Enteroaggregative *Escherichia coli*: A Review of Trends, Diagnosis, and Treatment

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Enteroaggregative *Escherichia coli* (EAEC) is an increasingly recognized cause of acute diarrhea among both children and adults. Travelers to developing regions such as India, Jamaica, and Mexico are at increased risk. Contamination of food and water plays a central role in transmission. The clinical presentation of EAEC infection is characterized by watery diarrhea, usually unaccompanied by blood or mucus. The gold standard for diagnosis is the HEp-2 cell adherence assay; the unique “stacked-brick” aggregative pattern of adherence is characteristic of this pathogen. However, because laboratory diagnosis is not routinely available, symptomatic infections are usually treated empirically. In most regions, EAEC strains are susceptible to the fluoroquinolones and rifaximin. [Infect Med. 2007; 24:100-110]

**Key words:** Enteroaggregative *Escherichia coli* ■ Diarrhea

The recognized pathotypes of diarrheagenic *Escherichia coli* are enterotoxigenic, enteroaggregative, enteropathogenic, enterohemorrhagic, enteroinvasive, and diffusely adherent *E. coli*. Enteroaggregative *E coli* (EAEC) has recently received increasing attention as an emerging enteric pathogen. The purpose of this article is to provide health care workers with an update on the epidemiology, clinical manifestations, diagnostic modalities, and treatment of EAEC infection.

**EPIDEMIOLOGY**
EAEC was first detected in 1987 in the stool of a Peruvian child with diarrheal illness. On examination of her stool, a characteristic “stacked-brick” adherence to HEp-2 cells was noted.¹ Since this observation, EAEC has been implicated in both acute and chronic diarrheal illness among adults and children in industrialized and developing countries.²

Travelers to developing regions such as India, Jamaica, and Mexico are at increased risk for EAEC infection. International travelers are at particular risk because they often have limited exposure to this pathogen at home and, thus, have diminished immunity to EAEC infection.³ North American and European travelers to developing regions such as Guadalajara, Mexico; Ocho Rios, Jamaica; and Goa, India, frequently experience diarrheal illness caused by EAEC infection.⁴ Collectively, in these 3 regions, EAEC was isolated in 26% of cases of diarrheal illness; this was second only to enterotoxigenic *E. coli*, which was isolated in 30% of cases.⁵ The rates of EAEC infection varied; the rate was 33% in travelers to Mexico, 26% in travelers to Jamaica, and 19% in travelers to India.

Case series and cohort field studies indicate that EAEC is endemic in some regions. In a study of children younger than 5 years in Vietnam, Nguyen and colleagues⁶ identified EAEC in 11.6% of stool samples from those presenting with diarrhea, compared with 4.4% of age-matched controls without diarrhea.
Pabst and colleagues,\textsuperscript{7} who investigated the prevalence of EAEC in Swiss children, found EAEC in 19 (10.2\%) of 187 specimens from children with diarrhea, compared with 3 (2.2\%) of 137 specimens from those without diarrhea. When the analysis was limited to children younger than 5 years, specimens were positive for EAEC in 11.9\% of those with diarrhea compared with 2.2\% of controls. This association was strongest in the younger children and was correlated with recent international travel.\textsuperscript{7}

EAEC has been isolated from 2\% to 68\% of patients with diarrhea and from 0\% to 15\% of controls from India, South America, Europe, and the Middle East.\textsuperscript{1,8,9} It has also been implicated in outbreaks of diarrheal illness in both adults and children. In 1993, a massive outbreak affected 2697 children in Japan, representing an attack rate of 40\%.\textsuperscript{10} The children consumed contaminated school lunches and began having symptoms an average of 40 to 50 hours later. At least 30 children suffered prolonged diarrhea.\textsuperscript{10}

