

## Review

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# Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection

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Enteroaggregative *Escherichia coli* (EAEC) is a subgroup of diarrhoeagenic *E. coli* (DEC) that during the past decade has received increasing attention as a cause of watery diarrhoea, which is often persistent. EAEC have been isolated from children and adults worldwide. As well as sporadic cases, outbreaks of EAEC-caused diarrhoea have been described. The definition of EAEC is the ability of the micro-organism to adhere to epithelial cells such as HEp-2 in a very characteristic 'stacked-brick' pattern. Although many studies searching for specific virulence factor(s) unique for this category of DEC have been published it is still unknown why the EAEC cause persistent diarrhoea. In addition, the aggregative property of EAEC causes a lot of problems in serotyping due to the cells auto-agglutinating. The gold standard for identification of EAEC includes isolation of the agent and an adherence assay using tissue culture, viz. HEp-2 cells. This assay is in most cases reliable; however, emergence of 'atypical' EAEC has been described in several publications. In addition, the HEp-2 assay is time consuming, demands a tissue culture lab and trained staff. Several molecular biological assays have been described, however, none show 100% specificity.

## Introduction

*Escherichia coli* is the type species of the genus *Escherichia* that contains mostly motile Gram-negative bacilli that fall within the family *Enterobacteriaceae*. It is the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life, and thereafter *E. coli* and the host derive mutual benefit for decades (Kaper *et al.*, 2004). However, there are several highly adapted *E. coli* clones that have acquired specific virulence factors, which increase their ability to adapt to new niches and allow them to cause a broad spectrum of diseases. Three general clinical syndromes can result from infection with pathogenic *E. coli* strains: enteric/diarrhoeal disease, urinary tract infection and sepsis/meningitis (Nataro & Kaper, 1998). As long as these bacteria do not acquire genetic elements encoding virulence factors, they remain benign commensals. Strains that acquire 'foreign' DNA encoding enterotoxins, adhesins or invasion factors become virulent and can cause either a plain, watery diarrhoea or inflammatory dysentery.

## Enteroaggregative *E. coli*

Among the *E. coli* causing intestinal diseases, there are six well-described categories: enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC) (Nataro & Kaper, 1998). These categories have virulence attributes that help bacteria to cause diseases by different

mechanisms. The EIEC, EHEC and ETEC are defined as *E. coli* strains possessing specific virulence attributes, including different toxins, invasion plasmid and colonization factors. Almost half a century ago, Ewing *et al.* (1963) determined that certain serotypes of *E. coli* were associated with outbreaks of diarrhoea. Strains belonging to these serotypes were referred to as EPEC. In 1979, Cravioto and colleagues described an *in vitro* assay based on the adhesion of the bacteria to HEp-2 cells showing that EPEC bind to the cells in a localized pattern (Cravioto *et al.*, 1979). A few years later it was shown that adherent non-EPEC strains were associated with diarrhoea. These strains were called 'enteroadherent *E. coli*' (Cravioto *et al.*, 1991; Mathewson *et al.*, 1985, 1987). At the same time, Nataro *et al.* (1987) recognized two different phenotypes among the enteroadherent strains, i.e. diffuse and aggregative. The finding of Nataro was the first description of EAEC. The aggregative adherence is characterized by a 'stacked-brick' formation of bacterial cells attached to the HEp-2 cells (Nataro & Kaper, 1998; Nataro *et al.*, 1998). The basic strategy of EAEC seems to comprise colonization of the intestinal mucosa, probably predominantly that of the colon, followed by secretion of enterotoxins and cytotoxins (Nataro *et al.*, 1998). Studies on human intestinal specimens indicate that EAEC induces mild, but significant, mucosal damage (Hicks *et al.*, 1996). EAEC strains characteristically enhance mucus secretion from the mucosa, with trapping of the bacteria in a bacterium-mucus biofilm (Nataro & Kaper, 1998). Two further observations support a role of mucus in EAEC pathogenesis: EAEC bound avidly in the rabbit *in vitro*

