MINIREVIEW

An overview of atypical enteropathogenic Escherichia coli

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Abstract

The enteropathogenic Escherichia coli (EPEC) pathotype is currently divided into two groups, typical EPEC (tEPEC) and atypical EPEC (aEPEC). The property that distinguishes these two groups is the presence of the EPEC adherence factor plasmid, which is only found in tEPEC. aEPEC strains are emerging enteropathogens that have been detected worldwide. Herein, we review the serotypes, virulence properties, genetic relationships, epidemiology, reservoir and diagnosis of aEPEC, including those strains not belonging to the classical EPEC serogroups (nonclassical EPEC serogroups). The large variety of serotypes and genetic virulence properties of aEPEC strains from nonclassical EPEC serogroups makes it difficult to determine which strains are truly pathogenic.

Introduction

Escherichia coli is one of the best known and best characterized bacterial species. Escherichia coli strains associated with the human host are classified as commensals, enteric pathogens [diarrheagenic E. coli (DEC)] or extraintestinal pathogens according to their set of virulence genes and clinical properties (Russo & Johnson, 2000; Kaper et al., 2004). The latter pathogens comprise various pathotypes, which can cause human and/or animal infections.

DEC strains have been classified into six different pathotypes: enterotoxigenic E. coli, enteroinvasive E. coli, enterogaugregative E. coli, diffusely adherent E. coli, enteropathogenic E. coli (EPEC) and Shiga toxin-producing E. coli (STEC) (reviewed by Kaper et al., 2004). The various E. coli pathotypes tend to be clonal groups that share O (somatic) and H (flagellar) antigens, which define their serogroups (O antigen only) or serotypes (O and H antigens) (reviewed by Kaper et al., 2004). The World Health Organization recognized that EPEC comprises strains of 12 O serogroups: O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158 (WHO, 1987), also known as the classical EPEC serogroups.

In 1995, EPEC was divided into two groups, typical EPEC (tEPEC) and atypical EPEC (aEPEC). The basic difference between the two groups is the presence of the EPEC adherence factor plasmid (pEAF) in tEPEC and its absence in aEPEC (Kaper, 1996). Trabulsi et al. (2002) described many other differential characteristics between tEPEC and aEPEC. By the time that the latter review was published, most studies encompassed aEPEC strains mainly belonging to the classical EPEC serogroups. However, various E. coli strains that fit the concept of aEPEC, but that belong to nonclassical EPEC serogroups, have been identified in the last several years. Thus, we can define aEPEC as E. coli strains that may or may not belong to the classical EPEC serogroups that produce the characteristic histopathologic lesion known as attaching and effacing on intestinal cells, but do not express the bundle-forming pilus (BFP) and lack Shiga-toxin genes.

Although the epidemiological association of aEPEC with diarrhea is still controversial, its high prevalence worldwide
and the involvement of some strains with diarrheal outbreaks support the concept that some aEPEC strains are diarrheagenic. The aim of this review is to gather information regarding serotypes, virulence properties, genetic relationships, epidemiology, reservoir and diagnosis of aEPEC strains, including those not belonging to the classical EPEC serogroups.

**Serotypes**

A vast serotype diversity of aEPEC strains, particularly when considering nonclassical EPEC serogroups, has been reported by several authors worldwide (Supporting Information, Table S1). Although most of these studies (62.5%) include strains isolated from preschool children both with and without diarrhea, some of them include strains from diarrheic adults. Regarding serogroups, 113 different types have been reported in a total of 746 strains, including the 12 classical EPEC serogroups. Approximately 81% of the aEPEC strains reported in Table S1 do not belong to the classical EPEC serogroups, and 26.6% of them are O nontypeable. The most frequent serogroup is O51 (4.7%), followed by O145 (4.3%), O26, O55 and O111 (2.5% each), and O119 (2.4%). The O-typeable strains reported so far belong to > 200 different serotypes, including many nontypeable and H nontypeable strains.