A meta-analysis of 41 case-control studies demonstrated an association between EAEC and acute diarrhea among children and adults living in developing and industrialized regions, adult travelers to developing regions, and HIV-infected patients in developing nations. The presence of EAEC identified with the HEp-2 cell adherence assay was found to be significantly associated with acute diarrheal illness among children residing in developing regions (odds ratio [OR], 1.58; 95\% confidence interval [CI], 1.36 - 1.83) and industrialized regions (OR, 1.23; 95\% CI, 1.03 - 1.48), adults with HIV infection residing in developing regions (OR, 6.43; 95\% CI, 2.91 - 14.16), adults residing in developing regions (OR, 7.15; 95\% CI, 1.96 - 26.04), and international travelers to developing regions (OR, 6.72; 95\% CI, 2.62 - 17.20) (Figure 1).\textsuperscript{2}

Although most EAEC studies have been conducted in developing regions, 2 epidemiologic studies have examined the prevalence of EAEC in the United States. The first prospective study was conducted among children in Cincinnati.\textsuperscript{11} Ten percent of children younger than 1 year who presented to the emergency department (ED) and approximately 5\% of inpatients with diarrhea had evidence of EAEC infection.\textsuperscript{11} The second prospective cohort study investigated the cause of diarrhea in patients who presented to EDs and clinics in New Haven, Conn, and Baltimore and found that EAEC was the single most common bacterial cause of diarrhea; it was isolated in 37 (4.5\%) of 823 case patients, versus 1.7\% of controls.\textsuperscript{12}

**PATHOGENESIS**

EAEC is transmitted by the fecal-oral route. Contamination of food and water play a central role in transmission. A volunteer study has shown that oral challenge with 10\textsuperscript{10} colony-forming units of EAEC causes diarrheal illness.\textsuperscript{5} Once EAEC is ingested, it can bind to the mucosa of the small and large intestines. EAEC that is bound to the intestinal mucosa stimulates epithelial cells to produce a thick mucus layer above the intact enterocyte brush border,\textsuperscript{13} and EAEC elicits inflammatory mediators that produce cytotoxic effects involving the intestinal mucosa.\textsuperscript{14}

Not all EAEC infections cause diarrheal illness. EAEC is a very heterogeneous bacterium. Many virulence genes have been identified. \textit{aggR}, the master regulator of EAEC virulence, controls the expression of adherence factors, a dispersin protein, and a cluster of genes encoded on the EAEC chromosome.\textsuperscript{15} As
a result of the importance of the aggR region, EAEC that carries aggR has been designated typical EAEC. Many of the EAEC genes encoded on its chromosome are homologues in other Gram-negative bacteria, and these genes have been proposed to constitute a type IV secretion system. Other genes have been identified, and their significance in virulence is being studied.

Adherence factors enable EAEC to adhere to the intestinal mucosa. Three AAF structural subunits have been identified: aggA encodes AAF/I,19 aggA encodes AAF/II,19 and agg-3 encodes AAF/III.20 AAF/I and AAF/II are regulated by transcriptional activator AggR.

Outer membrane proteins play an important role in EAEC adherence and hemagglutination of animal cells. A type IV pilus that contributes to plasmid conjugation, epithelial cell adherence, and adherence to abiotic surfaces has recently been described.16 There are probably more AAF structural subunits and adherence factors that are yet to be identified.

EAEC has a type III secretion system (T3SS), designated ETT2.22 Its specific role has not yet been characterized, but the type secretion systems typically export protein molecules across Gram-negative bacterial membranes directly into the host cells. This is accomplished via a variety of mechanisms, from simple one-component systems to complex multi-component pathways. There are 6 types of nonhomologous protein secretion systems. For EAEC, the T3SS is located on the glyU locus of prototype EAEC strain 042. The T3SS of EAEC and the presumed effectors are located on different chromosomal islands and are coordinately activated by EilA. EilA, like aggR, appears to serve as a virulence-related regulon for EAEC genes involved with adherence, aggregation, and the T3SS.22

Dispersin protein, encoded by aap, is an important secreted low molecular weight protein responsible for mediating dispersal of EAEC across the intestinal mucosa to allow for efficient adherence and aggregation. This protein is exported by an ATP-binding cassette transporter complex that is encoded by a genetic locus on the EAEC virulence plasmid pAA223. aap is regulated by the transcriptional activator AggR.