model (Wanke *et al.*, 1990) and volunteers fed EAEC strains excrete mucoid stools (Nataro *et al.*, 1995). The formation of a heavy biofilm may be related to the diarrhoeagenicity of the organisms and, perhaps, to its ability to cause persistent colonization and diarrhoea. In addition to forming a mucous biofilm, many EAEC strains induce cytotoxic effects on the intestinal mucosa. In animal models infected with EAEC in rabbit and rat ileal loops, light microscopy showed some destructive lesions (Vial *et al.*, 1988). EAEC also induced shortening of the villi, haemorrhagic necrosis of the villous tips, and a mild inflammatory response with oedema and mononuclear infiltration of the submucosa. Both light and electron microscopy showed adherent bacteria without the attaching and effacing lesion, which is characteristic of EPEC. The clinical features of EAEC diarrhoea are increasingly well defined in outbreaks, sporadic cases and the volunteer model. Typical illness is characterized by watery, mucoid, secretory diarrhoea with low-grade fever and little to no vomiting (Bhan *et al.*, 1989b; Paul *et al.*, 1994). However, up to one third of patients with EAEC diarrhoea had grossly bloody stools (Cravioto *et al.*, 1991).

## Epidemiology

### Developing countries

A growing number of studies have supported the association of EAEC with diarrhoea in developing countries, most prominently in association with persistent diarrhoea (Bhan *et al.*, 1989a, b, c; Fang *et al.*, 1995; Lima *et al.*, 1992). Several studies in children with diarrhoea have shown a significant difference in the prevalence of EAEC compared to the controls (Bhatnagar *et al.*, 1993; Bouzari *et al.*, 1994; Cravioto *et al.*, 1991; Gonzalez *et al.*, 1997; Nataro *et al.*, 1987). EAEC and persistent diarrhoea syndrome have been consistently associated (Fang *et al.*, 1995; Lima *et al.*, 1992; Wanke *et al.*, 1991). The increasing number of such reports and the rising proportion of diarrhoeal cases in which EAEC are implicated suggest that EAEC are important emerging agents of paediatric diarrhoea.

During the initial years after the discovery of EAEC there were a lot of doubts about the pathogenicity of this category of diarrhoeagenic *E. coli* (DEC) (Echeverria *et al.*, 1992; Gomes *et al.*, 1989). However, Nataro *et al.* (1995) showed that a reference strain of EAEC could cause diarrhoea in a volunteer study. In addition, a number of outbreaks have proven that at least some EAEC strains cause diarrhoea in humans. Furthermore, many case-control and cohort surveys of the past 15 years strongly suggest that EAEC is an important cause of diarrhoea in people of all ages in developing and industrialized countries. In a recent study from Vietnam by Vu Nguyen *et al.* (2006) it was shown that EAEC is more frequently associated with diarrhoea in children less than 2 years of age. In this study, 587 children of less than 5 years of age with diarrhoea and 249 age-matched healthy controls were examined for, among other pathogens, EAEC. Of all identified EAEC strains in the diarrhoeal group, 87 % were isolated from children less than 2 years of age. The corresponding figure for the control

group was 39 % (Vu Nguyen *et al.*, 2006). Many of the epidemiological surveys that identified EAEC as a diarrhoeal pathogen were done in developing countries. However, EAEC has been found to be associated with diarrhoea in developed countries as well.