**Pathogenesis**

**Attacking and effacing (A/E) lesion**

tEPEC and aEPEC have in common the ability to form A/E lesion, which is the main pathogenic mechanism in both groups. This lesion results from intimate bacterial adherence, local microvillus effacement and accumulation of polymerized actin and other elements of the cytoskeleton underneath adherent bacteria forming pedestal-like structures (Rothbaum et al., 1982; Moon et al., 1983). A/E lesion production has also been detected in enterohemorrhagic E. coli (EHEC), a subgroup of STEC strongly associated with severe human illnesses (reviewed by Nataro & Kaper, 1998). However, the LEE of O51:H40 aEPEC strains is located in the rabbit ileal loop model. Furthermore, Bai et al. (2008) have shown that although aEPEC strains of serotype O125:H6 trigger inefficient actin polymerization in vitro, these strains can form A/E lesions in human intestinal biopsies. These authors consequently proposed that strains unable to polymerize actin on in vitro cultured cells (FAS-negative strains) should be confirmed as nonpathogenic by alternative methods, such as infection of cultured human intestinal explants (in vitro organ culture).

**Locus of enterocyte effacement (LEE) region**

The genes necessary for the establishment of A/E lesions are located on the pathogenicity island (PAI) designated as the LEE (McDaniel et al., 1995). LEE encodes the structural components of a type III secretion system (T3SS), regulators, translocators, chaperones and effector molecules that alter diverse cell signaling processes (reviewed by Garmendia et al., 2005a). LEE also encodes the outer membrane adhesive protein intimin and its translocated receptor Tir (translocated intimin receptor) (Jerse et al., 1990; Kenny et al., 1997).

The LEE PAI of the clinical aEPEC isolates 3431-4/86 (O8:HNM), 0181-6/86 (O119:H9), B6 (O26) and 9812 (O128:H2) were sequenced and compared (Gärtner & Schmidt, 2004; Bielaszewska et al., 2007a). These LEE islands exhibited genetic organization analogous to that of the prototype tEPEC strain E2348/69 and other pathogenic E. coli strains (Bielaszewska et al., 2007a). Although the genes constituting the T3SS apparatus are highly conserved, the LEE-encoded effector proteins and the flanking regions of the LEE exhibit major differences.

LEE is inserted at different sites in the E. coli chromosome, downstream of the genes encoding the tRNA for selenocysteine (selC) or phenylalanine (pheU or phev) (McDaniel et al., 1995; Sperandio et al., 1998; Jores et al., 2001). The variable location of the LEE within different EPEC strains suggests that different strains may have acquired the LEE from different progenitors at various times (reviewed by Donnenberg & Whittam, 2001), or that LEE can be mobilized and reintegrated within individual E. coli strains (Tauschek et al., 2002). Furthermore, it has been shown that LEE insertion sites vary according to the clonal origin of the E. coli strains (Wieder et al., 1997). In most aEPEC strains, the LEE is inserted in selC or pheU (Sperandio et al., 1998; Vieira et al., 2001; Dulguer et al., 2003). However, the LEE of O51:H40 aEPEC strains is located in phev, as indicated by PCR analysis (Moreira et al., 2008).

**Intimin**

Intimin, which is encoded by the E. coli attaching and effacing (aae) gene, is required for intimate adhesion to epithelial cells and cytoskeletal reorganization (Jerse et al., 1990). The variable 280-amino acid C-terminal sequence of intimin (Int280) defines many different intimin subtypes (Frankel et al., 1994; Adu-Bobie et al., 1998; Lacher et al., 2006). Correlation between the expression of some intimin
subtypes and tissue tropism has been demonstrated in vivo (Phillips & Frankel, 2000). Although the host tissue distribution of EPEC strains is probably multifactorial, characterization of different intimin subtypes could provide information regarding tissue tropism (reviewed by Torres et al., 2005).

