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 6 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 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 colleagues[6] identified EAEC in 11.6% of stool samples from those presenting with diarrhea, compared with 4.4% of age-matched controls without diarrhea.

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Pabst and colleagues,[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.[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.[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%.[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.[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.[2] 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).[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.[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.[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 of case patients, versus 1.7% of controls.[12]

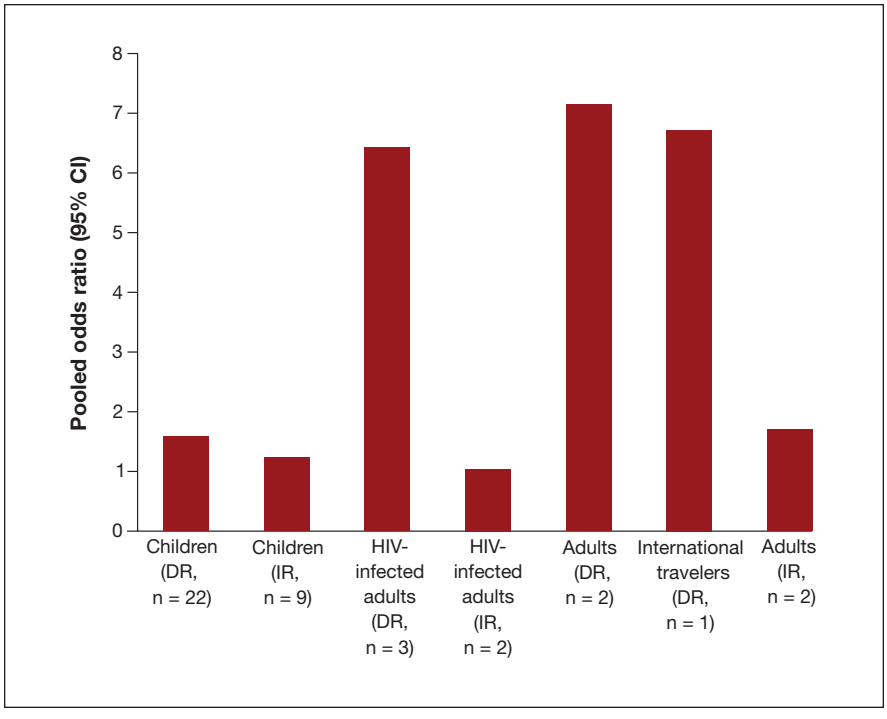

Figure 1 – *Enteroaggregative* Escherichia coli *has been associated with acute diarrhea among children and adults living in developing and industrialized regions, adult travelers to developing regions, and HIV-infected patients in developing nations. The odds ratios range from 1.58 in some pediatric populations to as high as 7.15 in some adult populations. (CI, confidence interval; n, number of published studies; DR, developing regions; IR, industrialized regions.)*

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^{10} colony-forming units of EAEC causes diarrheal illness.[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 mucous layer above the intact enterocyte brush border,[13] and EAEC elicits inflammatory mediators that produce cytotoxic effects involving the intestinal mucosa.[14]

Not all EAEC infections cause diarrheal illness. EAEC is a very heterogeneous bacterium. Many virulence genes have been identified. *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.[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.[16] Other genes have been identified, and their significance in virulence is being studied.

Adherence factors enable EAEC to adhere to the intestinal mucosa.[17] Three AAF structural subunits have been identified: *aggA* encodes AAF/I,[18] *aafA* 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.[21] 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 pAA2[23]; *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, suggesting that *aggR* 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 associates[25] 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-1β, 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 (fliC), 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 upregulated, 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 promoter has been identified as an important gene associated with diarrheal illness and greater levels of fecal IL-8 caused by EAEC infection.[36] Recently, a single nucleotide polymorphism with T/C substitution in exon15 (LTFEx15 codon 632 [T/C]) of the lactoferrin gene was associated with susceptibility to inflammatory 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 important in determining susceptibility to EAEC infection.

CLINICAL PRESENTATION

One of the most common clinical manifestations of EAEC infection is watery diarrhea, usually unaccompanied by blood or mucus. Typically, patients are afebrile.[15] Less commonly 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 period for illness ranges from 8 to 18 hours.[1,5,14]

These signs and symptoms are usually self-limited even without antibiotic therapy. However, persistent diarrhea (lasting more than 14 days) may occur in select populations, including HIV/AIDS patients and malnourished children in developing countries.[39,40] Patients infected with EAEC can be asymptomatic.[41] This mixed spectrum of clinical manifestations reflects the heterogeneous nature of EAEC strains bearing different 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 narrow the differential diagnosis for EAEC diarrhea. Typically, EAEC is identified among patients who have traveled to developing areas where EAEC is endemic.