Once EAEC binds to the mucosa of the small and large intestines, it stimulates the epithelial cells to produce a thick mucous layer above the enterocytes, which forms biofilm. This mucous layer surrounds the EAEC and may explain why persons infected with EAEC have mucoid stools. Most EAEC isolates produce biofilm, and biofilm formation is associated with the multiple EAEC genes: aggR, set1A, aatA, and irp2.24 EAEC isolates that possess the aap, astA, irp2, pet, and set1A genes in the aggR background produce more biofilm than aggR-lacking background.24 The aggR gene regulates other genes needed for biofilm formation in EAEC.24 Biofilm production by EAEC isolates from patients with traveler’s diarrhea has been associated with the carriage of virulence genes, particularly aggR, set1A, and aatA.24

Wakimoto and associates25 reported that the aggR-positive EAEC isolates showed significantly stronger biofilm formation than the aggR-negative EAEC isolates. Another study identified EAEC adhesions to be allelic in nature, and biofilm formation was shared by all members of the AAF family.26 Other genes have been associated with biofilm production among EAEC. Fis and yafK are mediated by AAF and have been identified as important genes in the biofilm production of EAEC strain 042, a prototype EAEC strain.27 It is clear that we still have to decipher the genetic basis of biofilm formation by EAEC, including quorum sensing in the GI tract.

EAEC isolates release toxins that bind to the intestinal mucosa and elicit inflammatory mediators that produce cytotoxic effects and intestinal secretion. The best-studied toxins are plasmid-encoded toxin (pet),28 EAEC heat-stable enterotoxin (EAST1),29 and Shigella enterotoxin 1 (set1A). These toxins are destructive to the tips and sides of intestinal villi and enterocytes. Host inflammatory mediators of EAEC infection include the production of intestinal cytokines and inflammatory markers, such as interleukin (IL)-1ra, IL-1B, IL-8, interferon-γ, lactoferrin, fecal leukocytes, and occult blood.30-32

In vitro studies suggest that many of these inflammatory responses are mediated by flagellin, (HIC), a major bacterial surface protein of EAEC.33 Flagellin binds to toll-like receptor 5 and induces transcription of proinflammatory cytokines such as IL-8. IL-8 is a neutrophil chemoattractant that facilitates intestinal secretion.34 Other in vitro studies indicate that other proinflammatory genes are up-regulated, including IL-6, tumor necrosis factor α, growth-related gene product (GRO)-α, GRO-γ, intracellular adhesion molecule-1, granulocyte-macrophage colony-stimulating factor, and IL-1ra.35

HOST SUSCEPTIBILITY

Genetics plays an important role in determining the host’s susceptibility to diarrheal illness. Two genes that are important in determining susceptibility to EAEC infection are the genes for IL-8 and lactoferrin. IL-8 is a proinflammatory chemokine that functions as a neutrophil chemoattractant, and lactoferrin is an important element of the intestinal immune system.

A single nucleotide polymor-
phism of the AA genotype in the –251 position of the IL-8 gene pro-
motor has been identified as an im-
portant gene associated with diar-
rheal illness and greater levels of fecal IL-8 caused by EAEC infec-
tion.36 Recently, a single nucleotide polymorphism with T/C substitu-
tion in exon15 (LTFEx15 codon 632 [T/C]) of the lactoferrin gene was as-
associated with susceptibility to in-
flammatory diarrhea caused by
EAEC in North Americans who
were traveling in Mexico.37 Much
work is still needed to identify other
host genetic factors that are impor-
tant in determining susceptibility to
EAEC infection.