Intimin subtypes α, β, γ, ζ, δ and ε appear to be the most frequent among aEPEC strains of different serotypes worldwide, while other intimin subtypes are less frequent (Gomes et al., 2004; Blanco et al., 2006a, b; Jenkins et al., 2006; Abe et al., 2007). aEPEC strains of a single serotype isolated in different geographic areas were shown to carry the same intimin subtype, for example, aEPEC serotype O51:H40 isolated in Brazil, Uruguay and Spain possessing intimin subtype θ (Blanco et al., 2006a, b; Moreira et al., 2008). Conversely, certain aEPEC serotypes carry different intimin subtypes, for example, O80:H26 carries either intimin subtype β or ε (Blanco et al., 2006a; Jenkins et al., 2006). The occurrence of aEPEC strains of a single serotype carrying different intimin subtypes confirms the diversity of aEPEC strains and reinforces the proposal that the LEE may have been acquired at different periods of time by different strains (Donnenberg & Whittam, 2001).

**Tir**

Tir is inserted in the plasma membrane of eukaryotic cells, exposing its middle segment at the cell surface. The amino and carboxy portions of Tir remain exposed in the cytosol, interacting with host proteins and other bacterial proteins (reviewed by Frankel & Phillips, 2008).

Ooka et al. (2007) have recently found two human aEPEC strains (serotypes O104:H12 and ONT:H19) that harbor new variant Tir proteins, which exhibit < 65% sequence identity to Tir molecules reported previously. While the Tir intimin-binding domains of these strains are highly conserved, the cytoplasmic terminal regions are highly divergent. As the functional analysis of these Tir variants is lacking, more information is required to better understand the implications of these findings in aEPEC pathogenesis.

**Non-LEE-encoded putative T3SS effectors**

Various effector proteins encoded outside the LEE, which are secreted by T3SS, have been described in EHEC O157, *Citrobacter rodentium* and tEPEC (Deng et al., 2004; Tobe et al., 2006; Iguchi et al., 2009). The contribution of these various putative effectors (e.g. NleA/EspI, NleB, NleC, NleD, NleE, NleF, NleG and NleH) to virulence is still under investigation (Gruenheid et al., 2004; Mundy et al., 2004; Marchés et al., 2005; Kelly et al., 2006; Li et al., 2006; reviewed by Dean & Kenny, 2009). Another non-LEE-encoded effector is the cycle-inhibiting factor (Gif), which is encoded by a lambdoid prophage in several tEPEC and EHEC strains and causes an irreversible cytopathic effect in host cells (Marchés et al., 2003; Taieb et al., 2006). EspG2, which is encoded on the EspC PAI, triggers the disruption of the microtubule network of the host cells (Shaw et al., 2005).

Studies on the frequency of these non-LEE effectors among aEPEC strains are scarce. In a case control study in Norwegian children, Afset et al. (2006) found that in aEPEC strains, the *nleB* and *nleE* genes were statistically associated with diarrhea. In addition, in a study conducted in Brazil, the Nle-encoding genes were more frequent in tEPEC than in aEPEC strains, with *espG2* and *nleC* being the most, and *nleD* the least frequent in both groups. The *espG2* and *nleC* genes occurred in 70% and 58% of 63 aEPEC strains, respectively (T.A.T. Gomes et al., unpublished data).

In contrast to the LEE of tEPEC, the LEE of EHEC is not sufficient for A/E lesion formation (Elliott et al., 1999). EHEC injects into the host cell its own adaptor protein, Tir cytoskeleton coupling protein, which connects EHEC Tir to N-WASP and promotes actin accumulation via Arp2/3 complex activation (Campellone et al., 2004; Garmendia et al., 2004). To date, two variants of the *tccP* gene have been described: *tccP* (carried on prophage CP-933U/Sp14) and *tccP2* (carried on prophage Sp4/CP-933 M). Studies concerning the characterization of aEPEC have shown the presence of *tccP* and *tccP2*, and have demonstrated that aEPEC strains can trigger actin polymerization by Tir-Nck (which depends on the phosphorylation of Y474) and/or Tir-Tccp pathways (Garmendia et al., 2005b; Ooka et al., 2007; Frankel & Phillips, 2008).