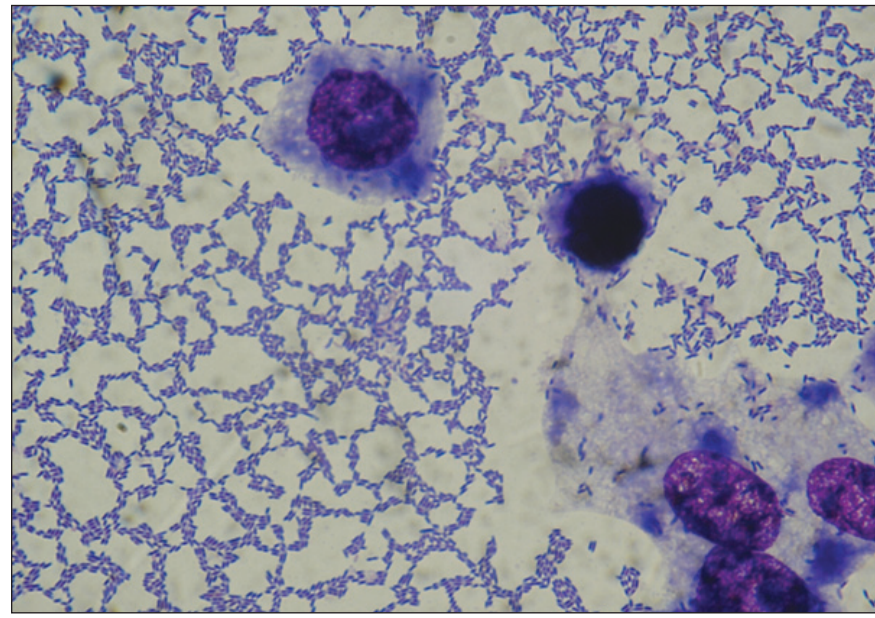

Figure 2 – *This HEp-2 cell adherence assay shows the "stacked-brick" aggregative adherence to other bacteria, the coverslip, and the HEp-2 cells. This pattern of adherence is characteristic of enteroaggregative* Escherichia coli.

The HEp-2 cell adherence assay remains the gold standard for diagnosing EAEC infection.[1] The unique "stacked-brick" aggregative pattern of adherence is characteristic of this pathogen. Essential components of this adherence pattern include binding of the bacteria to the glass surface, to the human epithelial cell borders, and to one another (Figure 2). Further characterization of this aggregative pattern has been recently described as typical honeycomb formation, 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 sensitivity (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 requirements and limited availability in reference laboratories. These limitations have led to the search for other diagnostic methods, including polymerase chain reaction (PCR) assays, DNA probes, and quantitative biofilm assays (Table).

Currently, there is a lack of consensus in the literature regarding which EAEC genes should be screened with PCR detection. Multiple genes have been investigated, including *aggR, aatA* (CVD432), *aggA, aafA,* and *astA*.[12,25,44] Unfortunately, the sensitivity of primers based on these genes appears to markedly vary given the heterogeneous presence 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 informative 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 colleagues[48] 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 diar-

Table – Diagnostic modalities for identifying enteroaggregative *Escherichia coli*

Diagnostic test	Study location	Sensitivity	Specificity
Formalin-preserved HEp-2 cell assay	Guadalajara, Mexico[43]	92% - 98%	100%
PCR assay (astA, aafA, aggA, aggR)	Zacatenco, Mexico[53] Barcelona, Spain[54] Mendoza, Argentina[55] Taichung, Taiwan[44]	86% - 94%	78% - 100%
Multiplex PCR assay (CVD432)	Sao Paulo, Brazil[56] Hanoi, Vietnam[6] Stockholm[6]	100%	100%
Real-time PCR assay	Zurich[47]	100%	100%
DNA probe (CVD432)	Tehran, Iran[46] Sao Paulo, Brazil[57]	15% - 89%	99%
Quantitative biofilm assay (OD_{570} > 0.2)	Kagoshima, Japan[25]	100%	99%

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.[52]

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.

Therapeutic agents mentioned in this article

Amoxicillin

Amoxicillin/clavulanic acid

Ampicillin

Azithromycin

Chloramphenicol

Ciprofloxacin

Co-trimoxazole

Nalidixic acid

Rifaximin

Sulfamethoxazole

Tetracycline

Trimethoprim

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. ❖

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