Characterization of Enteropathogenic and Enteroaggregative Escherichia coli Isolated from Diarrheal Outbreaks

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There are six categories of Escherichia coli that cause diarrhea: enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli, enteroaggregative E. coli (EAggEC), enteroinvasive E. coli, and diffusely adherent E. coli (21). EPEC causes characteristic attaching-and-effacing lesions (A/E), which can be observed by intestinal biopsy in both human patient (19) and animal (29) models. A/E is characterized by loss of microvilli, intimate adherence of bacteria between epithelial cell membranes (27, 30), and cytoskeletal changes such as actin polymerization directly beneath the adherent bacteria (15). Generally, EPEC causes infantile diarrhea in developing countries and sporadic diarrhea in developed countries (21). EAggEC, on the other hand, is an enteric pathogen defined by its distinctive aggregative or “stacked-brick” pattern of adherence to cultured human epithelial cells (22). EAggEC associates mainly with persistent diarrhea in developing countries (21). Only two reports in Japan have described diarrheal outbreaks caused by EAggEC or EPEC. Itoh et al. (11) reported the isolation of EAggEC from the stools of patients with severe diarrhea in elementary and junior high schools. Makino et al. (18) reported the isolation of EPEC from a mass outbreak. In this paper, we describe three cases of diarrheal outbreaks in Japan that might be significantly greater than is currently appreciated.

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Chromosomal DNA-embedded agarose plugs for pulsed-field gel electrophoresis (PFGE) analysis were prepared by using the CHEF Bacterial DNA Plug Kit (Bio-Rad, Hercules, Calif.) and were digested with XbaI (Nippon gene; Osaka, Japan) at a concentration of 30 U/plug for 4 h at 37°C. The plugs were applied to a 1% PFC Grade Agarose (Bio-Rad) gel. Electrophoresis was performed in 0.5× Tris-Borate EDTA buffer at 14°C using a CHEF DR-II PFGE apparatus (Bio-Rad) under the following conditions: voltage, 6 V/cm; block 1, 11 h, with initial switching time of 4 s to final switching time of 8 s; block 2, 9 h, with initial switching time of 8 s to final switching time of 50 s. The HEP-2 cell assay was performed following the method described by Craviotto et al. (4), with modifications involving 3 or 6 h of incubation (15). The E. coli isolates were examined for the presence of the following virulence genes by PCR: stx1 (Shiga toxin) and stx2 (16), eaE (E. coli attaching and effacing) (12), bfpA (bundle-forming pilus) (9), perA (EPEC plasmid-encoded regulatory region) (8), astA (EAggEC heat-stable enterotoxin) (28), aggR (transcriptional activator for EAggEC aggregative adherence fimbria I expression) (20), and pet (EAggEC plasmid-encoded heat-labile toxin) (6) using the primers listed in Table 1. EPEC E2348/69 and EAggEC 17-2 were kindly provided by James B. Kaper, and EAggEC 042 was kindly provided by James P. Nataro, University of Maryland School of Medicine, Baltimore, Md.

The diarrheal patients were junior high school students in case 1, adults who attended a party in case 2, and infants of a day care center in case 3. The only diarrheagenic bacterial pathogens isolated from the patients were three E. coli O126:NM isolates from four of nine patients in case 1, nine E. coli O111:NM isolates from 9 of 21 patients in case 2, and four E. coli O126:NM isolates from four of four patients in case 3. As shown in Fig. 1, E. coli strains isolated within the same case showed identical PFGE patterns, suggesting that the strains originated from the common infectious sources in the respective cases. These results indicated that these E. coli strains were the causative agents of the diarrheal outbreak cases. As shown in Fig. 2, E. coli O55:NM possessed eaeA and showed a localized HEP-2 cell adherence pattern only in the 6-h assays but was negative for bfpA and perA, indicating that E. coli O55:NM is a murine virulent EPEC. The E. coli O55:NM isolate was negative for aggR, astA, and pet. On the other hand, both E. coli O126:NM and O111:NM strains were negative for the eaeA, bfpA, and perA genes but positive for the aggR and astA genes and showed an aggregated HEP-2 cell adherence pattern, indicating that they should be classified as EAggEC, displaying features of the traditional EPEC serotypes. The E. coli O126:NM was pet positive, while E. coli O111:NM was negative for this gene. All three E. coli isolates were negative for both stx1 and stx2 genes.

At the Second International Symposium on EPEC (13), a consensus on the basic characteristics of EPEC infection was
reached, identifying them as the presence of A/E histopathology and the absence of Shiga toxin. The A/E phenotype is closely related to the localized adherence phenomenon displayed by EPEC (14). DNA probes and PCR primers have been developed and used for the evaluation of the three major characteristics of EPEC: A/E (12), the presence of a ca. 60-MDa plasmid designated EPEC adherence factor plasmid (EAF) (23), and lack of Shiga toxin (16). Some EPEC strains possess EAF-encoding bundle-forming pilus (BFP) (5). Typical EPEC strains possess the eaeA for A/E and the EAF or bfpA, while atypical EPEC strains possess the eaeA gene only, and there is some controversy over whether atypical EPEC strains are true diarrheagenic pathogens (13). On the other hand, EAggEC infection is diagnosed definitively by isolation from the stools of patients of E. coli showing the aggregative HEp-2 cell adherence pattern (21). EAggEC strains possess the aggA gene that encodes the aggregative adherent fimbria I (AAF/I) protein (24), the aggR gene for transcriptional activation of AAF/I expression (20), and the astA gene that encodes the enteroaggregative E. coli heat-stable enterotoxin I protein (28). In this study, we examined E. coli isolated from patients with diarrhea from three outbreak cases. Serotyping of E. coli isolates showed the pathogenic strains to be O55:NM, O111:NM, and O126:NM, representing traditional EPEC serotypes. Based on phenotypic and genotypic tests, the O55:NM strain was identified as an atypical EPEC; it showed localized adher-