**EPEC interaction with host cells**

Characteristically, after 3 h of incubation, tEPEC strains produce localized adherence (LA) to HeLa/HEp-2 cell surfaces, forming compact microcolonies (Fig. 1a) (Scaletsky et al., 1984). LA is mediated by the BFP, a type IV pilus encoded by pEAF, which interconnects bacteria within microcolonies, promoting their stabilization (Girón et al., 1991).

A variant LA pattern has been described in aEPEC strains, which is characterized by the presence of loose clusters of bacteria in few cells, and was named LA like (LAL) (Fig. 1b) (Rodrigues et al., 1996). This pattern is defined only in assays using prolonged incubation periods (6-h assays) and is the most frequent pattern among aEPEC strains (Pelayo et al., 1999; Scaletsky et al., 1999; Vieira et al., 2001; Dulguer et al., 2003; Gomes et al., 2004; Abe et al., 2007). LAL probably corresponds to the poor LA pattern as described previously by Knutton et al. (1991). Notably, many variations of the LA pattern can be found, in which most show a tendency toward the formation of loose clusters (Vieira et al., 2001; Hernandez et al., 2006).
Furthermore, some *E. coli* strains, fitting the concept of aEPEC, can express adherence patterns distinct from LAL, as diffuse adherence (DA) (Fig. 1c) or aggregative adherence (AA) (Vieira *et al.*, 2001; Dulguer *et al.*, 2003; Nunes *et al.*, 2003; Robins-Browne *et al.*, 2004; Abe *et al.*, 2007). In some DA-producing aEPEC strains, this pattern results from the expression of *daaC* and *afa*, which are sequences associated with the Dr family of adhesins (Beinke *et al.*, 1998; Vieira *et al.*, 2001; Keller *et al.*, 2002). Scaletsky *et al.* (2005) cloned a 15-kb genomic region of an aEPEC O26:H11 strain designated the locus for DA (*lda*), which encodes a nonfimbrial structure conferring DA phenotype, and whose expression is induced by bile salts (Torres *et al.*, 2007).


One very large protein called lymphocyte inhibitory factor (LifA) was originally characterized in EPEC strains as the toxin that inhibits lymphocyte activation (Klapproth *et al.*, 2000). An adhesin encoded by the O island 122 (O1-122) PAI termed EHEC factor adhesin 1 (Efa1) was identified in EHEC strains of serotype O111:HNM, showing a similarity of 99.9% with LifA (Nicholls *et al.*, 2000). Although Efa1/LifA has been implicated in the attachment of aEPEC strains to host cells (Badea *et al.*, 2003), its association with diarrheal diseases is controversial (Robins-Browne *et al.*, 2004; Afset *et al.*, 2006). In a recent study in Brazil, the efa1/lifA gene was found to be more frequent among tEPEC (60%) than among aEPEC (28%) strains (M.A.M. Vieira *et al.*, unpublished data).

Although we have recently identified many other putative *E. coli* adhesin-encoding genes (*paa, LpfA*O113, *iha, toxB* and *ldaG*) in a collection of aEPEC strains (T.A.T. Gomes *et al.*, unpublished data), the role of such adhesins remains to be established.

