TOXINS AND COLONIZATION FACTOR ANTIGENS OF ENTEROTOXIGENIC ESCHERICHIA COLI AMONG RESIDENTS OF JAKARTA, INDONESIA

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INTRODUCTION

Global mortality due to diarrhea among children less than five years of age during the period 1978–1987 has been estimated at 3.3 million deaths per year, with a case fatality rate of 0.3%.1 In Indonesia, diarrheal disease mortality rates have been similar, ranging from 1–18 per 1,000 children less than five years old, with the highest death rates among those younger than five years old, with the highest death rates among those younger than 12 months of age.2,3 While a number of enteropathogens have been associated with travelers’ diarrhea, colonization of the small intestinal mucosa by ETEC strains is mediated by antigenically specific fimbriae, also known as colonization factor antigens (CFA). The significance of this study arises from reports that active and passive immunization with ETEC strains harboring CFAs has previously been shown to induce protective immunity against diarrhea in animal models. The aim of this study was to determine toxin-associated CFAs of ETEC isolated from a diarrheal disease case-control study in Jakarta, Indonesia. Thirteen hundred and twenty-three diarrheic and control patients with lactose-fermenting colonies were screened by ganglioside GM1-ELISA for the LT/ST toxins. Two hundred and forty-six (19%) ETEC isolates identified by GM1-ELISA for the LT/ST toxins were screened for CFAs by Dot blot assay using monoclonal antibodies against CFA/I, II, and IV and against the putative colonization antigens (PCF) PCFO159, PCFO166, CS7, and CS17. Of the 246 ETEC isolates, 177 (72%) elaborated ST, 56 (23%) produced LT, while 13 (5%) elicited both the ST and LT toxins. CFA testing of the 246 ETEC isolates showed that 21 (8%) expressed CFA/I, 3 (1%) exhibited CFA/II, 14 (6%) elaborated CFA/IV, while 7 (3%) expressed PCFO159 and PCFO159 plus CS5. No CFAs or PCFs could be associated with 201 (82%) of the ETEC strains. This report documents the types of CFAs associated with ETEC strains in Jakarta, Indonesia. These data may help current research efforts on the development of CFA-based vaccines for humans against ETEC and provide additional information for future ETEC vaccine trials in Southeast Asia.

MATERIALS AND METHODS

Study population. Three hospitals (Harapan Kita, Friendship, and Sumber Waras) and two Pusat Kesehatan Masyarakat (PUSKESMAS) (Community Health Centers) in Ja-
overnight incubation at 37°C. MSB were subcultured onto MC and SS within 18-hr after inoculation into alkaline peptone water (APW) and mannitol selenite broth (MSB) for enrichment. Isolates from APW culture were used, including conventional screening sets of Kligler iron agar (KIA), motility-indole-ornithine (MIO) media, and sucrase semi-solid (SSS) agar. The presence of protozoan and helminthic parasites was determined by microscopic examination of fresh stools and 10% formalin concentration of specimens.22 Specimens stored at −20°C were screened for rotavirus antigen by Rotazyme enzyme immunoassay method (Abbott Laboratories, North Chicago, IL).

**Toxin and CFA determination.** Fecal specimens collected from patients were cultured on MacConkey agar plates for selection of *E. coli* isolates. After overnight culture, 5 *E. coli* lactose-fermenting colonies from each patient were picked and stored on nutrient agar stab cultures until toxin assay. No values between ETET cases and controls *P* < 0.05.

**RESULTS**

**Pathogens.** Of the 6,615 *E. coli* isolates processed from 1,323 patients, 246 patients (19%) were positive for ETET. Of the 246 ETET isolates, only 9 (4%) were positive for other pathogens (1 Shigella spp., 2 *Vibrio cholera*, 2 *Salmonella* spp.) (data not shown). No parasites or rotavirus were found.

**Toxins.** The distributions of toxins produced by the 246 ETET isolates are shown in Table 1. Both ST and LT toxins were produced by 5% of the isolates, and LT alone was produced by 23% of the isolates. Of the 1,169 symptomatic diarrhea cases in which ETET was isolated, 160 (13.7%) of the patient isolates were associated with ST, 53 (4.5%) of the patient isolates with LT, while 13 (1.1%) of the patient isolates were
attributed to both ST/LT. Of the 154 control isolates, 17 (11%) were associated with ST, 3 (2.0%) were associated with LT, while none of the ETEC from the controls were associated with both ST and LT (Table 1).

**CFA expression.** Of the 246 ETEC isolates, 21 (8.5%) expressed CFA/I, 14 (5.7%) elaborated CFA/II, 3 (1.2%) showed CFA/III, 3 (1.2%) expressed PCFO159 and CS5, while 4 (1.6%) elaborated PCFO166 only. Two hundred and one (82%) of the ETEC isolates showed no association with either CFA or PCs (Table 1).

**Association of toxins and CFAs.** The correlation between ST and LT expression with synthesis of CFA subtypes is illustrated in Table 1. As seen, a greater percentage of the ST/LT expression with synthesis of CFA subtypes is either CFAs or PCFs (Table 1).