### TABLE 1. PCR primers used in this study

<table>
<thead>
<tr>
<th>Designation</th>
<th>Location</th>
<th>Sequence (5' to 3')</th>
<th>Target gene</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>213–230</td>
<td>AGT-TAA-TGT-GGT-GGC-GAA</td>
<td>stx1</td>
<td>817</td>
<td>16</td>
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<tr>
<td>V5</td>
<td>1013–1029</td>
<td>GAC-TCT-TCC-ATC-TGC-TCC</td>
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<tr>
<td>V3</td>
<td>289–306</td>
<td>TTC-GGT-ATC-CTA-TTC-CCG</td>
<td>stx2</td>
<td>474</td>
<td>16</td>
</tr>
<tr>
<td>V4</td>
<td>745–762</td>
<td>TCT-CTG-GTC-ATT-GTA-TTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA-1</td>
<td>1846–1865</td>
<td>AAA-CAG-GTG-AAA-CTG-TTG-CC</td>
<td>easA</td>
<td>454</td>
<td>This study</td>
</tr>
<tr>
<td>EA-2</td>
<td>2280–2299</td>
<td>CTC-TGC-AGA-ACC-TGC-TGC</td>
<td>easA</td>
<td>454</td>
<td>This study</td>
</tr>
<tr>
<td>EP-1</td>
<td>2773–2793</td>
<td>AAT-GGT-GCT-TTC-CTG-TGC</td>
<td>bfpA</td>
<td>324</td>
<td>9</td>
</tr>
<tr>
<td>PerAS</td>
<td>522–541</td>
<td>TGT-CAT-CTG-CTG-TTC-CTT</td>
<td>perA</td>
<td>354</td>
<td>This study</td>
</tr>
<tr>
<td>PerAAS</td>
<td>856–875</td>
<td>GGC-AAT-GTT-CCT-TTG-CTT</td>
<td>perA</td>
<td>354</td>
<td>This study</td>
</tr>
<tr>
<td>EAST-1S</td>
<td>63–82</td>
<td>GCC-ATC-AAC-ACA-GTA-TAT</td>
<td>astA</td>
<td>106</td>
<td>This study</td>
</tr>
<tr>
<td>EAST-1AS</td>
<td>149–168</td>
<td>GAG-TGG-GCT-TGG-GTG-CC</td>
<td>astA</td>
<td>106</td>
<td>This study</td>
</tr>
<tr>
<td>AggRks1</td>
<td>100–120</td>
<td>GTA-TAC-ACA-GAA-GAA-GGA</td>
<td>aggR</td>
<td>254</td>
<td>26</td>
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<tr>
<td>AggRks2</td>
<td>353–334</td>
<td>ACA-GAA-CTG-TCA-GCA-TCA</td>
<td>aggR</td>
<td>254</td>
<td>26</td>
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<tr>
<td>PetS</td>
<td>557–576</td>
<td>TCA-TTT-CCA-GCA-CCT-CCT</td>
<td>pet</td>
<td>442</td>
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<td>PetAS</td>
<td>979–998</td>
<td>CTC-CGA-CAG-TAT-TTC-GTA</td>
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![FIG. 1. PFGE patterns of the diarrheal isolates. Lanes: M, lambda molecular weight ladder; 1 to 3 (case 1, E. coli O126:NM); 4 to 12 (case 2, O111:NM isolates); 13 to 16 (case 3, O55:NM isolates); A to E (sporadic isolates of respective organisms).](image-url)
ence and possessed the eaeA without the EAF. However, E. coli isolates from the other two cases were identified as EAggEC, because they showed aggregative adherence and possessed astA and aggR but not eaeA or EAF. In general, EPEC infection is primarily a disease of infants younger than two years old (17), and EAggEC is associated with persistent diarrhea (1). Several outbreaks of diarrhea due to EPEC have been reported in the United States, the United Kingdom, Finland, and other developed countries. These outbreaks frequently occur in day care centers (2, 25) and occasionally occur in pediatric wards (10). However, reports of outbreaks due to atypical EPEC are infrequent. Recently, Hedberg et al. (10) reported an outbreak caused by an atypical EPEC among adults who ate at a gourmet buffet in the United States. This atypical EPEC strain was unique, because its serotype was O39:NM, which did not belong to any of the traditional EPEC serotypes, and it was positive for astA along with eaeA but negative for the EAF. Our present data, along with Hedberg’s observation, suggest that atypical EPEC is a diarrheic pathogen. Furthermore, the number of reports describing outbreaks due to EAggEC is increasing (3, 7). In Japan, there are only two reports describing outbreaks of diarrhea involving EPEC and EAggEC (11, 18). Based on our cases and the two reports just mentioned, the contribution of EPEC and EAggEC to the human disease burden in Japan might be significantly greater than is currently appreciated.

REFERENCES


22. Nataro, J. P., J. B. Kaper, R. Robins-Browne, V. Prado, P. Vial, and M. M.