The invasion of intestinal epithelial cells has been considered a critical virulence characteristic of several important enteric pathogens. Although EPEC is considered an extracellular pathogen, some *in vivo* and *ex vivo* studies have shown the presence of these pathogens inside human enterocytes (Polotsky *et al.*, 1977; Ulshen & Rollo, 1980; Pedroso *et al.*, 1993). Moreover, the invasion ability of EPEC strains has been demonstrated *in vitro* using different cell lines, including HeLa, HEP-2 and Caco-2 cells (Andrade *et al.*, 1989; Donnenberg *et al.*, 1989; Francis *et al.*, 1991). Regarding aEPEC strains, whereas some studies have shown that they invade epithelial cells efficiently (Scaletsky *et al.*, 1996; Rosa *et al.*, 2001; Hernandez *et al.*, 2008), other studies have not (Pelayo *et al.*, 1999; Robins-Browne *et al.*, 2004). The pathogenic role of the invasiveness of aEPEC strains is unknown. As invasive organisms may be protected from destruction by the immune system and some antibiotics that do not penetrate eukaryotic cells, invasion could contribute to the permanence of certain aEPEC strains in the intestine, resulting in the persistent diarrhea reported in recent studies (Afset *et al.*, 2004; Nguyen *et al.*, 2006).

**Diversity of virulence properties**

In general, aEPEC strains may carry genes encoding virulence factors of other DEC pathotypes more often than tEPEC strains (Trabulsi *et al.*, 2002). Among 59 aEPEC strains of nonclassical EPEC serogroups tested for the presence of 15 virulence DNA sequences of various DEC pathotypes, Vieira *et al.* (2001) observed 17 different combinations of specific virulence sequences (genetic profiles), which reflected the heterogeneity of the group. Extending...
the analysis of Vieira et al. (2001) to additional putative virulence genes, and including strains from other geographic regions in Brazil, Gomes et al. (2004) demonstrated that the aEPEC strains of nonclassical EPEC serogroups are even more diverse. Interesting genetic combinations were observed, for example, extraintestinal pathogenic E. coli and EAEC strains, and tEPEC (bfpA) and EHEC (ehx) genes. In a survey of aEPEC strains isolated in another geographic region of Brazil, genes encoding the DEC autotransporter proteins Pet, Pic, Sat, EspC and EspP were detected. Except for espC, detected in 29.2%, the other genes were of low prevalence (sat/11.1%, pet/5.5%, espP/4.2% and pic/1.4%) (W.P. Elias et al., unpublished data). These proteins include virulence factors such as toxins, proteases and adhesins (Henderson et al., 2004), but their role in aEPEC pathogenesis remains to be established.

Because genes encoding virulence factors are located on transmissible plasmids, PAIs, transposons or bacteriophages, the emergence of different combinations of virulence gene sequences found in aEPEC strains is not surprising. These sequences could have been transferred horizontally to these strains in the intestine and/or environment. In addition, some of the strains are probably tEPEC that have lost pEAF (or part of it), or EHEC that have lost stx phase sequences during infection.

Escherichia coli of serogroup O26 can bear serotypes that cause haemolytic-uremic syndrome (EHEC strains) or aEPEC associated with uncomplicated diarrhea. Bielaszewska et al. (2007b) compared the presence of the O1-122, which is present in highly virulent EHEC strains, among aEPEC O26 and EHEC O26 clinical isolates. Most of them contained O1-122 virulence genes (efa1/lifA, nleB, nleE and ent) and shared a highly conserved PAI, suggesting that aEPEC O26 and EHEC O26 are closely related. In fact, based on serotypes, non-stx virulence profiles and multilocus sequence types (MLST), Bielaszewska et al. (2008) showed in a study in Germany that most aEPEC strains associated with bloody diarrhea are probably EHEC that had lost Stx-encoding phages (EHEC-LST) during infection. Indeed, recent work in our laboratory has shown the presence of various STEC virulence genes among 117 aEPEC strains, i.e. paa (49.6%), lpfA5113 (26.5%), iha (25.6%), ehx, toxB, kat and espP (6.0% each), and IdAG (2.6%). Interestingly, all ehx-positive strains also carried kat and espP, which are located in the EHEC plasmid (T.A.T. Gomes et al., unpublished data).

