

Review

A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*David B. Huang,¹ Alakananda Mohanty,² Herbert L. DuPont,^{1,2,3,4} Pablo C. Okhuysen^{2,3} and Tom Chiang⁵

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Enteroaggregative *Escherichia coli* (EAEC) is an increasingly recognized enteric pathogen. It is a cause of both acute and persistent diarrhoea among children, adults and HIV-infected persons, in both developing and developed countries. In challenge studies, EAEC has caused diarrhoeal illness with the ingestion of 10^{10} c.f.u. Outbreaks of diarrhoeal illness due to EAEC have been reported, and linked to the ingestion of contaminated food. Diarrhoeal illness due to EAEC is the result of a complex pathogen–host interaction. Some infections due to EAEC result in diarrhoeal illness and elicit an inflammatory response, whereas other infections do not result in a symptomatic infection. Many putative virulence genes and EAEC strains that produce biofilm have been identified; however, the clinical significance of these genes and of biofilm production has yet to be defined. A –251 AA single nucleotide polymorphism (SNP) in the interleukin (IL)-8 promoter region is reported to increase host susceptibility to EAEC diarrhoea. Ciprofloxacin and rifaximin continue to be an effective treatment in persons infected with EAEC. This review is intended to provide an updated review for healthcare workers on EAEC, an emerging enteric pathogen.

General considerations

Diarrhoeagenic *Escherichia coli* is categorized into the following six pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC). Other diarrhoeagenic *E. coli* pathotypes have been proposed, such as cell detaching *E. coli* (CDEC); however, their significance remains uncertain (Abdouch-Fabrega *et al.*, 2002; Clarke, 2001). Each of the pathotypes has distinguishing characteristics related to epidemiology, pathogenesis, clinical manifestations and treatment. EAEC is the most recently identified and described diarrhoeagenic *E. coli*. This bacterium was described in 1987, and identified in a child from Chile with persistent diarrhoea (Nataro, 2005).

Abbreviations: ABC, ATP-binding cassette; AAF, aggregative adherence fimbriae; CI, confidence interval; DAEC, diffusely adherent *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; IL, interleukin; OMP, outer-membrane protein; OR, odds ratio.

EAEC has caused large diarrhoeal outbreaks in Europe, the UK, Switzerland and Japan. EAEC is also a common bacterial cause of diarrhoea among travellers to developing countries, and among children and HIV-infected persons residing in developing and developed regions of the world (Ruttler *et al.*, 2002; Nataro *et al.*, 2006). The virulence of EAEC strain 042 has been established in volunteers, who develop diarrhoea following an experimental challenge with an inoculum of 10^{10} c.f.u. (Nataro *et al.*, 1995). A high inoculum required for disease development suggests a food or water vehicle as the likely mode of disease transmission for EAEC strains. A study of Mexican tabletop sauces identified 44% of sauces from Guadalajara, Mexico, to contain viable EAEC, compared to 0% of sauces in restaurants in Houston, TX (Adachi *et al.*, 2002b).

EAEC is increasingly recognized as an emerging enteric pathogen. EAEC is a cause of persistent diarrhoea and malnutrition in children and HIV-infected persons living in developed countries, is the second most common cause of travellers' diarrhoea (ETEC is the most common cause), and

is a common cause of acute diarrhoeal illness in children and adults (4·5 %) presenting to emergency departments and inpatient units in the USA (Cohen *et al.*, 2005; Nataro *et al.*, 2006). EAEC is also considered a potential bioterrorism agent (National Institutes of Health category B) (Huang & DuPont, 2004; Huang *et al.*, 2004b). The objective of this review is to provide an update on this increasingly recognized emerging enteric pathogen.

