REVIEW

Enteropathogenic *Escherichia coli*: foe or innocent bystander?

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) remain one the most important pathogens infecting children and they are one of the main causes of persistent diarrhoea worldwide. Historically, typical EPEC (tEPEC), defined as those isolates with the attaching and effacement (A/E) genotype (*eae*$^+$), which possess *bfpA*$^+$ and lack the *stx*$^-$ genes are found strongly associated with diarrhoeal cases. However, occurrence of atypical EPEC (aEPEC; *eae*$^+$ *bfp*A$^-$ *stx*$^-$) in diarrhoeal and asymptomatic hosts has made investigators question the role of these pathogens in human disease. Current epidemiological data are helping to answer the question of whether EPEC is mainly a foe or an innocent bystander during infection.

Keywords: Asymptomatic pathogenic *Escherichia coli*, atypical EPEC, diarrhoea, enteropathogenic *Escherichia coli*, epidemiology
Article published online: 28 January 2015

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Introduction

Diarrhoea is one of the global leading causes of death in children <5 years of age, especially in low-income countries [1,2], accounting for 800 000 fatalities per year worldwide. Enteropathogenic *Escherichia coli* (EPEC is a major cause of infantile diarrhoea in developing countries (Fig. 1). It was first described in 1955 when a number of *E. coli* strains, epidemiologically associated with outbreaks in 1940s and 1950s, were described [3]. A hallmark phenotype of EPEC is the induction of a distinctive histopathology known as the attaching and effacing (A/E) lesion, which is characterized by the effacement of the intestinal microvilli and the intimate attachment of the bacteria to the host epithelial surface [4]. After entering the gastrointestinal tract, EPEC adhere to the mucosa of the small and large intestines and at least three steps for pathogenesis have been described [5]. The initial step includes adherence to the host cell. After attachment, a type III secretion system would be used to inject virulence factors in the host cell. Finally, an intimate bacterial attachment and pedestal formation is observed. The initial definition of EPEC indicated that this pathotype is part of the diarrhoeagenic *E. coli* strains that have the ability to produce the A/E lesion without producing Shiga toxin (*stx*$^-$) [6,7].

Currently, the EPEC pathotype is subdivided into typical EPEC (tEPEC) and atypical EPEC (aEPEC) strains. This classification is initially based on the presence of EPEC adherence factor plasmid (pEAF) [8]. The *bfp* and *per* are two important loci encoded on the plasmid, with *bfp* encoding the type IV bundle-forming pilus (BFP), which promotes bacterial microcolony formation [9]. The *per* operon encodes a transcriptional activator called the plasmid-encoded regulator of the Locus for Enterocyte Effacement (LEE) pathogenicity island [10,11]. Typical EPEC strains are more homogeneous in their virulence traits than aEPEC. Most of the typical strains produce the virulence factors encoded by the LEE region and EAF plasmid [8]. Atypical EPEC might possess enteroaggregative heat-stable toxin (EAST1) and other potential virulence factors not encoded in the LEE, such as a haemolysin [8]. EPEC belongs to specific O:H serotypes and at least 13 O groups are representative of these strains: O26, O39, O55, O86, O88, O103, O111, O114, O119, O125ac, O126, O127, O128ab, O142,