CLINICAL PRESENTATION
One of the most common clinical
manifestations of EAEC infection is
watery diarrhea, usually unaccom-
panied by blood or mucus. Typically,
patients are afebrile.15 Less com-
monly reported associated symptoms
and signs include low-grade fever;
vomiting; abdominal pain; and the
presence of fecal blood, mucus, or
leukocytes.8,12,32,38 The incubation pe-
riod for illness ranges from 8 to 18
hours.1,5,14

These signs and symptoms are
usually self-limited even without an-
tibiotic therapy. However, persistent
diarrhea (lasting more than 14 days)
may occur in select populations, in-
cluding HIV/AIDS patients and
malnourished children in develop-
ing countries.39,40 Patients infected
with EAEC can be asymptomatic.41
This mixed spectrum of clinical man-
ifestations reflects the heterogeneous
nature of EAEC strains bearing dif-
ferent virulence properties as well as
differing host immunity.

DIAGNOSIS
The differential diagnosis of EAEC-
associated diarrhea does not differ
from that of viral, other bacterial,
or parasitic enteric pathogens that
cause community-acquired and
traveler’s diarrhea. Physical findings
are nonspecific and do not help nar-
row the differential diagnosis for
EAEC diarrhea. Typically, EAEC is
identified among patients who have
traveled to developing areas where
EAEC is endemic.

The HEp-2 cell adherence assay
remains the gold standard for diag-
nosing EAEC infection.1 The unique
“stacked-brick” aggregative pattern
of adherence is characteristic of this
pathogen. Essential components of
this adherence pattern include bind-
ing of the bacteria to the glass sur-
face, to the human epithelial cell bor-
ders, and to one another (Figure 2).
Further characterization of this ag-
gregative pattern has been recently
described as typical honeycomb for-
mation, lack of honeycombing, and
aggregative adherence with lined-up
cells.25,42

The use of formalin-fixed HEp-2
cells has been demonstrated to be a
viable option for the storage of HEp-
2 cells for future use, with a sensitiv-
ity (94% to 98%) and a specificity
(100%) that are similar to those of the
traditional assay.43 Limitations of the
HEp-2 cell assay include time re-
quirements and limited availability
in reference laboratories. These limi-
tations have led to the search for
other diagnostic methods, including
polymerase chain reaction (PCR) as-
says, DNA probes, and quantitative
biofilm assays (Table).

Currently, there is a lack of con-
sensus in the literature regarding
which EAEC genes should be
screened with PCR detection. Multi-
ple genes have been investigated, in-
cluding aggR, aatA (CVD432), aafA,
and astA.12,25,44 Unfortunately,
sensitivity of primers based on
these genes appears to markedly
vary given the heterogeneous pres-
ence of virulence genes in different
EAEC strains.

PCR detection of aggR may not
prove to be an appropriate initial
screening test for EAEC, but it is in-
formative because it can identify
typical EAEC, which is postulated to
have a more pathogenic role than EAEC lacking aggR.35 Multiplex PCR assays for multiple EAEC genes may help overcome the diverse genetic composition of EAEC strains with improved sensitivity, but problems with specificity continue to necessitate confirmation with the HEp-2 cell assay.45,46 Real-time PCR has been demonstrated to be at least as sensitive as conventional PCR for the detection of EAEC.47

Another diagnostic tool is a DNA probe to the pCVD432 (aatA) gene sequence. Although this test is specific (99%), its sensitivity is variable (15% to 89%).35,41 This test may help identify more virulent typical EAEC strains because a positive result can be considered a surrogate marker for the presence of aggR.15

The biofilm assay using a microtiter plate has potential as a preliminary screening tool in developing countries because it is relatively quick. However, the test’s sensitivity (77%) and specificity (100%) require the HEp-2 cell assay for more accurate detection of EAEC.25