Epidemiology

Numerous epidemiological studies of EAEC have been conducted. In a meta-analysis of published studies, EAEC was a cause of acute diarrhoeal illness in a median of 15 % of children living in developing countries and 4 % of children living in industrialized countries (Huang *et al.*, 2006). These and other studies have examined the role of EAEC as a cause of diarrhoeal illness among other populations, in different regions of the world (Sanders *et al.*, 2004; Sarantuya *et al.*, 2004; Cohen *et al.*, 2005). The populations best studied include children, adults and HIV-infected persons, living in developing and developed regions, and international travellers to developing countries. Many of these epidemiological studies conflict with regard to the pathogenicity of EAEC strains (Vernacchio *et al.*, 2006), and many of these studies were not able to determine the importance of EAEC as a cause of diarrhoeal illness.

The data that support the virulence of EAEC come from a volunteer challenge study of EAEC prototype strain 042; outbreaks reported in Europe, the UK, Switzerland and Japan; large case-series and cohort studies of children, adults and HIV-infected persons living in developing and developed countries; and cases among international travellers to developing countries. In a volunteer challenge study, four groups of five volunteers were fed with one of four EAEC strains (042, 17-2, 34b and JM 221), each at a dose of 10¹⁰ c.f.u. (Nataro *et al.*, 1995). Strain 042 caused diarrhoea in one group (three of five adults), whereas the other three strains failed to cause diarrhoea. These results support case-control and cohort-study findings of heterogeneity of EAEC in virulence. Four outbreaks of gastroenteritis due to EAEC occurred in the UK in 1994, occurring at different locations and at different times, and involving

19, 10, 51 and 53 persons, respectively. These illnesses were characterized by vomiting, diarrhoea and low-grade fever (Smith *et al.*, 1997). In 1993, a massive outbreak of diarrhoea due to EAEC serotype O:H10 occurred in Japan. A total of 2697 children from 16 schools developed symptoms of abdominal pain, nausea and diarrhoea after presumably consuming contaminated school lunches (Itoh *et al.*, 1997).

Several large population-based case-series of persons infected with EAEC have been described (Zamboni *et al.*, 2004; Dulguer *et al.*, 2003; Kang *et al.*, 1995; Bhatnagar *et al.*, 1993; Wilson *et al.*, 2001; Knutton *et al.*, 2001; Huppertz *et al.*, 1997; Svenungsson *et al.*, 2000). Table 1 shows pooled odds ratios (ORs) and confidence intervals (CIs) from a meta-analysis of epidemiologic studies of EAEC infections, identified by the HEP-2 cell-adherence assay, by population and region of the world (Huang *et al.*, 2006). The OR represents the ratio of patients with diarrhoea from whom EAEC was isolated compared with those with asymptomatic EAEC infection. From the studies, EAEC was associated with acute diarrhoeal illness among children residing in developing and industrialized regions, HIV-infected adults residing in developing regions, adults residing in developing regions, and international travellers to developing regions. Limited studies have examined the role of EAEC in acute diarrhoeal illness among HIV-infected adults and non-HIV-infected adults residing in industrialized regions. Additional studies that are able to examine the role of EAEC in acute diarrhoea are needed.

It is likely that outbreaks of diarrhoea and diarrhoeal illness due to EAEC are underdiagnosed. Underdiagnosis of EAEC occurs because many public health and healthcare workers are not familiar with EAEC as a possible cause of diarrhoeal illness, and/or because limited research facilities with trained laboratory technicians are available to regularly perform assays to identify EAEC.

Like ETEC, EAEC is transmitted by the faecal–oral route. Risk factors for EAEC include travel to developing countries, ingestion of contaminated food and water, poor hygiene, host susceptibility, and possibly immunosuppression (HIV infection) (Huang & DuPont, 2004; Huang *et al.*, 2004b).

Table 1. Epidemiology of EAEC infection among different populations living in different geographic areas of the world (Huang *et al.*, 2006)

n, Number of studies.