Studies concerning aEPEC virulence have aimed at identifying diarrhea-associated virulence genes or phenotypic characteristics. Scaletsky et al. (1999) showed that aEPEC expressing LAL were significantly associated with diarrhea (Table 1). In a study of infectious intestinal disease in England, the characterization of aEPEC strains by serotyping, intimin subtyping and antimicrobial resistance typing showed no significant differences among aEPEC strains isolated from patients vs. healthy controls (Jenkins et al., 2006). Using DNA microarray analyses to search for diarrheal-associated genes, Afset et al. (2006) found that O1-122 genes (efa1/lifA, set/ent, nleB and nleE) and certain genes located elsewhere (lpfA, paa, ehxA and ureD) were associated with diarrhea. In another study, Dulguer et al. (2003) demonstrated a strong association between the enterogaggregative E. coli heat-stable 1 (East-1) encoding-gene (astA) and diarrhea in aEPEC strains isolated in different regions of Brazil. However, whether these virulence-associated genes are expressed in aEPEC is yet to be demonstrated.

**Phylogeny**

Afset et al. (2008) compared the phylogenetic ancestry (groups A, B1, B2 and D) and virulence properties (by DNA microarray analysis) of aEPEC strains to search for an association between ancestry and diarrhea. The strains were serotyped and analyzed by MLST and pulsed-field gel electrophoresis (PFGE). These authors concluded that aEPEC strains are heterogeneous both phylogenetically and by virulence profile.

The investigation of the genetic diversity of aEPEC strains of serotype O51:H40 and their relatedness to tEPEC and EHEC by enterobacterial repetitive intergenic consensus PCR showed a much closer relationship between these strains and EHEC strain EDL933 (68% similarity) than to tEPEC strain E2348/69 (30% similarity) (Moreira et al., 2008).

**Epidemiology**

Recent epidemiological studies suggest an increasing identification of aEPEC in both developed and developing countries (reviewed by Trabulsi et al., 2002, Table 1). Some of these studies have shown an association of aEPEC with childhood diarrhea (Afset et al., 2004; Cohen et al., 2005; Moreno et al., 2008; Estrada-Garcia et al., 2009), whereas others have not (Vieira et al., 2001; Nunes et al., 2003; Nataro et al., 2006; Spano et al., 2008; D.M. Girão, pers. commun.). The studies in Table 1 show the prevalence of aEPEC worldwide and were performed at different times. Although some studies have shown an association of aEPEC strains with acute childhood diarrhea (Scaletsky et al., 1999; Franzolin et al., 2005; Araujo et al., 2007), recent works have implicated aEPEC as agents of persistent diarrhea (Afset et al., 2004; Nguyen et al., 2006).

Despite the controversial data regarding the epidemiological association of aEPEC strains with diarrhea, the occurrence of aEPEC strains in diarrheic patients of various ages and in patients with AIDS (Gassama-Sow et al., 2004; Gomes et al., 2004) indicate that some aEPEC strains with certain genetic combinations are true enteropathogens.
Table 1. Epidemiological studies on aEPEC

<table>
<thead>
<tr>
<th>Location</th>
<th>aEPEC</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Comments*</th>
<th>References</th>
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<tr>
<td>USA (Seattle)</td>
<td></td>
<td>3.8</td>
<td>ND</td>
<td>eae⁺ EAF⁻</td>
<td>Bokete et al. (1997)</td>
</tr>
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<td>Rosa et al. (1998)</td>
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<td>E. coli with LAL</td>
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<td>8.0</td>
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<td>Paciorek (2002)</td>
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<td>2.0</td>
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<td>Scaletsky et al. (2002)</td>
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<td>ND</td>
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<td>11.0</td>
<td>eae⁺ EAF⁻</td>
<td>Regua-Mangia et al. (2004)</td>
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<td>2.3</td>
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<td>Robins-Browne et al. (2004)</td>
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<td>10.0</td>
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<td>Sarantuya et al. (2004)</td>
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<td>4.4</td>
<td>eae⁺ bfpA⁻</td>
<td>Nguyen et al. (2005)</td>
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<td>Estrada-Garcia et al. (2005)</td>
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<td>Bueris et al. (2007)</td>
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<td>Mexico (Mexico City)</td>
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<td>1.2</td>
<td>eae⁺ bfpA⁻</td>
<td>Paniagua et al. (2007)</td>
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<td>Vietnam (Hanoi)</td>
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<td>3.6</td>
<td>eae⁺ EAF bfpA⁻</td>
<td>Hien et al. (2007)</td>
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<td>Kozub-Vitkovski et al. (2008)</td>
</tr>
<tr>
<td>Vietnam (Hanoi)</td>
<td></td>
<td>10.4</td>
<td>6.45</td>
<td>eae⁺ bfpA⁻</td>
<td>Hien et al. (2008)</td>
</tr>
<tr>
<td>Brazil (Espírito Santo)</td>
<td></td>
<td>5.5</td>
<td>6.9</td>
<td>eae⁺ bfpA⁻</td>
<td>Spano et al. (2008)</td>
</tr>
</tbody>
</table>

