPEC can cause intestinal secretion and diarrhea (27). The mechanism of production of diarrhea by EPEC may be the production of an enterotoxin which is different from those produced by enterotoxigenic E. coli (14, 15) or a mechanism related to adhesion and colonization of the small intestine (2, 3, 29; A. Darfeuille, B. Lafeuille, B. Joly, and R. Cluzel, Trop. Dis. Bull. 80:392, 1983).

A workshop on EPEC was held in 1982 at the National Institutes of Health, Bethesda, Md. Participants stated that EPEC had persisted as an important cause of sporadic diarrhea in developing countries. In their opinion, outbreaks of EPEC diarrhea appear to have stopped only because hospitals stopped serotyping (4). The Centers for Disease Control, Atlanta, Ga., currently recommends serotyping of E. coli isolated from all diarrheal outbreaks in which E. coli is suspected to be the cause (4).

Interest has been renewed in various parts of the world to restudy the problem of EPEC diarrhea. The present study was undertaken to study EPEC diarrhea in Bangladesh, where only one previous study was done, in 1966 (I. Huq and S. Rizvi, Abstr. 5th Annu. Med. Symp., Jinnah Post-Graduate Medical Center, Karachi, Pakistan, p. 41, 1966).

The EPEC strains were also tested for enterotoxin production and enteroinvasiveness to evaluate pathogenicity.

MATERIALS AND METHODS

A total of 104 infants and children up to the age of 8 years who were admitted to Dhaka Shishu Hospital with acute diarrhea were studied from October 1982 to September 1983. All patients admitted on Tuesday and Saturday (except holidays) from 7:30 a.m. to 2:00 p.m. who had not taken antibiotics but had diarrhea were chosen for the study. Seventy-four age- and sex-matched concurrent healthy children who had had no diarrheal complaints during the previous month were included in the study as controls.

Stool examination. Two rectal swabs and a stool specimen were collected from every patient. All stool specimens were examined under the microscope within 1 h of collection for any ova, cysts, pus cells, erythrocytes, and macrophages. Immediately after collection, one rectal swab was inoculated onto MacConkey agar, salmonella-shigella agar, and Monsur’s medium for the isolation of E. coli, salmonellae, shigellae, and vibrios. After inoculation, the swab was brought to the laboratory in Carry Blair medium and cultured in Skirrow’s medium to test for Campylobacter spp. (30). The second swab was kept in phosphate-buffered saline and stored at −20°C for the detection of rotavirus antigen by an enzyme-linked immunosorbent assay technique. Also found in this study but not reported here were campylobacter, shigella, vibrio, and rotavirus pathogens. Standard bacteriological techniques were used to isolate and identify E. coli (30).

From the primary MacConkey agar plate, five to six lactose-fermenting colonies morphologically resembling E. coli were stocked individually on a blood agar base slant. These colonies were biochemically confirmed as E. coli and then serogrouped with commercially prepared antisera (Difco Laboratories, Detroit, Mich.). E. coli giving positive agglutination with any of the five polyvalent antisera (Table 1) were subsequently grouped with the constituent monovalent antisera by slide agglutination.

The EPEC strains were tested for the production of heat-labile and heat-stable enterotoxins. Heat-labile enterotoxin was detected by the Chinese hamster ovary...
Table 1. Antiser used for grouping EPEC

<table>
<thead>
<tr>
<th>Type of antiserum</th>
<th>Polyvalent</th>
<th>Monovalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly A</td>
<td>O26:K60, O55:K59, O111:K58, O127a:K63</td>
<td></td>
</tr>
<tr>
<td>Poly B</td>
<td>O86a:K61, O119:K69, O124:K72, O125:K70, O126:K71, O128:K67</td>
<td></td>
</tr>
<tr>
<td>Poly C</td>
<td>O18a, O18c:K77; O20a, O20b:K84; O28:K73; O44:K74; O112a, O112c:K66</td>
<td></td>
</tr>
<tr>
<td>Poly D</td>
<td>Not available</td>
<td></td>
</tr>
<tr>
<td>Poly E</td>
<td>Not available</td>
<td></td>
</tr>
</tbody>
</table>

(CHO) cell assay (9, 12), and heat-stable enterotoxin was detected by the infant mouse assay (9). All the EPEC were then studied for invasiveness by the Sereny test (12, 16). A Shigella flexneri strain was used as a positive control, and a saline wash of a sterile blood agar plate was used as the negative control. Any sign of keratoconjunctivitis in the animals within 4 days was regarded as positive for invasiveness.

The Z test was applied for statistical analysis.

RESULTS

The mean age (± standard error) was 10 ± 2 months for the patients and 11 ± 2 months for the controls; 65% were younger than one year old, and 90% were younger than three years old. The sex ratio (male:female) was 1.4:1 and 2:1 for the patients and controls, respectively.

E. coli were isolated in 91 (87.50%) diarrheal and 73 (98.65%) control specimens. Because of overgrowth of Klebsiella, Enterobacter, and Citrobacter cells, no E. coli could be isolated from the rest of the specimens.

A total of 30 E. coli strains isolated from diarrheal specimens gave positive agglutination with polyvalent antisera (poly A, B, C, D, and E). As the monovalent antisera of poly D and poly E were not available and as poly D and E contain both EPEC and non-EPEC antisera, we could not group six E. coli strains which gave positive agglutination with poly D and E. Therefore, these six strains were excluded from the EPEC group. Thus, EPEC was isolated from 24 (23.1%) diarrheal cases and 6 (8.1%) controls. This difference in the rate of EPEC isolation in the test and control groups is statistically highly significant (P < 0.01).