**TREATMENT**

Many EAEC infections are self-limited.31 Symptomatic infections are usually treated empirically because laboratory diagnosis is not routinely available. EAEC susceptibility varies by region. In most regions, EAEC strains are susceptible to the fluoroquinolones, azithromycin, rifaximin, amoxicillin/clavulanic acid, and nalidixic acid.48,49 In a study conducted by Sobieszczanska and colleagues,50 EAEC strains identified in the stools of Polish children with diarrhea were resistant to ampicillin, tetracycline, trimethoprim, sulfamethoxazole, and chloramphenicol. In Thailand, EAEC strains were resistant to several antibiotics routinely used for gastroenteritis, including co-trimoxazole and amoxicillin. Most of the strains, however, were sensitive to the fluoroquinolones.51

Three clinical trials have been conducted on the treatment of EAEC diarrheal illness; 2 were conducted among travelers to developing countries and 1 was conducted among HIV-infected patients in a developing country. Glandt and colleagues48 compared the clinical responses to ciprofloxacin treatment with responses to placebo among 29 US travelers to Jamaica and Mexico who had EAEC diarrhea. Sixteen travelers were treated with ciprofloxacin, 500 mg bid for 3 days, and 13 received placebo. The patients who were treated with ciprofloxacin had a significantly reduced duration of diarrhea compared with the controls (35 vs 56 hours).

A multicenter trial that included US travelers to Guatemala; Kenya; and Guadalajara, Mexico,49 compared the clinical responses to rifaximin with responses to placebo among 43 patients with EAEC diarrhea. Thirty patients were treated with rifaximin (200 or 400 mg bid for 3 days), and 13 received placebo. The patients treated with rifaximin had a significantly shorter duration of illness than those who received placebo (22 vs 72 hours). In a double-blind, placebo-controlled, crossover treatment trial involving 24 HIV-infected patients with EAEC diarr-

**Table – Diagnostic modalities for identifying enteroaggregative Escherichia coli**

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Study location</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin-preserved HEp-2 cell assay</td>
<td>Guadalajara, Mexico</td>
<td>92% - 98%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR assay (astA, aafA, aggA, aggR)</td>
<td>Zacatenco, Mexico</td>
<td>86% - 94%</td>
<td>78% - 100%</td>
</tr>
<tr>
<td>multiplex PCR assay (CVD432)</td>
<td>Sao Paulo, Brazil</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Real-time PCR assay</td>
<td>Zurich</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>DNA probe (CVD432)</td>
<td>Tehran, Iran</td>
<td>15% - 89%</td>
<td>99%</td>
</tr>
<tr>
<td>Quantitative biofilm assay (OD570 &gt; 0.2)</td>
<td>Kagoshima, Japan</td>
<td>100%</td>
<td>99%</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction.
rhea, those treated with ciprofloxacin (500 mg bid for 7 days) reported 50% fewer bowel movements and a 42% decrease in other enteric symptoms compared with those who received placebo.

These 3 clinical studies indicate that the fluoroquinolones, especially ciprofloxacin, 500 mg bid for 3 to 7 days, and rifaximin, 200 or 400 mg bid for 3 days, may be the antimicrobial treatments of choice for symptomatic EAEC infections.

CONCLUSION

EAEC is recognized as an emerging cause of diarrhea. It has been implicated in acute and chronic diarrhea among travelers, children, adults, and HIV-infected persons in both industrialized and developing countries. Volunteer studies, outbreak reports, case-control studies, and clinical trials in industrialized and developing countries have shown an association of EAEC with acute diarrhea. The pathogenesis is complex, and EAEC has a host of heterogeneous virulence factors.

Currently, identification of EAEC is not routinely performed. In the near future, efficient ways of identifying EAEC will allow for standardized laboratory testing. Since EAEC infection is usually a self-limited disease, management should be individualized. Most EAEC infections can be treated conservatively with oral hydration. Clinical trials have shown that EAEC diarrhea is responsive to treatment with fluoroquinolones and rifaximin.

REFERENCES


Therapeutic agents mentioned in this article

Amoxicillin
Amoxicillin/clavulanic acid
Ampicillin
Azithromycin
Chloramphenicol
Ciprofloxacin
Co-trimoxazole
Nalidixic acid
Rifaximin
Sulfamethoxazole
Tetracycline
Trimethoprim

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