### Developed countries

In a Scandinavian case-control study the prevalence and the association of EAEC with diarrhoea was greater than for EPEC (Bhatnagar *et al.*, 1993). Another study conducted in east London showed that EAEC could be recovered from children with acute and persistent diarrhoea (Chan *et al.*, 1994). A clear association of EAEC with diarrhoea in children in Germany was shown by Huppertz *et al.* (1997) who recovered EAEC from 16 (2 %) of 798 children with diarrhoea but none from 580 healthy controls. Other European studies in children (Knutton *et al.*, 2001; Presterl *et al.*, 1999) also indicate that EAEC may be a leading cause of diarrhoeal disease in developed as well as developing countries. In a Serbian neonatal ward an outbreak of EAEC diarrhoea was described by Cobeljic *et al.* (1996) where 19 babies were affected and 3 of these patients got diarrhoea. One EAEC strain resistant to multiple antibiotics was implicated. In the largest reported outbreak so far, 2697 (40.6 %) Japanese children who ate infected school lunches had severe diarrhoea and EAEC was found in 10 % of cases (Itoh *et al.*, 1997). Several other outbreaks both in children and in adults have been described in the UK (Smith *et al.*, 1997; Spencer *et al.*, 1999), India (Pai *et al.*, 1997) and France (Boudailliez *et al.*, 1997; Morabito *et al.*, 1998).

In a 1 year prospective Swedish study on enteropathogens in adult patients with diarrhoea and healthy control subjects, 105 of 760 patients with diarrhoea were positive for DEC. EAEC was present in 16 cases and was the second most common isolate among DEC surpassed only by ETEC (Svenungsson *et al.*, 2000). Other studies have shown that this pathogen is an important cause of travellers' diarrhoea (Adachi *et al.*, 2001; Gascón *et al.*, 1998; Schultsz *et al.*, 2000). However, documented reports are less common because EAEC is not sought in many studies. Travellers to all developing countries are at risk and if EAEC were sought in all laboratories, infection by this pathogen could explain over 25 % of cases for which no pathogen is recovered (Adachi *et al.*, 2001).

### Immunocompromised

Diarrhoea is an important cause of morbidity in the immunocompromised. A wide range of pathogens is implicated in AIDS-associated diarrhoea, and in many cases a causative agent is not found. Case reports from Mayer & Wanke (1995) described that EAEC could be recovered from diarrhoeal stools from AIDS patients. There have been other reports after epidemiological surveillance among HIV-positive patients (Mayer & Wanke, 1995).

## Virulence factors

In order to cause diarrhoeal disease, EAEC adheres to intestinal mucosa, forms a mucoid biofilm and induces toxic effects on the intestinal mucosa that result in diarrhoea. The exact mechanism of pathogenesis is not fully understood; however, adhesins, toxins and several other factors have been implicated. Certain strains carry a high molecular weight plasmid associated with the aggregative adherence (Law *et al.*, 1998; Vial *et al.*, 1988), on which a number of virulence genes are located. These are (i) heat stable toxin-1 (EAST-1) (Savarino *et al.*, 1991), (ii) aggregative adherence fimbriae I and II (AAF/I and AAF/II) (Nataro *et al.*, 1992; Rich *et al.*, 1999), as well as the AAF/III (Bernier *et al.*, 2002). Also located on the plasmid are (i) transcriptional activator gene (Nataro *et al.*, 1994, 1998), (ii) anti-aggregation protein gene (Sheikh *et al.*, 2002), and (iii) anti-aggregation protein transporter gene (Baudry *et al.*, 1990). In addition, the plasmid-encoded toxin (Eslava *et al.*, 1998) and a cryptic ORF known as *shf* (Czeczulin *et al.*, 1999) have been described. Other virulence factors that are believed to be associated with EAEC are 18 and 30 kDa outer-membrane adhesins (Chart *et al.*, 1997; Debroy *et al.*, 1995; Grover *et al.*, 2001). In addition, different pathogenicity islands have been identified within the EAEC group, including *Shigella she* pathogenicity island, containing enterotoxin and mucinase genes (Henderson *et al.*, 1999), and *Yersinia* high-pathogenicity island, containing the yersiniabactin siderophore gene (Schubert *et al.*, 1998). Strains that carry the high molecular weight plasmid represent an important subgroup (Cerna *et al.*, 2003; Elias *et al.*, 2002) and may be regarded as 'typical EAEC'. However, EAEC is a heterogeneous group of *E. coli* (Czeczulin *et al.*, 1999) and certain strains, although adherent to the HEp-2 cells in the stacked-brick mode, lack the high molecular weight plasmid and can be called 'atypical EAEC' (Cobeljic *et al.*, 1996; Elias *et al.*, 2002; Gioppo *et al.*, 2000; Itoh *et al.*, 1997).

