Brief Report

The Epidemiological and Clinical Characteristics of Diarrhea Associated with Enteropathogenic, Enteroaggregative and Diffuse-Adherent Escherichia coli in Egyptian Children

by Salwa F. Ahmed,1 Hind I. Shaheen,1 Ibrahim Adib Abdel-Messih,1 Manal Mostafa,1 Shannon D. Putnam,1 Karim A. Kamal,1 Abdel Nasser El Sayed,2 Robert W. Frenck Jr,1 John W. Sanders,1 John D. Klena,1 and Thomas F. Wierzba1
1Research Science Directorate, US Naval Medical Research Unit No. 3, Cairo, Egypt
2Ministry of Health and Population, Cairo, Egypt

Correspondence: Salwa F. Ahmed, Commanding Officer, U.S. Naval Medical Research Unit No. 3 (NAMRU-3), PSC 452 Box 5000, code 302 FPO AE 09835-9998, USA. Tel: 011-20-2-2-342-1375. Fax: 011-20-2-2-342-1382. E-mail <salwa.fouad.eg@med.navy.mil>.

Summary

A total of 220 enteroadherent Escherichia coli were identified from 729 Egyptian children with diarrhea using the HEp-2 adherence assay. Enteropathogenic E.coli (EPEC = 38) was common among children <6 months old and provoked vomiting, while diffuse-adhering E.coli (DAEC = 109) induced diarrheal episodes of short duration, and enteroaggregative E.coli (EAEC = 73) induced mild non-persistent diarrhea. These results suggest that EPEC is associated with infantile diarrhea in Egyptian children.

Key words: enteroaggregative E.coli, enteropathogenic E.coli, diffuse-adherent E.coli, Egypt, diarrhea.

Three phenotypes of enteroadherent E.coli strains have been described based on mannose-resistant adherence patterns to HEp-2 cells [1]. Enteropathogenic E.coli (EPEC) display a localized adherence pattern (LA) [1], and have been previously associated with infantile diarrhea in developing countries [2]. The association of enteroaggregative E.coli (EAEC), which exhibit aggregative adherence (AA) and form a stacked brick-like arrangement [1], with pediatric diarrhea remains controversial due to their frequent isolation from control subjects [3–5]. Similar to EAEC, contradictory results have been reported [3, 6, 7] regarding the pathogenicity of diffuse-adhering E.coli (DAEC), which display a pattern of diffuse adherence (DA).

We conducted this study to assess the prevalence of these organisms among Egyptian children with diarrhea and study their clinical and epidemiological significance. Pediatric cases seeking care were evaluated at two Fever hospitals, located on the Nile River Delta, and southeast of Alexandria, Egypt [8]. At the pediatric clinic of each hospital, every fifth child <60 months of age presenting with dysentery (episode lasting <14 days with visible blood or mucus in the stool), acute (episode lasting <14 days without visible blood), or persistent diarrhea (episode with or without blood lasting >14 days) was selected for enrollment [8]. After obtaining written informed consent, a detailed clinical history and a stool specimen were collected [8]. From each diarrheal case, five putative E.coli colonies were tested by a ganglioside enzyme-linked immunosorbent assays (GM1-ELISA) for enterotoxigenic E.coli (ETEC) [8]. Three of the putative E.coli colonies, negative for either toxin were selected for the phenotypic identification of enteroadherent E.coli [9] using the HEp-2 cell adherence assay [2]. ETEC was isolated from 148 cases (20%), and no E.coli was recovered from 126
of the 729 children. Of the remaining 455 cases, we screened 1365 E. coli isolates using the HEp-2 assay. Adherence patterns were assigned based on the observation of three independent readers. Odds ratios were used to measure the strength of association between a selected pattern of adherence and an epidemiological or clinical characteristic [8].

Using HEp-2 assay, 73 cases (16%) yielded E. coli which was phenotypically indistinguishable from EAEC (Table 1). In 53 of these cases, EAEC was the sole pathogen identified. Thirty-eight children (8%) yielded E. coli phenotypically similar to enteropathogenic E. coli (EPEC). In 26 (6%) of these EPEC cases, no co-pathogen was identified. From 109 cases (24%), DAEC were recovered; 81 cases had this organism as the sole pathogen. Thirty-two cases displayed no specific pattern of adherence, while 10 cases resulted in the complete detachment of HEp-2 cells [7]. There were an additional 119 cases (26%) in which the E. coli did not adhere to HEp-2 cells, and 74 cases with mixed phenotypes [10].

Cases with enteric pathogens identified in the 729 diarrheal cases and which comprise the ‘others’ group (Tables 1 and 2) were excluded from analyses in case the pathogen coexists with any of the three patterns of adherence [8].