Population studied	Pooled OR	95 % CI
Children residing in developing countries (<i>n</i> =22)	1·58	1·36–1·83
Children residing in industrialized nations (<i>n</i> =9)	1·23	1·03–1·48
HIV-infected adults residing in developing regions (<i>n</i> =3)	6·43	2·91–14·16
HIV-infected adults residing in industrialized regions (<i>n</i> =2)	1·04	0·64–1·69
Adults residing in developing regions (<i>n</i> =2)	7·15	1·96–26·04
International travellers to developing regions (<i>n</i> =1)	6·72	2·62–17·20

Although EAEC has been identified as a cause of acute and chronic diarrhoea, it is unclear if HIV-infected persons are more likely to develop symptomatic EAEC infection in comparison to non-HIV-infected persons. It is also uncertain if EAEC represents an opportunistic infection (Gassama-Sow *et al.*, 2004).

Pathogenesis

The pathogenesis of EAEC is complex, and EAEC strains are very heterogeneous (Elias *et al.*, 2002). Human and animal studies indicate that EAEC is able to bind to jejunal, ileal and colonic epithelium. Electron microscopy of infected small- and large-intestinal mucosa, from children between 3 and 190 months, cultured with several different EAEC strains, reveals bacteria in a thick mucus layer above the intact enterocyte brush border (Hicks *et al.*, 1996). In the colon,

EAEC elicits inflammatory mediators and produces cytotoxic effects such as microvillus vesiculation, enlarged crypt openings, and increased epithelial cell extrusion (Harrington *et al.*, 2005). Numerous putative virulence factors, a yersiniabactin system, a complex carbohydrate-specific lectin (Basu *et al.*, 2004), enterotoxins and cytotoxins have been identified, but the clinical implication of these factors remains unclear (Table 2) (Jenkins *et al.*, 2005; Jiang *et al.*, 2002; Moon *et al.*, 2005; Hu *et al.*, 2005). The best-studied virulence factor is AggR, the master regulator of EAEC virulence, which controls expression of adherence factors, a dispersin protein, and a large cluster of genes encoded on the EAEC chromosome (Nataro, 2005). EAEC pathogenesis involves three stages: (1) adherence to the intestinal mucosa by aggregative adherence fimbriae (AAF) and adherence factors; (2) production of mucus by bacteria and the host cell forming a biofilm on the surface of

Table 2. Putative EAEC virulence genes and virulence factors

Gene	Description	References
Master regulator gene		
<i>aggR</i>	Master regulator of a package of EAEC plasmid virulence genes, including genes encoding aggregative adherence factors, fimbriae AAF/I and AAF/II, a dispersin protein, and a large cluster of genes inserted on a pathogenicity island at the PheU locus	Nataro (1994, 2005); Jenkins <i>et al.</i> (2005); Sheikh <i>et al.</i> (2002)
Fimbriae		
<i>aggA</i>	Encodes AAF/I and haemagglutination of erythrocytes	Nataro <i>et al.</i> (1993, 1994)
<i>aafA</i>	Encodes AAF/II, which mediates adherence to colonic mucosa	Czeczulin <i>et al.</i> (1997)
<i>agg3</i>	Encodes AAF/III	Bernier <i>et al.</i> (2002)
Enterotoxins		
<i>astA</i>	Encodes the enteroaggregative heat-stable toxin, which has physical and mechanistic similarities to <i>E. coli</i> STa enterotoxin	Menard & Dubreuil (2002); Menard <i>et al.</i> (2004)
<i>pet</i>	A 108 kDa autotransporter protein that functions as a heat-labile enterotoxin and cytotoxin	Eslava <i>et al.</i> (1998); Navarro-Garcia <i>et al.</i> (1999)
OMPs		
OMP	Provides adherence capabilities of EAEC and haemagglutination of erythrocytes	Suzart <i>et al.</i> (1999); Monteiro-Neto <i>et al.</i> (2003)
Dispersin transporter		
<i>aatA</i>	Encodes an ABC protein responsible for transporting the dispersin protein out of the outer membrane of EAEC	Nishi <i>et al.</i> (2003); Iwashita <i>et al.</i> (2006)
Secreted proteins		
<i>aap</i>	Encodes a 10 kDa secreted protein named dispersin, and is responsible for 'dispersing' EAEC across the intestinal mucosa. Dispersin was immunogenic in a human EAEC challenge.	Nataro <i>et al.</i> (1995); Sheikh <i>et al.</i> (2002)
<i>pic</i>	Encodes a 109 kDa serine protease autotransporter protein that catalyses gelatin degradation. The Pic protein has mucinase activity and is capable of causing haemagglutination of erythrocytes.	Henderson <i>et al.</i> (1999); Behrens <i>et al.</i> (2002)
Yersiniabactin system		
<i>irp2</i>	Encodes an iron-uptake system mediated by the siderophore yersiniabactin that plays a role in iron transport and regulation	Schubert <i>et al.</i> (1998)
Lectin		
Lectin	A complex carbohydrate that shows cross-reactivity to the binding subunit of cholera toxin, and induces morphological changes in HEp-2 cells and fluid accumulation in the rabbit ileal loop.	Basu <i>et al.</i> (2004)