## Identification – serotyping and molecular biology

However, none of the above-described gene(s) is conserved among all of the EAEC and many are not unique to this category of DEC. Recently, Jenkins *et al.* (2006a) used several different probes to characterize EAEC strains. The results show that the majority of the HEp-2 positive strains were also positive for the anti-aggregation protein transporter gene described by Baudry *et al.* (1990) against which Schmidt and colleagues developed a PCR assay in 1995 (Schmidt *et al.*, 1995). Overall, 143 EAEC strains were analysed and 128 (90%) were positive for the anti-aggregation protein transporter gene (Jenkins *et al.*, 2006a). However, 10% of the strains verified by HEp-2 assay were negative in the PCR assay. This makes it difficult to provide a genotypic definition for EAEC and to design specific molecular biological assays for the detection of this pathotype. In addition, serotyping of EAEC is a problem. Due to their aggregative phenotype, many of the strains auto-agglutinate and are often described

in the literature as non-typable or as O-rough. It is also well established that the EAEC group is highly heterogeneous. Huppertz *et al.* (1997) analysed EAEC from German children and found that of 14 typable isolates all belonged to different serotypes. In another study in the UK, 97 EAEC strains were serotyped to 40 different O-types. However, the remaining 121 EAEC isolates were non-typable (Food Standards Agency, 2000). In a study by Jenkins *et al.* (2006a), 93 out of 143 EAEC strains could be serotyped and belonged to as many as 47 different serotypes. This shows that serotyping, although useful in the characterization of other DEC, is of little value in the diagnosis of EAEC. As mentioned earlier, the HEp-2 adherence test is the gold standard for the identification of EAEC. This test requires specialized facilities and can therefore only be conducted in reference laboratories, and strict adherence to protocol is required (Haider *et al.*, 1992; Vial *et al.*, 1990). Several attempts have been made to develop a molecular biological assay for the identification of EAEC. In 1990, Baudry and colleagues developed a DNA probe, CVD432, that was found to be 89% sensitive and 99% specific for EAEC (Baudry *et al.*, 1990). Based on this probe, a PCR assay was developed by Schmidt *et al.* in 1995. This assay has been widely used since then. In search for additional virulence factors, several other assays have been described, none of which with has as high sensitivity and specificity as the one described in 1995. The problems in identification of EAEC have been clearly highlighted very recently (Jenkins *et al.*, 2006b). The authors set up a multiplex PCR targeting three different genes: (i) the anti-aggregation protein transporter gene described by Baudry *et al.* (1990), (ii) the EAST gene (Savarino *et al.*, 1991) and (iii) a chromosomal gene present in the *pheU* pathogenicity island designated *aggR*-activated island (Jenkins *et al.*, 2006b). The authors used this multiplex PCR to identify EAEC from patients in the community suffering from diarrhoea. They found that twice as many isolates were positive against at least one of the primers as compared to the HEp-2 adherence assay alone.

## Conclusion

The HEp-2-adherence assay and/or the CVD432 standard probe represent the best means at present for detecting EAEC. However, it is clear that more sensitive, specific and practical methods are needed in order to improve the diagnosis of infected cases with EAEC and to understand the disease.

## Note from the editor-in-chief

A review on EAEC by Huang and colleagues was published in the *Journal of Medical Microbiology* October 2006 issue (Huang *et al.*, 2006) that concentrated on the epidemiology and pathogenic mechanisms of the bacterium. More recently a paper by Jenkins and colleagues (Jenkins *et al.*, 2006b) has been published drawing attention to the difficulties in diagnosing EAEC infection by molecular methods. This mini review by Andrej Weintraub discusses the problem further.

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