No demographic differences were noted among children with EAEC- vs. non–EAEC-associated diarrhea (Table 1). In the present study, EAEC did not appear to cause severe clinical symptoms. Less

![Table 1](image)

**Table 1**  
*Distribution of age, gender, season and study site for diarrhea associated with enteroaggregative (EAEC), enteropathogenic (EPEC) and diffuse-adherent E. coli (DAEC) among study children*

<table>
<thead>
<tr>
<th>Variables</th>
<th>EAEC (n = 53)</th>
<th>Non-EAEC (n = 607)</th>
<th>OR*</th>
<th>p-value</th>
<th>EPEC (n = 26)</th>
<th>Non-EPEC (n = 659)</th>
<th>OR*</th>
<th>p-value</th>
<th>DAEC (n = 81)</th>
<th>Non-DAEC (n = 552)</th>
<th>OR*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>10 (19%)</td>
<td>96 (16%)</td>
<td>1.4</td>
<td>0.4</td>
<td>9 (35%)</td>
<td>104 (16%)</td>
<td>3.0</td>
<td>0.04</td>
<td>14 (17%)</td>
<td>92 (17%)</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>6–11</td>
<td>22 (41%)</td>
<td>247 (46%)</td>
<td>1.1</td>
<td>0.8</td>
<td>9 (35%)</td>
<td>305 (46%)</td>
<td>1.1</td>
<td>0.9</td>
<td>38 (47%)</td>
<td>250 (45%)</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>12–59</td>
<td>21 (40%)</td>
<td>264 (43%)</td>
<td>1.0</td>
<td></td>
<td>8 (31%)</td>
<td>250 (38%)</td>
<td>1.0</td>
<td></td>
<td>29 (26%)</td>
<td>210 (29%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Warm season</td>
<td>37 (70%)</td>
<td>438 (72%)</td>
<td>1.2</td>
<td>0.6</td>
<td>21 (81%)</td>
<td>464 (70%)</td>
<td>1.3</td>
<td>0.6</td>
<td>60 (74%)</td>
<td>392 (71%)</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>22 (42%)</td>
<td>287 (47%)</td>
<td>1.0</td>
<td></td>
<td>7 (27%)</td>
<td>303 (46%)</td>
<td>0.4</td>
<td>0.1</td>
<td>39 (48%)</td>
<td>262 (47%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Abu-Homsb</td>
<td>25 (47%)</td>
<td>313 (52%)</td>
<td>1.1</td>
<td>0.8</td>
<td>17 (65%)</td>
<td>326 (49%)</td>
<td>0.5</td>
<td>0.1</td>
<td>43 (53%)</td>
<td>282 (51%)</td>
<td>1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Odds ratio adjusted for all other variables in the model using multivariate logistic regression, p-value.

**Table 2**  
*Adjusted ratios for the association between selected clinical characteristics of diarrhea and prevalence of enteroaggregative (EAEC), enteropathogenic (EPEC) and diffuse-adherent E. coli (DAEC) among study children*

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>EAEC (n = 53)</th>
<th>Non-EAEC (n = 607)</th>
<th>OR*</th>
<th>p-value</th>
<th>EPEC (n = 26)</th>
<th>Non-EPEC (n = 659)</th>
<th>OR*</th>
<th>p-value</th>
<th>DAEC (n = 81)</th>
<th>Non-DAEC (n = 552)</th>
<th>OR*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of stools</td>
<td></td>
<td></td>
<td>7 ± 4</td>
<td>8 ± 5</td>
<td>0.9, 0.02</td>
<td>7 ± 5</td>
<td>8 ± 5</td>
<td>1.0, 0.6</td>
<td>7 ± 4</td>
<td>7 ± 5</td>
<td>1.0, 0.8</td>
<td></td>
</tr>
<tr>
<td>Diarrhea days &gt; 4</td>
<td>14 (26%)</td>
<td>246 (41%)</td>
<td>0.5</td>
<td>0.03</td>
<td>9 (33%)</td>
<td>260 (40%)</td>
<td>0.6</td>
<td>0.3</td>
<td>22 (27%)</td>
<td>225 (41%)</td>
<td>0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Mucoid stools</td>
<td>39 (71%)</td>
<td>379 (63%)</td>
<td>1.7</td>
<td>0.1</td>
<td>22 (81%)</td>
<td>404 (64%)</td>
<td>1.9</td>
<td>0.2</td>
<td>51 (64%)</td>
<td>354 (64%)</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Bloody stools</td>
<td>5 (9%)</td>
<td>38 (6%)</td>
<td>1.5</td>
<td>0.5</td>
<td>3 (11%)</td>
<td>43 (7%)</td>
<td>2.4</td>
<td>0.2</td>
<td>7 (9%)</td>
<td>33 (6%)</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Persistent diarrhea</td>
<td></td>
<td></td>
<td>5 (9%)</td>
<td>37 (6%)</td>
<td>1.5, 0.4</td>
<td>2 (7%)</td>
<td>42 (6%)</td>
<td>0.9, 0.8</td>
<td>6 (8%)</td>
<td>33 (6%)</td>
<td>1.3, 0.7</td>
<td></td>
</tr>
<tr>
<td>History of fever</td>
<td>41 (77%)</td>
<td>532 (88%)</td>
<td>0.4</td>
<td>0.03</td>
<td>26 (93%)</td>
<td>568 (86%)</td>
<td>1.3</td>
<td>0.7</td>
<td>67 (83%)</td>
<td>480 (87%)</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>42 (67%)</td>
<td>449 (74%)</td>
<td>1.0</td>
<td>0.9</td>
<td>24 (92%)</td>
<td>492 (75%)</td>
<td>4.4</td>
<td>0.05</td>
<td>64 (79%)</td>
<td>407 (74%)</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Dehydration%</td>
<td>10 (27%)</td>
<td>169 (46%)</td>
<td>0.4</td>
<td>0.01</td>
<td>8 (42%)</td>
<td>175 (43%)</td>
<td>1.0</td>
<td>0.9</td>
<td>23 (44%)</td>
<td>148 (43%)</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>% Hospitalized</td>
<td>11 (39%)</td>
<td>268 (41%)</td>
<td>1.0</td>
<td>0.9</td>
<td>11 (39%)</td>
<td>268 (41%)</td>
<td>1.0</td>
<td>0.9</td>
<td>29 (36%)</td>
<td>226 (41%)</td>
<td>0.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Odds ratio adjusted for all other variables in the model using multivariate logistic regression, p-value.