The prevalence of ETEC isolated from the stool samples (ETEC)-associated diarrhea was 19% in children. This result is similar to that observed in a 1982 study of diarrhea among residents of Kebon Bawang, an urban, densely populated section of North Jakarta. In that study, ETEC was found in 16% of diarrheal cases identified by active community surveillance, with 50% of ETEC cases in children less than two years of age. The current study shows a high proportion of children with ETEC diarrhea; more than 50% in children 0–5 years and 15.4% of ETEC cases were observed among children who were 0–2 years of age. In Indonesia, diarrheal disease mortality rates have been similar, ranging from 1–18/1,000 in children <5 yrs, with the highest death rates among those less than 12 months of age. The current study shows that ETEC-associated diarrhea occurred most frequently among children less than 1 yr of age, supporting previously reported findings in Indonesia.

**DIFFUSION**

Twenty-three percent of the ETEC isolates from this study expressed LT toxin, with or without concomitant ST, a rate that was not similar to that in a community-based study in Kebon Bawang, Jakarta (71% of these strains produced LT). However, in studies done in Brazil and Bangladesh, LT-producing ETEC was seen in 25–52% of the cases.

The association of toxin production with severity of disease is relevant to the formulation and evaluation of vaccine can-

![Table 2a](image)

**Table 2a**

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr</th>
<th>&gt;1-5 yr</th>
<th>&gt;5-12 yr</th>
<th>&gt;12-25 yr</th>
<th>&gt;25-60 yr</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>ST</td>
<td>16 (29)</td>
<td>23 (41)</td>
<td>13 (24)</td>
<td>15 (27)</td>
<td>3 (27)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>LT</td>
<td>5 (9)</td>
<td>11 (20)</td>
<td>7 (13)</td>
<td>13 (24)</td>
<td>1 (9)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>1 (2)</td>
<td>4 (7)</td>
<td>3 (5)</td>
<td>–</td>
<td>2 (18)</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>56 (25)</td>
<td>55 (25)</td>
<td>11 (5)</td>
<td>15 (7)</td>
<td>83 (38)</td>
<td>220 (100)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr</th>
<th>&gt;1-5 yr</th>
<th>&gt;5-12 yr</th>
<th>&gt;12-25 yr</th>
<th>&gt;25-60 yr</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>ST</td>
<td>1 (20)</td>
<td>3 (60)</td>
<td>1 (33)</td>
<td>2 (33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LT</td>
<td>–</td>
<td>1 (20)</td>
<td>–</td>
<td>1 (33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ST/LT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>5 (25)</td>
<td>3 (15)</td>
<td>0 (0)</td>
<td>5 (25)</td>
<td>7 (35)</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

**Table 2b**

Control group: age/sex distribution of patients with enterotoxigenic *Escherichia coli* (ETEC)-associated diarrhea (%)

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr</th>
<th>&gt;1-5 yr</th>
<th>&gt;5-12 yr</th>
<th>&gt;12-25 yr</th>
<th>&gt;25-60 yr</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>ST</td>
<td>1 (20)</td>
<td>3 (60)</td>
<td>1 (33)</td>
<td>2 (33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LT</td>
<td>–</td>
<td>1 (20)</td>
<td>–</td>
<td>1 (33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ST/LT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>5 (25)</td>
<td>3 (15)</td>
<td>0 (0)</td>
<td>5 (25)</td>
<td>7 (35)</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

**Notes:**

ST = heat-stable toxin.

LT = heat-labile toxin.

F = female.

M = male.
**TOXIN AND COLONIZATION FACTORS OF ESCHERICHIA COLI**

**TABLE 3**

Clinical symptoms associated with enterotoxigenic Escherichia coli (ETEC) diarrhea patients (%)

<table>
<thead>
<tr>
<th>ETEC</th>
<th>No. of episodes in 24 hr</th>
<th>Loose</th>
<th>Watery</th>
<th>Mucus</th>
<th>Bloody</th>
<th>Vomiting</th>
<th>Nausea</th>
<th>Abd. cramps</th>
<th>Fever</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&lt;10</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>122</td>
<td>84 (47)</td>
<td>38 (21)</td>
<td>14 (8)</td>
<td>101 (57)</td>
<td>47 (27)</td>
<td>38 (21)</td>
<td>59 (33)</td>
<td>87 (49)</td>
</tr>
<tr>
<td>LT</td>
<td>46</td>
<td>43 (24)</td>
<td>3 (2)</td>
<td>7 (4)</td>
<td>37 (21)</td>
<td>13 (7)</td>
<td>11 (6)</td>
<td>18 (10)</td>
<td>13 (7)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>9</td>
<td>9 (5)</td>
<td>–</td>
<td>2 (1)</td>
<td>6 (3)</td>
<td>1 (1)</td>
<td>5 (3)</td>
<td>2 (1)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>177*</td>
<td>136 (76)</td>
<td>41 (23)</td>
<td>23 (13)</td>
<td>144 (81)</td>
<td>61 (35)</td>
<td>54 (30)</td>
<td>79 (44)</td>
<td>56 (31)</td>
</tr>
</tbody>
</table>

*Cases with complete clinical data.
ST = heat-stable toxin.
LT = heat-labile toxin.
ST/LT = heat-stable and heat-labile toxin.

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