the enterocytes; and (3) release of toxins and elicitation of an inflammatory response, mucosal toxicity and intestinal secretion (Nataro, 2005; Huang *et al.*, 2004a; Harrington *et al.*, 2005).

EAEC adherence to the intestinal mucosa requires AAF and adherence factors (Moreira *et al.*, 2003). Three AAF structural subunits encoded by *aggA* (AAF/I), *aafA* (AAF/II) and *agg-3* (AAF/III) on the 60–65 MDa pAA plasmid have been described. *aggA* encodes AAF/I, and is responsible for the aggregative phenotype and human erythrocyte haemagglutination of some EAEC strains (Nataro *et al.*, 1993). *aafA* encodes AAF/II, which allows EAEC to adhere to the intestinal mucosa (Czeczulin *et al.*, 1997). Both *aggA* and *aafA* are regulated by the transcriptional activator AggR. AAF/III functions as an adhesin, and is encoded by *agg-3*, which has a sequence closely related to that of the *agg* and *aaf* operon of DAEC (Bernier *et al.*, 2002). Other adherence factors have been described. Three membrane-associated proteins (MAPs), of 18, 20 and 58 kDa, are believed to play an important role in EAEC adherence to and haemagglutination of animal cells (Spencer *et al.*, 1998). One study has characterized the outer-membrane protein (OMP) profiles of EAEC strains from children with diarrhoea from Sao Paulo, Brazil, and has observed a heterogeneity in OMP profiles, suggesting that EAEC strains are very heterogeneous (Monteiro-Neto *et al.*, 2003).

AggR regulates the expression of a secreted low-molecular-weight protein known as dispersin (*aap*) (Sheikh *et al.*, 2002). *Aap* lies immediately upstream of *aggR* in EAEC strain 042. Dispersin is a 10.2 kDa protein that has been identified in 80 % of EAEC isolates from one laboratory (Sheikh *et al.*, 2002). This protein is exported by an ATP-binding cassette (ABC) transporter complex which is encoded by a genetic locus on the EAEC virulence plasmid pAA2 (Nishi *et al.*, 2003). The locus consists of a cluster of five genes (designated *aat-PABCD*), including homologues of an inner-membrane permease (AatP), an ABC protein (AatC) and an OMP TolC (AatA). AatA localizes to the outer membrane independently of its ABC partner. Dispersin appears to require the Aat complex for outer-membrane translocation, but not for secretion across the inner membrane. In a similar manner to the dispersin gene (*aap*), transcription of the *aat* cluster is dependent on AggR, a regulator of a package of virulence genes in EAEC (Jenkins *et al.*, 2005). Dispersin is responsible for mediating dispersal of EAEC across the intestinal mucosa to allow for efficient adherence and aggregation. This protein neutralizes the negatively charged LPS of the EAEC surface, allowing the positively charged AAF to splay out from the bacterium. In a volunteer challenge study, dispersin has been shown to be highly immunogenic, suggesting that it is a potential vaccine candidate (Nataro *et al.*, 1995). Undoubtedly, other potential AAF and adherence factors exist, and they are currently being investigated.

