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%. 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 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%. 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 of age.

Enterotoxigenic Escherichia coli (ETEC) poses a serious health problem among children and adults in developing countries. 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-enzyme-linked immunosorbent assay (GM1-ELISA) for heat-labile (LT) and heat-stable (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.

Abstract. Infection caused by enterotoxigenic Escherichia coli (ETEC) poses a serious health problem among children and adults in developing countries. 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-enzyme-linked immunosorbent assay (GM1-ELISA) for heat-labile (LT) and heat-stable (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. Samples were subcultured onto MacConkey (MC) and SS within 18 hr after inoculation into alkaline peptone water (APW) and procedures were used, including conventional screening sets of MSB isolates. V. cholerae, Salmonella, Shigella, Campylobacter, and Vibrio cholerae. Laboratory processing included streaking onto MacConkey agar (MC), Salmonella-Shigella (SS) agar, thiosulfate citrate bile salts sucrose agar (TCBS), Campylobacter blood agar plate (CBAP), as well as inoculation into alkaline peptone water (APW) and mannitol selenite broth (MSB) for enrichment. Isolates from MSB were subcultured onto MC and SS within 18 hr after overnight incubation at 37 °C for Salmonella spp. isolation. APW was subcultured onto TCBS for Vibrio spp. isolation. Campylobacter blood agar plates were incubated at 42 °C for 72 hr in a microaerophilic atmosphere. Cultures were examined for the following agents: Salmonella, Shigella, Campylobacter, Escherichia coli, and V. cholerae. Standard procedures were used, including conventional screening sets of KLIGLER iron agar (KIA) agar, motility-indole-ornithine (MIO) media, and sucrose semi-solid (SSS) agar. The presence of protozoa and helminthic parasites was determined by microscopic examination of fresh stools and 10% formalin concentration of specimens. 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 detection 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 toxigenicity of the isolates could be determined historically as further evidence of diarrhea illness. When diarrhea was reported, a stool specimen was obtained by the nurse. If diarrhea was serious, intravenous rehydration therapy was immediately instituted. Dehydration was diagnosed clinically based on the presence of dysentery, malaise, or decrease in skin turgor.

Within 2-hr of passage, the stool samples from ill and control cases were delivered to the NAMRU-2 microbiology laboratory where numbers were assigned.

**Bacterial strains and cultivation.** Although ETEC was the organism of primary interest, the study included standard microbiological methods to identify *Salmonella, Shigella, Campylobacter,* and *Vibrio cholerae.* Laboratory processing included streaking onto MacConkey agar (MC), Salmonella-Shigella (SS) agar, thiosulfate citrate bile salts sucrose agar (TCBS), Campylobacter blood agar plate (CBAP), as well as inoculation into alkaline peptone water (APW) and mannitol selenite broth (MSB) for enrichment. Isolates from MSB were subcultured onto MC and SS within 18 hr after overnight incubation at 37 °C for Salmonella spp. isolation. APW was subcultured onto TCBS for Vibrio spp. isolation. Campylobacter blood agar plates were incubated at 42 °C for 72 hr in a microaerophilic atmosphere. Cultures were examined for the following agents: Salmonella, Shigella, Campylobacter, Escherichia coli, and V. cholerae. Standard procedures were used, including conventional screening sets of KLIGLER iron agar (KIA) agar, motility-indole-ornithine (MIO) media, and sucrose semi-solid (SSS) agar. The presence of protozoa and helminthic parasites was determined by microscopic examination of fresh stools and 10% formalin concentration of specimens. Specimens stored at −20 °C were screened for rotavirus antigen by Rotazyme enzyme immunoassay method (Abbott Laboratories, North Chicago, IL).

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

**Toxins.** The distributions of toxins produced by the 246 ETEC isolates are shown in Table 1. Both ST and LT toxins were produced by 5% of the isolates, ST alone was produced by 72% of the isolates, and LT alone was produced by 23% of the isolates. Of the 1,169 symptomatic diarrhea cases in which ETEC 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 PCF (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 and LT ETEC detected was associated with CFA/I. There were not enough of the other CFA subtypes to derive any meaningful correlation.

Age and sex distribution of LT- and ST-synthesizing ETEC isolates. Age and sex of 240 of the 246 patients from whom ETEC strains were isolated were available for analysis and are illustrated in Table 2a and 2b. As seen, a high proportion of the ST (43%, 74/172), LT (69%, 38/55), and ST/LT (61%, 8/13) synthesizing ETEC isolates were from patients <5 yrs of age, although 50% of the ETEC was identified from children 0–5 years of age. There did not appear to be any sex difference in the frequency of ST- and ST/LT-synthesizing ETEC isolates across the various ages of the patients. However there did appear to be approximately twice as many males with LT-only ETEC isolates (15% in males and 8% in females). The reason for this male predominance for LT-synthesizing ETEC is not clear at present, and it is possible that the sample size may not be large enough to derive conclusions from such a finding. It should be noted that there were 131 as compared to 109 cases of ETEC from males versus females which could reflect the LT data described above.

Complete clinical data available for 177 cases with ETEC were evaluated for symptoms. Of the 177 ETEC cases, 41 (23%) experienced more than 10 episodes of diarrhea in a 24 hr period. Overall, watery diarrhea was the most frequently reported clinical finding in this study, occurring in 144 (81%) of the ETEC cases. It should be noted that as many as 57% of the watery-diarrhea cases were caused by ST only. Abdominal pain was the next most commonly reported symptom with 118 (67%) of the patients reporting this finding, followed by vomiting in 79 (45%) cases (Table 3).

**DISCUSSION**

The prevalence of ETEC isolated from the stool samples of diarrheic patients was 19%. 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.

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**

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr n = 36</th>
<th>&gt;1-5 yr n = 35</th>
<th>&gt;5-12 yr n = 11</th>
<th>&gt;12-25 yr n = 15</th>
<th>&gt;25-60 yr n = 83</th>
<th>Total n = 220</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>16 (29)</td>
<td>13 (24)</td>
<td>3 (27)</td>
<td>5 (33)</td>
<td>41 (49)</td>
<td>78 (35)</td>
</tr>
<tr>
<td>LT</td>
<td>5 (9)</td>
<td>7 (13)</td>
<td>1 (9)</td>
<td>2 (13)</td>
<td>3 (4)</td>
<td>18 (8)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>1 (2)</td>
<td>4 (7)</td>
<td>3 (5)</td>
<td>2 (18)</td>
<td>2 (2)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Total no. (%)</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>
</tbody>
</table>

**Table 2b**

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr n = 36</th>
<th>&gt;1-5 yr n = 35</th>
<th>&gt;5-12 yr n = 11</th>
<th>&gt;12-25 yr n = 15</th>
<th>&gt;25-60 yr n = 83</th>
<th>Total n = 220</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>1 (20)</td>
<td>1 (33)</td>
<td>2 (33)</td>
<td>–</td>
<td>1 (20)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>LT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 (20)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total no. (%)</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>

ST = heat-stable toxin.
LT = heat-labile toxin.
F = female.
M = male.
TOXIN AND COLONIZATION FACTORS OF ESCHERICHIA COLI

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

<table>
<thead>
<tr>
<th>ETEC</th>
<th>No. episodes of diarrhea 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>
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<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>38 (21)</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>5 (3)</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.

LT = heat-labile toxin.
ST/LT = heat-stable and heat-labile toxin.

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Disclaimer: The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the U.S. Navy, Department of Defense, or Department of Human and Health Services, nor of the Ministry of Health, Republic of Indonesia.

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