*Epidemiological studies were selected based on the following criteria: detection of fecal Escherichia coli strains presenting eae and lacking pEAF genes (EAF sequence and or bfpA), except for two studies detecting E. coli strains expressing the LAL phenotype.

1Studies that demonstrate a statistical association of aEPEC strains with diarrhea.
ND, not determined.

Several outbreaks have implicated aEPEC as the causative agent of diarrhea (Viljanen et al., 1990; Hedberg et al., 1997; Trabulsi et al., 2002). In one outbreak involving > 100 adults, the implicated pathogen was E. coli O39:HNM (Hedberg et al., 1997). Another diarrhea outbreak involving > 600 people implicated an O111 aEPEC strain (Viljanen et al., 1990). A waterborne diarrheal outbreak affecting 41 of 75 students (ages 12–15) was reported in Akita Prefecture, Japan (Yatsuyanagi et al., 2003). Among the 41 individuals with clinical symptoms, seven carried aEPEC isolates of serotype ONT:H45. Interestingly, all three outbreak-associated E. coli strains (O39:NM, O111 and ONT:H45) also carried astA. As astA has been detected in commensal E. coli strains as well as other DEC pathotypes (Savarino et al., 1996), the virulence role of East-1 is questionable.

Another outbreak involving aEPEC was described in infants at a daycare center in Japan. The only diarrheagenic bacterial pathogens isolated from the patients were four aEPEC O55:HNM isolates from four patients, which showed identical PFGE patterns, suggesting that the strains originated from common infectious sources (Yatsuyanagi et al., 2002).

**Transmission and reservoir**

A large number of reports on the isolation of aEPEC from a wide range of diarrheic and healthy animal species have been published. In most of the cases, these aEPEC strains have been identified in the course of characterization of A/E E. coli isolates among animals used for food production, such as cattle, sheep, goat, pig and poultry (Aidar et al., 2000; Aktan et al., 2004; Stephan et al., 2004; Blanco et al., 2005; Krause et al., 2005; Leonil et al., 2005; Yuste et al., 2006; Ishii et al., 2007; Wani et al., 2007). Moreover, the detection of aEPEC in domestic animals (dog and cat), horse, deer and marmoset has also been described (Beaudry et al., 1996; Goffaux et al., 2000; Carvalho et al., 2003; Nakazato et al., 2004; Krause et al., 2005; Ishii et al., 2007; A. Cerqueira, pers. commun.).
Although there is no evidence of direct transmission from animals to humans, some aEPEC strains isolated from animals belong to serogroups implicated in human diseases, for example, O26, O103, O119, O128 and O142 (Aidar et al., 2000; Carvalho et al., 2003; Aktan et al., 2004; Nakazato et al., 2004; Blanco et al., 2005; Krause et al., 2005; Leomil et al., 2005; Yuste et al., 2006), suggesting that these animals may represent important reservoirs of aEPEC, which can be transmitted to humans.