The second stage of EAEC pathogenesis involves production of a mucus layer by the bacteria and the intestinal mucosa.

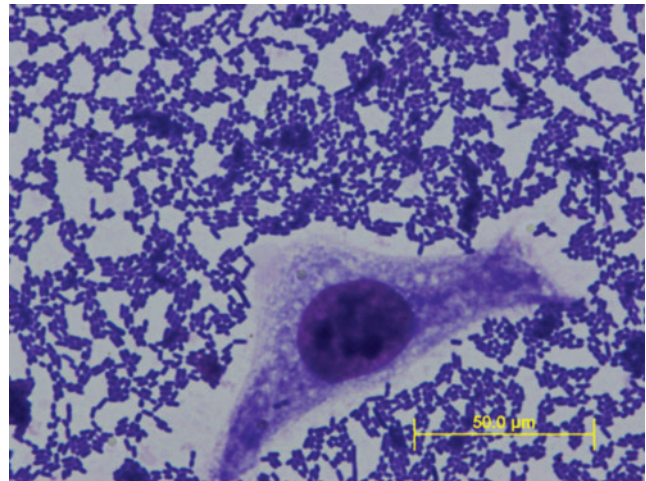


Fig. 1. HEp-2 cell-adherence assay of EAEC, showing the aggregative 'stacked-brick' pattern.

Animal and *in vitro* culture studies show that EAEC survives within the mucus layer, explaining why individuals infected with EAEC, especially children in developing countries with pre-existent malnutrition, may develop mucoid stools, malnutrition, and persistent colonization with prolonged diarrhoea. One study has identified biofilm production in 48 of 62 (77 %) EAEC strains from Japanese children with diarrhoea, using a quantitative biofilm assay, suggesting that this assay may be a useful and convenient screening tool for EAEC (Wakimoto *et al.*, 2004). Transposon mutagenesis studies suggest that biofilm production by EAEC strain 042 is dependent on two genes. *Fis* is a chromosomal gene encoding a DNA-binding protein involved in growth-phase-dependent regulation, and *yafK* encodes a secreted 28 kDa protein (Sheikh *et al.*, 2001). Both genes are mediated by AAF and likely reflect its interaction with the intestinal mucosa. Molecular epidemiologic studies are ongoing to determine the clinical impact of infection with EAEC strains that produce biofilm, and to investigate the genetic markers that identify biofilm-producing EAEC.

The third stage of EAEC pathogenesis involves release of EAEC toxins, and elicitation of an inflammatory response, mucosal toxicity and intestinal secretion. Numerous EAEC toxins have been described. Both animal and human studies show that EAEC toxins are destructive to the tips and sides of intestinal villi and enterocytes. Several toxins are part of this process. The three toxins best studied are plasmid-encoded toxin (Pet) (Navarro-Garcia *et al.*, 2001), EAEC heat-stable enterotoxin (EAST1) (Menard & Dubreuil, 2002), and *Shigella* enterotoxin 1 (ShET1) (Behrens *et al.*, 2002). Pet is a cytopathic serine protease autotransporter that functions as an enterotoxin and a cytotoxin (Dutta *et al.*, 2002). Intracellular expression of Pet is accompanied by cleavage of spectrin within the cytoskeleton of intestinal microvilli. *In vitro* studies show that purified toxin induces cell elongation and rounding, followed by exfoliation of cells

from the substratum. These effects are accompanied by loss of actin stress fibres and electrophysiologic changes (Sui *et al.*, 2003). EAST1 is encoded by *astA* and is a heat-stable protein similar to the heat-stable toxin of ETEC. EAST1 was originally detected in EAEC strains. However, EAST1 has subsequently been identified in ETEC, EHEC, EPEC and DAEC.

