Enteroaggregative *Escherichia coli* Strains as a Cause of Traveler’s Diarrhea: A Case-Control Study

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To elucidate the importance of enteroaggregative *Escherichia coli* (EAggEC) strains as a cause of traveler’s diarrhea in Spanish travelers, a prospective case-control 1:1 study was done in a university hospital clinic for travelers. EAggEC strains were isolated from 23 of 165 case-patients and from 4 of 165 controls ($P = .0003$). In 16 patients, this was the only isolate recovered. Six of the EAggEC-positive isolates from the case-patients and 2 from the controls were positive for the enteroaggregative stable toxin type 1 gene. Other enteropathogens were also isolated. *Shigella* and enterotoxigenic *E. coli* strains showed significant differences between cases and controls ($P = .0023$ and $P < .0001$, respectively). Geographic distribution of the EAggEC strains was homogeneous, and the clinical symptom, secretory diarrhea, did not differ statistically with that for the enterotoxigenic *E. coli* strains. EAggEC strains are a cause of secretory diarrhea in Spaniards traveling to developing countries.

Enteroadherent *Escherichia coli* strains have been involved in the etiology of traveler’s diarrhea in North American travelers to Mexico [1]. Nataro et al. [2] have described a new *E. coli* adherence pattern, called aggregative adherence, in Hep-2 cells. Strains with aggregative adherence have also been isolated in children with diarrhea in developing countries [3, 4]. In some studies, however, the association between the presence of enteroaggregative *E. coli* (EAggEC) strains in stools and diarrhea was not proved [5, 6]. The current prospective case-control study was done to determine the role of EAggEC strains as a cause of traveler’s diarrhea in Spanish travelers to developing countries.

**Patients, Materials, and Methods**

**Patients.** Case-patients were recruited from the traveler’s clinic of the Tropical Medicine Department of the Hospital Clinic, University of Barcelona, from May 1996 to October 1996. Patients were defined as travelers who had diarrhea between 12 h after arriving in and 5 days after departing from a country that they had visited for <3 months. Diarrhea was defined as three or more episodes of watery stools within a 24-h period, with or without other symptoms, or as the occurrence of unformed stools accompanied by vomiting, nausea, tenesmus, fever, abdominal cramps, chills, or prostration.

Controls were recruited on a voluntary basis among relatives or among travel companions of the case-patients. Controls were defined as travelers who did not have diarrhea during or after a short trip (at least 7 days).

Cases and controls were matched by the area visited, and all subjects filled out an epidemiologic questionnaire including questions about the subject’s name, sex, age, trip duration, area visited, alimentary risk factors for diarrheal diseases, and sleeping facili-
ties. In addition, the clinical history of the patients with diarrhea was obtained, and a physical examination was done.

Stool samples from cases and controls were sent to the Laboratory of Microbiology and processed for bacterial and parasitologic studies. The laboratory technicians had no knowledge of whether the samples were from cases or controls. The treatment of patients with diarrhea was based on results of the clinical and microbiologic tests.

**Bacteriologic studies.** All stool samples were inoculated on blood agar, *Salmonella-Shigella* agar, MacConkey agar, cefsulodin-irgasan-novobiocin (CIN) agar, and thiosulfate-citrate-bile salts-sucrose agar. These media were incubated at 37°C for 24–48 h. For *Salmonella* enrichment, feces were inoculated in selenite F broth, incubated at 37°C for 18 h, and subcultured on *Salmonella-Shigella* agar. All plates were examined, and colonies suspected of corresponding to enteropathogenic bacteria were identified by use of standard microbiologic methods and commercial antisera. To isolate *Yersinia* species, CIN medium was used, and we also inoculated the stools into 10 mL of tryptose broth, which was incubated for 3 weeks at 4°C and then subcultured on CIN agar. *Campylobacter* blood-free medium was used to isolate *Campylobacter* species; it was incubated for 48 h at 42°C under microaerophilic conditions.

All suspected colonies of *E. coli* were screened biochemically and tested with polyvalent antisera in order to establish the serotype. If they presented a positive reaction with these polyvalent antisera, colonies were tested with monovalent antisera against the following groups: O111, O55, O26, O86, O119, O127, O125, O126, and O128.

We screened *E. coli* for enterohemorrhagic *E. coli* by plating the bacteria on sorbitol-MacConkey agar. All non–sorbitol-fermenting colonies were tested in an agglutinating assay with O157 and H7 antisera. Two colonies of each morphologically different type of *E. coli* strain were studied by polymerase chain reaction (PCR) to detect plasmid DNA for EAggEC (the ipah chromosomal gene for enteroinvasive *E. coli* strains), enteroaggregative stable toxin type 1 gene (EAST1), VT1 and VT2 genes for verotoxigenic *E. coli* strains (the eae and bfp genes and EAF for enteropathogenic *E. coli* [EPEC]). EPEC were considered positive when at least one of these characteristics was found.