*Mean number of liquid stools 24 h before presentation.

*Diarrhea lasts > 14 days.

*Percent of cases who were reported by parent of having fever >38°C.

*Dehydration ranged from mild to severe according to the WHO criterion, mild dehydration characterized most of cases regardless of type of enteroadherent E. coli isolated, while very few cases (n = 10) suffered from severe dehydration.
number of children with EAEC had diarrhea for >4 days (26 vs. 41%, \( p = 0.03 \)), less likely to be dehydrated (27 vs. 46%, \( p = 0.01 \)) or have fever (77 vs. 88%, \( p = 0.03 \)) than children with any other cause of diarrhea (Table 2).

We did not find an association between EAEC and mucoid stool, bloody or persistent diarrhea (Table 2). These results were consistent with other published reports [11–13].

Earlier, we have reportedCryptosporidium associated with persistent diarrhea [14]. Eighteen cases with EAEC (data not shown) were positive for C.parvum as copathogen. Others suggested an association between EAEC and persistent diarrhea but did not test for cryptosporidium, which may have been a confounder in these studies [3, 4, 15]. Percent of children with dehydration associated with EAEC was comparable with that reported in a hospital study in France [4], but differed from that reported by a Swiss group where 50% of children with EAEC were noted to be dehydrated [10].

Several reports have suggested that EAEC virulence varies geographically [4, 11, 16–18]; however, we cannot exclude the possibility that the difference is due to statistical variation, clinical procedures and/or differences within study populations.

Children <6 months of age were three times more likely to be infected with EPEC than those 12–59 months old, \( p = 0.04 \) (Table 1). This association was not identified in children 6–12 months, \( p = 0.9 \) (Table 1). The association of age with infection has been reported elsewhere [12, 13, 19, 20].

Vomiting was 4.4 times \( (p = 0.05) \) more common among children with EPEC than among children with non-EPEC diarrhea (Table 2). Despite the increased reporting of vomiting, EPEC was as frequently associated with dehydration as non-EPEC-associated diarrhea, which might suggest that the hydration therapies delivered to children of this young age with diarrhea needs to be further monitored.

Oligonucleotide primers specific for genes associated with virulence in diarrheagenic E.coli have been described [19]; however, our interest was the overall phenotypic class of diarrheagenic E.coli present in Egyptian children. As EPEC was the only phenotype associated with the younger age group and associated with some specific clinical characteristics, we targeted these cases to detect the eae gene that characterized EPEC strains [21]. DNA extracted from the stools of EPEC cases was tested by PCR [22] and a product of \( \sim 881 \) bp corresponding to the eae gene was amplified.

The association of DAEC with pediatric diarrhea remains questionable. DAEC has been observed in the stools of diarrheal and asymptomatic children at similar rates [6], and a challenge study with DAEC failed to produce diarrhea [23]. DAEC is also frequently found in association with other enteric pathogens [2]. Overall, DAEC cases were less likely to be ill for >4 days (27 vs. 41%) than all other diarrheal cases (OR\(^a\) 0.5, \( p = 0.01 \); Table 2). In conclusion, these data suggest that EPEC appears to be associated with infantile diarrhea and results in an increased level of vomiting in children <6 months old. In contrast, diarrhea associated with DAEC or EAEC was less severe and was characterized by a shorter duration than other diarrheal illnesses.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, Department of Defense, the United States Government or the Egyptian Ministry of Health and Population. The study (Protocol#2000.0002) was reviewed and approved by the Institutional Review Boards of the US Naval Medical Research Unit No. 3 and the Egyptian Ministry of Health and Population in compliance with all Federal regulations governing the protection of human subjects. Informed consents were obtained from all adult participants and from parents or legal guardians of minors.

This article fits the description stipulated by the new U.S. Copyright Act of a ‘United States Government work’. The authors are employees of the U.S. Government and this work was prepared as part of their official duties. Title 17 U.S.C. 105 provides that ‘Copyright protection of this work was prepared as part of their official duties. Title 17 U.S.C. 105 provides that ‘Copyright protection of this work was not available for any work of the United States Government work as a work prepared by a military service member or employee of the United States Government as part of that person’s official duties.’

References