The host inflammatory response to EAEC infection is dependent on the host innate immune system and the EAEC strain. The role of putative virulence genes and clinical outcomes is unclear. EAEC carrying 'virulence' genes are not always associated with disease; however, virulence factors are associated with increased levels of faecal cytokines and inflammatory markers, such as interleukin (IL)-1 α , IL-1 β , IL-8, interferon (INF)- γ , lactoferrin, faecal leukocytes, and occult blood (Jiang *et al.*, 2002; Huang *et al.*, 2004b). IL-8 is an important pro-inflammatory chemokine involved in EAEC pathogenesis. IL-8 is responsible for recruiting neutrophils to the epithelial mucosa without mucosal injury, and facilitates intestinal fluid secretion (Kucharzik *et al.*, 2005; Sansonetti *et al.*, 1999; Madara *et al.*, 1993). Travellers to Mexico who developed symptomatic illness due to EAEC infection excreted high concentrations of faecal IL-8 compared to travellers who did not develop diarrhoea due to EAEC infection (Jiang *et al.*, 2003). In addition to IL-8, intestinal epithelial cells infected with EAEC 042, the prototype strain, have been shown to upregulate the following genes: IL-6, tumour necrosis factor (TNF)- α , growth-related gene product (GRO)- α , GRO- γ , intercellular adhesion molecule (ICAM)-1, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-1 α . These cellular responses are primarily mediated by flagellin (*fliC*), a major bacterial surface protein of EAEC (Harrington *et al.*, 2005). Flagellin causes IL-8 release from several epithelial cell lines by binding to Toll-like receptor 5 (TLR5). TLR5 signals through P38 mitogen-activating protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) to induce transcription of pro-inflammatory cytokines from epithelial and monocytic cells (Khan *et al.*, 2004). MAPK is a member of a family of stress-related kinases that influences a diverse range of cellular functions, including host inflammatory responses to microbial products (Khan *et al.*, 2004).

It is clear that multiple factors contribute to EAEC-induced inflammation, and further characterization of the nature of EAEC pro-inflammatory factors will greatly advance the understanding of this emerging pathogen.

Clinical manifestations of EAEC infection

The clinical symptoms of EAEC infection vary from one study to another (Table 3). Although not all EAEC infections result in symptomatic illness (Adachi *et al.*, 2002a), most studies suggest that EAEC infection results in gastrointestinal disease. The most commonly reported symptoms are watery diarrhoea with or without blood and mucus, abdominal pain, nausea, vomiting, and low-grade fever. EAEC can cause both an acute and a chronic (>14 days) diarrhoeal illness. Malnourished hosts, especially children living in developing countries, may be unable to repair mucosal damage and thus may become prone to persistent or chronic diarrhoea. The incubation period of EAEC diarrhoeal illness ranges from 8 to 18 h. The myriad variations of clinical symptoms of EAEC infection are due to factors such as host genetic susceptibility, host immune response, heterogeneity of virulence among EAEC strains, and the amount of bacteria ingested by the infected host.

Host susceptibility

Clinical manifestations of EAEC diarrhoea vary from individual to individual, depending upon the genetic composition of the host (Jiang *et al.*, 2003). The distribution of IL-8 genotypes in symptomatic and asymptomatic subjects infected with EAEC has been studied. Differences in the allele frequencies of T to A polymorphisms in the promoter region of IL-8 located -251 bp upstream of the transcription start site have been identified (Jiang *et al.*, 2003). An AA (OR 208.5; 95 % CI 28.5–1525.4) or AT (OR 14.3; 95 % CI 1.98–105.7) genotype at the -251 position in the promoter region of IL-8 results in greater chances of developing symptomatic EAEC infection compared with those of the TT genotype (Jiang *et al.*, 2003). An AA genotype is also associated with increased concentrations of faecal IL-8 than those of the AT and TT genotypes. Single nucleotide polymorphisms (SNPs) in regulatory and codon