The PCR technique described by Schmidt et al. [7] was used to detect EAggEC. To amplify the fragment of the *astA* gene, which codes for EAST1 from nt 132–241, we used two oligonucleotide primers that were designed on the basis of a nucleotide sequence described by Savarino et al [8], 5'-ATGCCATCACA-CAGTATAT-3' and 5'-GCCAGTGACGCTTTTGTAGT-3'. In brief, half a colony of each isolate was suspended in 25 μL of distilled sterile water and boiled for 10 min. After a short centrifugation (30 s to 13,000 g), 25 μL of a reaction mixture containing 20 mM Tris-HCl (pH 8.8), 100 mM KCl, 3.0 mM MgCl2, gelatin (1% wt/vol), 400 μM dNTPs, and 1 μM of the appropriate primers together with 2.5 U of Taq polymerase (Life Technologies Gibco BRL, Gaithersburg, MD) was added. The mixture was overlaid with oil and amplified using the following PCR program: 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min. Amplified DNA products were resolved by electrophoresis in agarose gels (2% wt/vol) containing 0.5 mg ethidium bromide/L.

**Parasitologic studies.** Fresh specimens were examined directly to view vegetative forms. The visualized amoebic cysts were confirmed by Hiedenhain staining. Stools were examined by a concentration method according to the merthiolate-iodine-formalin technique, and they were stained with Kinyoun carbolfuchsin [9].

**Statistical analysis.** The sample size was 165 cases and 165 controls. All data were introduced into a Dbase III Plus program (Ashton Tote, CA) and analyzed with a significance level of 5% by use of the Epi Info (version 6.04; CDC, Atlanta) and SPSS-Win (version 6.1.3; SPSS, Chicago) software programs. The statistical McNemar test was used.

**Results**

Of the 165 case-patients and 165 controls, 58.78% and 10.3%, respectively, had at least 1 enteropathogen. We found >1 enteropathogen in 19 case-patients, and 1 patient had 3 microorganisms. EAggEC strains were isolated from 23 cases and 4 controls (*P* = .0003). Other isolated enteropathogens (and the number of patients and controls, respectively, that harbored them) were ETEC (25, 3), *Shigella* species (12, 1), *Giardia lamblia* (11, 3), Entamoeba histolytica (5, 0), *Cyclospora cayetanensis* (6, 1), *Aeromonas hydrophila* (5, 0), enteroinvasive *E. coli* (5, 1), *Campylobacter* species (3, 1), EPEC (3, 2), *Salmonella* species (3, 0), *Vibrio fluvialis* and *Vibrio cholerae* (2, 0), *ETEC* (2, 1), *Blastocystis hominis* (2, 2), *Enterobius vermicularis* (2, 0), *Plesiomonas shigelloides* (1, 0), and *Yersinia* species (1, 1). Statistical results are shown in table 1. ETEC, EAggEC, and *Shigella* species were the 3 bacterial isolates with univarient statistical significance.

Several case-patients with these 3 bacterial isolates had a polymicrobial pattern: 3 in the ETEC group (2 EAggEC and 1 EAggEC-positive *V. fluvialis*), 7 in the EAggEC group (2 ETEC, 2 *cayetanensis*, 1 *Shigella* species, 1 *ETEC*, and 1 *ETEC*-positive *V. fluvialis*), and 5 in the *Shigella* group (2 *G. lamblia*, 1 EAggEC, 1 enteroinvasive *E. coli*, and 1 *P. shigelloides*).

The geographic distribution of EAggEC strains and other enteropathogens frequently isolated from case-patients is shown in table 2. The symptoms found among the 16 patients with only EAggEC strains were unformed stools (16 cases), abdominal cramps (9), borborygmi (8), vomiting (5), flatulence (5), tenesmus (5), and fever (3). Three patients had no symptom other than diarrhea. The EAggEC strain-related diarrhea was watery (13 cases), intermittent (7), chronic (>14 days in 5), and contained blood (2) and mucus (1). The clinical presentation and the duration of the diarrhea was not statistically significantly different than that for ETEC diarrhea.

None of the EAggEC strains were positive for the classic serotypes tested. Six of 23 EAggEC strains from the case-patients and 2 of 4 strains from the control group were EAST1 positive. Seven non-EAggEC strains from cases (1 VT1, 1 EPEC O126:B16, and 5 other nonpathogenic strains)
Table 1. Statistical results for the most frequently isolated enteropathogens from travelers.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>% positive isolates with diarrhea</th>
<th>% of cases with pathogen</th>
<th>% of controls with pathogen</th>
<th>P value* (95% CI) Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAggEC</td>
<td>85.2</td>
<td>13.9</td>
<td>2.4</td>
<td>.0003 5.75 (1.96–22.87)</td>
</tr>
<tr>
<td>Shigella species</td>
<td>92.3</td>
<td>7.3</td>
<td>0.6</td>
<td>.0023 12 (1.77–512.85)</td>
</tr>
<tr>
<td>ETEC</td>
<td>89.3</td>
<td>15.2</td>
<td>1.8</td>
<td>&lt;.0001 8.3 (2.54–43.11)</td>
</tr>
</tbody>
</table>

NOTE. EAggEC = enteroaggregative E. coli; ETEC = enterotoxigenic E. coli; CI = confidence interval.
* McNemar’s test.

and 5 non-EAggEC strains from controls were EAST1 positive.