http://dx.doi.org/10.1016/j.cmi.2015.01.015

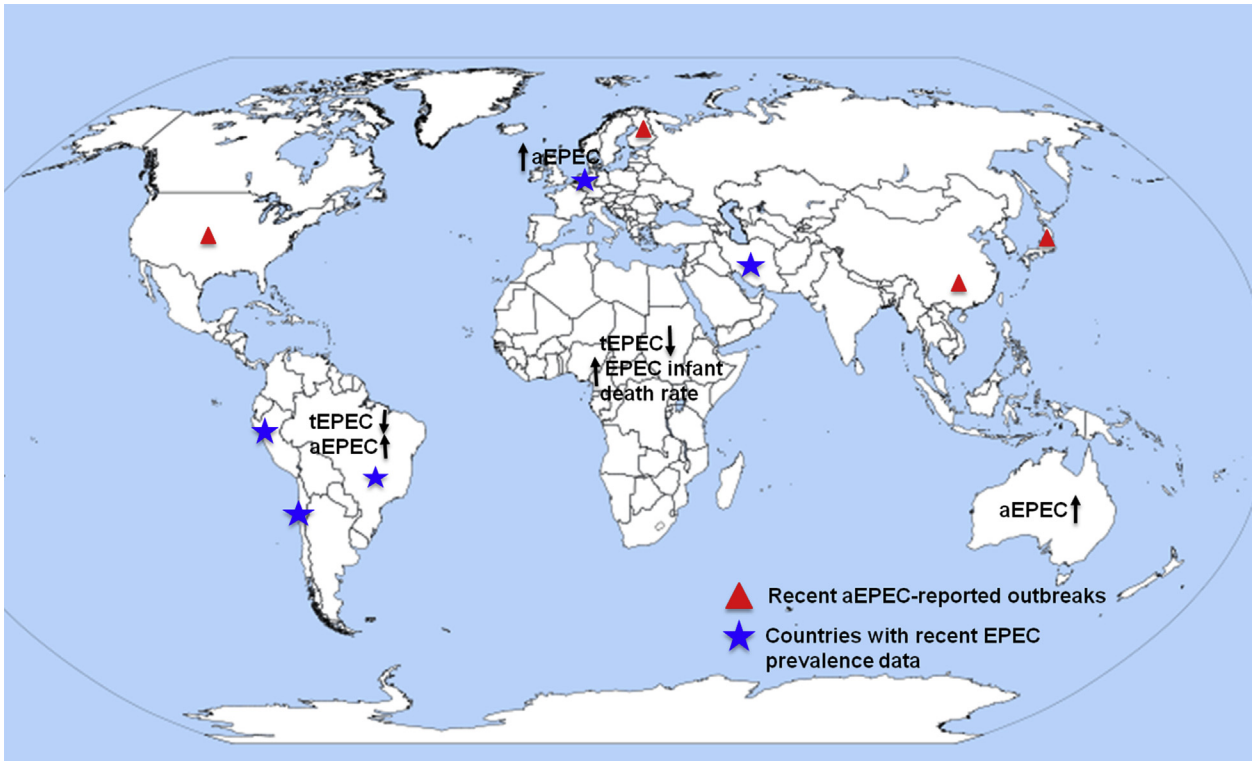

FIG. 1. Distribution of recent worldwide epidemiological studies of enteropathogenic *Escherichia coli* (EPEC). The arrows represent the increase or decrease of typical EPEC (tEPEC) and atypical EPEC (aEPEC) incidence per geographical region. The blue stars depict those countries with increased EPEC prevalence reported in recent years. The red triangles represent countries with recent reported aEPEC outbreaks.

O145, O157 and O158 [12]. Some aEPEC strains (e.g. O55:H7) are more closely related to LEE-positive Shiga toxin-producing *E. coli* (e.g. STEC O157:H7) in their genetic characteristics and virulence properties. The tEPEC and aEPEC strains also have different adherence patterns. While tEPEC strains display the localized adherence pattern, the atypical strains can produce a localized-like adherence, a diffuse adherence, or an aggregative adherence pattern [8]. Typical EPEC are rarely found in animals and humans are the major reservoir [8]; aEPEC are present in both healthy and diseased animals and humans [13].

Diarrhoeal cases caused by EPEC varied from subclinical to fatal infections [14]. The tEPEC strains can cause abundant secretory diarrhoea with mucus and significant losses of water and electrolyte in the faeces [15]. In addition, EPEC may lead to severe malabsorption of nutrients, which would progress to nutritional aggravation and persistence of diarrhoea [16]. Studies in volunteers demonstrated that a large bacterial inoculum (10^9 to 10^{10}) during short incubation periods (12 to 24 h) is able to induce diarrhoea in adults [17]. For aEPEC, the role as a diarrhoeagenic pathogen in disease is controversial. The pathogenesis of aEPEC seems to be related to the serotypes of aEPEC [18]. For example, in the case of aEPEC O128:H2, after administration to 15 adult volunteers, none of them became ill [17]. It has also been shown that a tEPEC O127:H6 strain without EAF plasmid was less virulent for adult volunteers than the wild-type strain [19]. However, there are also plenty of reports showing that aEPEC causes outbreaks linked to diarrhoea (Fig. 1). In a Japanese daycare centre, the only diarrhoeagenic pathogen isolated from patients was aEPEC O55:HNM and these clones showed indistinguishable pulsed field gel electrophoresis patterns [20]. *Escherichia coli* O111:B4 was responsible for a diarrhoeal outbreak including 611 pupils and 39 adults in Finland [21]. *Escherichia coli* O39:NM was also associated with an outbreak involving more than 100 adults in the USA [22]. An aEPEC EC3605 caused an outbreak in 75 students (ages 12 to 15 years) in Japan [23] and another aEPEC strain O127a:K63 was isolated from a 2010 food-poisoning outbreak involving 112 adults in China. This strain displayed multidrug resistance to quinolones and extended spectrum cephalosporins [24].