Table 3. Clinical manifestations of EAEC infection

Clinical symptoms	Range	References
Watery diarrhoea	0–76 %	Nataro (2005); Adachi <i>et al.</i> (2002a); Glandt <i>et al.</i> (1999); Infante <i>et al.</i> (2004); Kahali <i>et al.</i> (2004)
Abdominal pain/cramping	45.5 %	Kahali <i>et al.</i> (2004)
Nausea \pm vomiting	41.1–64.5 %	Kahali <i>et al.</i> (2004); Pabst <i>et al.</i> (2003)
Fever (>38.5 °C)	18–19.8 %	Kahali <i>et al.</i> (2004); Pabst <i>et al.</i> (2003)
Stool characteristics		
Diarrhoea with blood	3.3–31.1 %	Regua-Mangia <i>et al.</i> (2004); Bouckennooghe <i>et al.</i> (2000)
Diarrhoea with mucus	4.1–55.2 %	Regua-Mangia <i>et al.</i> (2004); Bouckennooghe <i>et al.</i> (2000)
Diarrhoea with faecal leukocytes	28.9 %	Regua-Mangia <i>et al.</i> (2004); Bouckennooghe <i>et al.</i> (2000)

regions of other chemokines and cytokines are an area of active investigation. In addition to determining host susceptibility to EAEC infection, SNPs are important in studying the epidemiology, risk assessment and pathogenesis of EAEC. These factors may define populations that benefit from therapeutic interventions, such as prophylactic antibiotic therapy or vaccination.

Diagnosis

The gold standard for identifying EAEC is the HEp-2 cell-adherence assay (Nataro & Kaper, 1998). This assay identifies EAEC by its 'stacked brick' aggregating phenotype (Fig. 1). Variations in the assay have been described. A formalin-fixed HEp-2 cell-adherence assay is reported to be sensitive (98 %) and specific (100 %) compared to the traditional assay, while reducing the risk of contamination (Miqdady *et al.*, 2002). It remains to be determined if this assay will improve the efficiency of EAEC identification and be routinely used in diagnostic laboratories. The HEp-2 cell-adherence assay is currently performed only in research settings, and is labour intensive (Huang *et al.*, 2004b). A clump formation test has also been described to be useful in the identification of EAEC (Iwanaga *et al.*, 2002).

Other diagnostic tools for identifying EAEC have been reported (Table 4) (Bouzari *et al.*, 2005; Ruiz-Blazquez *et al.*, 2005). A DNA probe from the pAA plasmid of EAEC is specific for EAEC strains, but has variable sensitivity (Scaletsky *et al.*, 2002). A PCR, a multiplex PCR, and a real-time PCR assay to detect EAEC virulence genes and a plasmid region corresponding to the DNA probe, are also available (Amar *et al.*, 2004). A problem with using DNA probes and PCR assays to identify EAEC is that EAEC strains are very heterogeneous, and this may account for the varying sensitivity of these techniques (Sarantuya *et al.*, 2004).

Treatment

EAEC infections are usually self-limiting and responsive to oral rehydration therapy (Huang *et al.*, 2004b). Antimicrobial therapy for travellers' diarrhoea and paediatric diarrhoea should be based on an individual basis, and remains largely an empirical treatment (DuPont & Ericsson, 1993). Antimicrobial susceptibility patterns of

EAEC strains vary by geographic region. Some studies have reported EAEC to have moderate- to high-level resistance to ampicillin, tetracycline, trimethoprim, sulfamethoxazole and chloramphenicol (Sobieszczanska *et al.*, 2003). One study suggests that integrons that include the *dfxA5*, *aadA1a*, *drA13* and *oxa5* cassette may be responsible for antibiotic resistance in EAEC (Gassama *et al.*, 2004). In most regions of the world, EAEC strains are susceptible to fluoroquinolones, azithromycin, rifaximin, amoxycillin/clavulanic acid, and nalidixic acid (Glandt *et al.*, 1999; Infante *et al.*, 2004).