Discussion

Statistical analysis showed that Shigella, EAggEC, and ETEC strains caused diarrhea. Results for other well-known enteropathogens, such of G. lamblia, E. histolytica, and C. cayetanensis, were not significant; a larger sample would be needed to evaluate them. Nonetheless, the aim of this study was to evaluate the potential of EAggEC strains for causing diarrhea, and the sample was, therefore, calculated for this purpose.

The control subject in whom C. cayetanensis was detected had no diarrhea but did present with upper abdominal pain.

In one study performed in travelers to Latin America, intestinal colonization by EAggEC strains was common (27.1% in a placebo group), but its association with diarrhea was low (3.2% in the placebo group) [10]. In our study, EAggEC strains were the second most frequently isolated enteropathogen among travelers with diarrhea (13.9%); however they were isolated in only 4 travelers without diarrhea (2.4%). These results are in agreement with other published studies [3, 4].

EAggEC strains were first described according to phenotypic characteristics (E. coli strains adhering to Hep-2 cells with an aggregative pattern) and a lack of toxin secretion. The mechanism of EAggEC diarrhea is poorly understood. EAggEC adhere poorly to jejunal mucosa but well to colonic mucosa, Peyer’s patches, and lymphoid and M cells [11]; some strains induce shortened villi in rabbit and rat intestinal loops, hemorrhagic necrosis of the villus tips, and a mild inflammatory response with edema and mononuclear infiltration of the submucosa [12]. In our travelers, the characteristics of the diarrhea seemed more secretory than inflammatory and were indistinguishable from ETEC diarrhea. These results agree with those of Nataro et al [13], who also described heterogeneity in the virulence factors of EAggEC strains.

In this study, 6 (26%) of 23 EAggEC strains from cases and 2 of 4 EAggEC strains from controls were EAST1 positive. These results show that even if most patients had had a secretory diarrhea, EAST1 could only have been responsible for 26% of the cases. On the other hand, the presence of EAST1 in 2 of 4 EAggEC strains from the control group means that the sole presence of EAST1 is not sufficient to develop diarrhea. Likewise, 7 non-EAggEC strains from cases and 5 nonpathogenic E. coli strains from controls were EAST1 positive. These results agree with a study in which the authors found positive hybridization with an EAST1 DNA probe for some enterohemorrhagic E. coli, ETEC, and EPEC strains, as well as E. coli strains from asymptomatic children [14]. There were no statist-

Table 2. Geographic distribution of isolates from case patients traveling to developing countries.

<table>
<thead>
<tr>
<th>Area</th>
<th>ETEC</th>
<th>EAggEC</th>
<th>Shigella species</th>
<th>Giardia lamblia</th>
<th>Entamoeba histolytica</th>
<th>Cyclospora cayetanensis</th>
<th>No. of patients tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Africa</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>West Africa</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Central Africa</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>South Africa</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mahgreb</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
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<td>Middle East</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Indian subcontinent</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>Central America</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td>South America</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>23</td>
<td>12</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>165</td>
</tr>
</tbody>
</table>

NOTE. ETEC = enterotoxigenic E. coli; EAggEC = enteroaggregative E. coli.
cally significant differences between EAST1-positive strains from cases and controls ($P = .15$). Further studies are necessary to determine whether other virulent factors play a role in the pathogenicity of the EAggEC strains.

In one study, there were no statistical differences in the clinical signs between patients with only EAST1-positive EAggEC strains and patients with only EAST1-negative EAggEC strains. Given the low numbers (3 and 13, respectively) in these groups, more studies are necessary to compare the clinical impact of these strains.

EAggEC strains were isolated from all geographic areas except East and South Africa and Maghreb. However, given the low numbers of patients from these areas, no geographic differences could be established regarding EAggEC.

EAggEC strains are described as a cause of persistent diarrhea in children in several tropical countries [15, 16]. Five of 23 EAggEC strains caused chronic diarrhea (>14 days), but statistical differences were not found for diarrhea duration between the ETEC, Shigella, and EAggEC strains. Our study differs from the above-mentioned studies (children coming from the same geographic area) in that patients were adult travelers coming from different ecologic areas.

Several studies have found links between some serotypes and the aggregative pattern. Strains belonging to the O44:H18 [17] serogroup are the main related group, as well as some strains belonging to O111 and O126 [18]. In one study analyzing 40 EAggEC strains [12], the authors found that 12 belonged to recognized serotypes and 15 were rough strains, and in 12 other strains, it was not possible to determine the serotype. In our study, none of the EAggEC strains was positive for the classic serotypes tested; however, it would be interesting in further studies to investigate the other serotypes in these strains from different geographic areas.

On the basis of this study, we conclude that EAggEC strains are a cause of secretory diarrhea in travelers to temperate and tropical areas. More studies are needed to investigate the pathogenesis of these EAggEC strains.

References