Detection and diagnosis

Traditionally, EPEC strains have been identified by the expression of certain serotypes epidemiologically linked to infantile diarrhea. However, as highlighted above, recent studies have shown that aEPEC belong to a large diversity of serotypes, many of which belong to nonclassical EPEC serogroups (Table S1). Furthermore, a substantial percentage of the strains are O and/or H nontypeable and many are nonmotile. In addition, some E. coli strains belonging to the classical EPEC serogroups can harbor virulence properties not associated with EPEC but with other DEC pathotypes (Campos et al., 2004). Therefore, phenotypic and genotypic assays based on the presence (or absence) of certain virulence properties should be used for EPECidentification. Table 2 summarizes the most important properties that may distinguish tEPEC and aEPEC strains.

The phenotypic methods to identify tEPEC and distinguish them from aEPEC include the determination of the LA adherence pattern on HeLa/HEp-2 cells and demonstration of BFP expression by immunologic assays (Fig. 1 and Table 2).

The genotypic assays include PCR and multiplex PCR, as well as genetic probes, to detect pEAF (EAF probe), bfpA (encoding the major pilin subunit of BFP) and eae genes (or other conserved LEE-genes) (Baldini et al., 1983; Jerse et al., 1990; Girón et al., 1993; Aranda et al., 2007; Müller et al., 2007). EPEC strains have been identified by the presence of the LEE region and absence of the stx genes (stx-negative), which distinguish them from EHEC. tEPEC and aEPEC strains are differentiated mainly by the presence of the EAF sequence and/or the bfpA gene in the former group. However, aEPEC strains from serotypes O119:H2 and O128:H2 react with the bfpA probe but lack a true pEAF. Instead, these strains have a large plasmid defective in the bfp operon and, consequently, do not produce BFP, the reason for their classification as aEPEC (Gonçalves et al., 1997; Bortolini et al., 1999). Conversely, some O142:H6 strains that do not react with the EAF probe but produce BFP, probably have a defective but still functional pEAF and are thus classified as tEPEC (Ghilardi et al., 2003). Based on this information, Trabulsi et al. (2002) suggested that the best distinguishing characteristic for tEPEC and aEPEC strains would be production or nonproduction of BFP, respectively. To further confirm the potential pathogenicity of an aEPEC strain, it is necessary to demonstrate its ability to produce A/E lesion on epithelial cells. Nevertheless, the use of such methods is restricted to research laboratories.

It is important to emphasize that in bloody diarrheal cases, isolates characterized as aEPEC could comprise EHEC-LST strains (Bielszewska et al., 2008). It may be difficult to distinguish between both groups, unless stx-positive and stx-negative isolates of the same serotype and identical genotypic characteristics are detected in the same patient.

Conclusions

The heterogeneous nature of strains fitting the concept of aEPEC is probably responsible for their controversial association with human diarrhea. However, the significantly higher prevalence in patients than in healthy controls in certain geographical regions and the occurrence of diarrheal outbreaks strongly confirm that aEPEC strains are truly diarrheagenic pathogens.

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Table 2. Important characteristics of tEPEC and aEPEC

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>tEPEC</th>
<th>aEPEC</th>
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<tr>
<td>A/E lesion formation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>stx genes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EAF probe sequence</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>bfpA</td>
<td>+</td>
<td>–/+/*</td>
</tr>
<tr>
<td>BFP expression</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Adherence pattern</td>
<td>LA</td>
<td>LA/LAL/DA/AA/LA/NA</td>
</tr>
</tbody>
</table>

*aIncomplete bfp operon (Bortolini et al., 1999).
1Adherence pattern in HeLa/HEp-2 cell: LA, localized adherence; LAL, LA like; DA, diffuse adherence; AA, aggregative adherence; NA, nonadherent.

The LA phenotype in aEPEC strains is independent of BFP expression.
References


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Serotypes identified among atypical EPEC strains isolated from humans.

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