Two clinical trials have been conducted evaluating treatment of EAEC diarrhoea in travellers (Table 5). The first trial evaluated the clinical response of travellers with EAEC diarrhoea to ciprofloxacin (500 mg twice a day for 3 days) (Glandt *et al.*, 1999), and the second trial evaluated the clinical response to rifaximin (200 and 400 mg twice a day for 3 days), a poorly absorbed antimicrobial agent (Infante *et al.*, 2004). In the first trial, 29 of 64 (45 %) US travellers to Jamaica and Mexico developed diarrhoea due to EAEC. Sixteen of the patients were treated with ciprofloxacin and 13 with placebo. The patients treated with ciprofloxacin had a significant reduction in the duration of post-treatment diarrhoea (35 versus 56 h), and a non-significant reduction in the mean number of unformed stools passed during the 72 h after enrolment compared to patients who received placebo (six episodes versus eight episodes). The second trial was multicentred, and included 43 of 137 (32 %) US travellers to Guatemala, Kenya and Mexico who developed diarrhoea due to EAEC. Thirty of the patients were treated with rifaximin and 13 with placebo. The patients treated with rifaximin had a significant reduction in the duration of post-treatment diarrhoea compared to placebo (22 versus 72 h).

Conclusion

EAEC is an increasingly recognized cause of diarrhoea. It is a cause of acute and chronic diarrhoea among children, adults and HIV-infected persons. EAEC has been responsible for diarrhoea in a volunteer study and in numerous outbreaks and case-control studies from both the developing and the developed world. EAEC causes gastrointestinal disturbance by a complex host-pathogen

Table 4. Available diagnostic tools for the identification of EAEC

Diagnostic tool	Source	Sensitivity	Specificity	References
Formalin-fixed HEp-2 cell-adherence assay	Bacteria	94–98 %	100 %	Miqdady <i>et al.</i> (2002)
Real-time PCR (LightCycler assay)	Bacteria	100 %	100 %	Bischoff <i>et al.</i> (2005)
DNA probe (CVD432)	Bacteria	15–89 %	99 %	Bouzari <i>et al.</i> (2005); Scaletsky <i>et al.</i> (2002)
PCR (<i>aggR</i> , <i>aap</i> , <i>aggA</i> , <i>aafA</i> , <i>astA</i>)	Bacteria	86–94 %	78–100 %	Cerna <i>et al.</i> (2003); Ruiz-Blazquez <i>et al.</i> (2005); Ruttler <i>et al.</i> (2002); Tsai <i>et al.</i> (2003)
Multiplex PCR (CVD432)	Bacteria	100 %	100 %	Aranda <i>et al.</i> (2004); Nguyen <i>et al.</i> (2005)
Clump formation test	Bacteria	82–100 %	89–100 %	Iwanaga <i>et al.</i> (2002)

Table 5. Clinical trials evaluating treatment of diarrhoea due to EAEC in travellers

TLUS, time to last unformed stool; NA, not applicable.

	Study one (Mexico, Jamaica)*		Study two (Mexico, Guatemala, Kenya)†	
Treatment	Ciprofloxacin (<i>n</i> =38)	Placebo (<i>n</i> =26)	Rifaximin (<i>n</i> =30)	Placebo (<i>n</i> =13)
Dosage	500 mg twice a day for 3 days	Twice a day for 3 days	200 or 400 mg twice a day for 3 days	Twice a day for 3 days
Median TLUS (h)	35·3	55·5	22·0	72·0
Microbiological cure	NA	NA	NA	NA

*Glandt *et al.*, 1999.

†Infante *et al.*, 2004.

interaction that involves host genetic susceptibility, heterogeneity of virulence among EAEC strains, and the amount of bacteria ingested by the infected host. Although identification of EAEC is currently limited to research laboratories, new and more efficient ways of identifying EAEC are being developed to allow for standardized laboratory testing on a regular basis. EAEC infections are usually self-limiting, and should be managed on an individual basis. However, two clinical trials have shown that EAEC diarrhoea in travellers is responsive to ciprofloxacin and rifaximin therapy. This review describes the importance of EAEC as an enteric pathogen, updates the epidemiology, pathogenesis, diagnosis and treatment of EAEC, and highlights the importance of further study of EAEC to advance our understanding of this increasingly recognized emerging enteric pathogen.

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