Twelve EPEC serogroups were isolated from diarrheal cases, with serogroup O20a, O20c:K61 (33.3%) predominating (Table 2). Of 24 diarrheal patients from whom EPEC was isolated, 17 (70.8%) excreted organisms of a single classical serogroup; the remaining 7 patients (29.1%) excreted organisms of multiple classical serogroups in their stool.

Two of the EPEC strains produced enterotoxins. One strain, from serogroup O26:K60, produced heat-stable enterotoxin, and another, from serogroup O18a, O18c:K77, produced heat-labile enterotoxin. No EPEC strains were found to be enteroinvasive by the Sereny test.

DISCUSSION

EPEC is an important cause of infantile epidemic diarrhea and is a likely cause of endemic diarrhea (1, 18). Workers in Western countries have isolated EPEC in 7 to 30% of diarrheal cases (4, 10, 11, 25). Studies from India detected EPEC in 8 to 24% of diarrheal cases in children (7, 13, 19, 24, 26). The only study reported thus far in Bangladesh (formerly East Pakistan), in 1966, isolated EPEC in 6% of the diarrheal children compared with 0.4 to 0.6% of the controls (Huq and Rizvi, 5th Annu. Med. Symp., 1966).

In the present study, EPEC was isolated from 24 (23.1%) diarrheal patients compared with 6 (8.1%) controls. The difference in this rate of EPEC isolation in diarrheal and control groups is statistically highly significant (P < 0.01). From 8 of these 24 cases, no pathogen other than EPEC was isolated. These findings strongly implicate EPEC as the cause of diarrhea in the children. Our results are in agreement with those of others in India and other countries (10, 13, 17, 25). The only study conducted earlier in this country also indicated a significant difference in the rate of EPEC isolation in test and control groups. However, the rate of EPEC isolation in the present study was higher than that of the previous study (Huq and Rizvi, 5th Annu. Med. Symp., 1966). Variation in the rates of EPEC isolation in various studies conducted in the same locality at different times are not unlikely and have been reported by many authors (10, 13, 17, 19, 23, 25, 26).

There appears to be little similarity between the serogroups isolated in the present study and those isolated in 1966 (Huq and Rizvi, 5th Annu. Med. Symp., 1966). In 1966, only 7 EPEC serogroups were isolated, and among those O26:K60, O127:B8, and O128:B12 were the predominant strains. In the present study, 12 different EPEC serogroups were isolated, and serogroups O20a, O20c:K61; O20a, O20b:K84; O26:K60; and O18a, O18c:K77 were the predominant strains. This variation in the prevalence of specific EPEC serogroups in a particular locality is not unexpected in view of the long interval since the previous study. Similar observations were also made by others (19, 23, 26).

The controversy over the importance of EPEC is primarily limited to its occurrence in endemic community-acquired diarrhea. Most authorities agree that serotyping is a useful tool to identify diarrheagenic strains associated with epidemics, especially epidemics occurring in the hospital. However, the routine practice of serogrouping E. coli strains from sporadic cases has been questioned. Our study, showing a significant difference in isolation rates for patients and controls, suggests that EPEC causes community-acquired diarrhea in Bangladesh. These findings are similar to those of Toledo et al. from Brazil (28).

The use of slide agglutination with commercially available

Table 2. Serogroups of EPEC isolated from diarrheal children and their enterotoxin production

<table>
<thead>
<tr>
<th>EPEC serogroup</th>
<th>No. of isolates (%)</th>
<th>Type of toxin produced*</th>
</tr>
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<tbody>
<tr>
<td>O20a, O20c:K61</td>
<td>8 (33)</td>
<td>Nil</td>
</tr>
<tr>
<td>O20a, O20b:K84</td>
<td>5 (21)</td>
<td>Nil</td>
</tr>
<tr>
<td>O26:K60†</td>
<td>4 (17)</td>
<td>ST</td>
</tr>
<tr>
<td>O18a, O18c:K77</td>
<td>4 (17)</td>
<td>LT</td>
</tr>
<tr>
<td>O86a:K61</td>
<td>3 (13)</td>
<td>Nil</td>
</tr>
<tr>
<td>O125:K70</td>
<td>3 (13)</td>
<td>Nil</td>
</tr>
<tr>
<td>O31:K58</td>
<td>2 (8)</td>
<td>Nil</td>
</tr>
<tr>
<td>O55:K59</td>
<td>1 (4)</td>
<td>Nil</td>
</tr>
<tr>
<td>O124:K72</td>
<td>1 (4)</td>
<td>Nil</td>
</tr>
<tr>
<td>O126:K71</td>
<td>1 (4)</td>
<td>Nil</td>
</tr>
<tr>
<td>O127a:K63</td>
<td>1 (4)</td>
<td>Nil</td>
</tr>
<tr>
<td>O128:K67</td>
<td>1 (4)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

* ST, Heat stable; LT, heat labile.
† Only one of the four strains with these serogroups produced toxin.
antisera is not ideal for identifying EPEC. It is, however, the method most commonly used in clinical laboratories. More sensitive and specific simple methods are needed to identify EPEC; nevertheless, even with this slide agglutination procedure, clear differences in the isolation rate were observed.

Classical EPEC strains producing enterotoxin were reported by Goldschmidt and Dupont (8); however, most of their isolates did not produce enterotoxin. Likewise, two of the isolates found in this study produced enterotoxin, but the majority did not, as determined by the CHO or infant mouse assays.

We could not detect by the Sereny test any enteroinvasive E. coli in our study. Although serotype O124, a known enteroinvasive strain, was isolated, it proved to be noninvasive. The mechanism of EPEC diarrhea remains unclear. In general, our strains were neither enterotoxigenic nor invasive. We did not test for adhesive property or for production of shigalike toxin, both of which may be related to the mechanism of diarrhea. Further work is needed to define these mechanisms.

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LITERATURE CITED