Some aEPEC are strongly associated with acute disease, but some strains are also associated with persistent diarrhoea [25–28]. Santona et al. identified 28 aEPEC strains among 402 *E. coli* strains isolated from the faeces of children with acute diarrhoea in Italy [29]. A study carried out in Australia compared aEPEC-infected patients with patients infected with

other diarrhoeal agents [28]. They found that patients infected with aEPEC experienced mild, non-dehydrating, non-inflammatory diarrhoea that was not associated with fever, vomiting or abdominal pain; however, the duration of diarrhoea was longer. Strains of aEPEC were also found to be the most common pathogens among children with persistent diarrhoea (diarrhoea lasting for more than 14 days) in Australia (43%) and Norway (22%) [5]. The exact mechanism of how EPEC causes diarrhoea is not well established. However, it is postulated that the extensive disruption of the intestinal microvilli may lead to a decrease in absorptive surfaces, affecting absorptive channels, thereby contributing to diarrhoea [30]. Other mechanisms that may participate in the diarrhoeal process include the effect of type III secretion system effectors on the intestinal cell. The type III secretion system effectors Tir, Map, EspF and EspG play a role in water and ion channel transport activity of intestinal epithelium. Also, EspF, EspG and Map disrupt the tight junctions and enhance intestinal permeability, which may lead to diarrhoea [31].

Some aEPEC isolates have been linked to bloody diarrhoea. A study in Germany from January 1995 to June 2007 found that aEPEC strains were isolated from 18 (15.3%) of 118 patients with bloody diarrhoea and from 141 (1.3%) of 10 550 patients with non-bloody diarrhoea. Bielaszewska et al. found that these strains were originally STEC O103 isolates that lost the Shiga toxin phage [32]. Finally, aEPEC may become the precursor for *stx*-positive isolates. Sekse et al. showed that aEPEC O103:H25 can be converted to STEC by an *stx* bacteriophage infection and become more virulent [33].

Recent epidemiological reports of EPEC

Currently, EPEC is estimated to be responsible for 5–10% of paediatric diarrhoea in developing countries such as Brazil, Chile, Peru and Iran [5]. For many decades, tEPEC have been considered to be strongly associated with infantile diarrhoea in developing countries. In several studies conducted in Latin America, tEPEC was found to be the main cause of endemic diarrhoea in children <1 year of age. The frequency of tEPEC infection drops with increase in age group and adults rarely experience tEPEC episodes [5]. This may be due to development of immunity or the loss of receptors interacting with some specific adhesins. Although tEPEC were major agents of acute diarrhoea in infants until the 1990s, there is a clear decline in many of these countries [8]. The Global Enteric Multicenter Study was a population-based case–control study including seven countries in Africa and Asia with the goal to identify the aetiology, burden and mortality of acute moderate to severe diarrhoea in children <5 years of age [34]. At most Global Enteric Multicenter Study study sites, tEPEC strains were not among the leading pathogens that cause acute moderate and severe diarrhoea. The reasons for the decline in cases are not known, but may be linked to improvements in public health measures such as active interventions, therapy, sanitary conditions and control of hospital infections [8,12]. However, tEPEC infection seems to be associated with a 2.8-fold increased risk of death among infants ages 0–11 months [34].

Atypical EPEC continue to be frequently detected in both developing countries and industrialized countries [35]. They are often associated with diarrhoea and in some countries they outnumber tEPEC infections. Studies from 13 developing countries showed that aEPEC isolates were responsible for 78% (131/169) of all EPEC cases in children <5 years old [5]. Wheeler et al. identified 142 aEPEC strains and only one tEPEC among 2774 samples isolated from symptomatic children in the UK [36,37]. In another study, 61 EPEC strains were isolated from stool samples of symptomatic persons from 2008 to 2011 in Australia [38], where 95.1% (58/61) were aEPEC. In 2009, aEPEC strain O76 was associated with a nursery outbreak in Finland [39]. Further, Sakkejha et al. studied 109 EPEC isolates detected in England from 2010 to 2012, with 93% of the patients reporting diarrhoeal episodes and 32% bloody diarrhoea. The study found that aEPEC were more common and were associated with a wider variety of serogroups than tEPEC [40].

Overall, according to 266 studies published between 1990 and 2002, EPEC are still among the most important pathogens causing diarrhoea [35]. As such, in 2014 a European, multicentre, prospective quarterly point-prevalence study of community-acquired diarrhoea (EUCODI) showed that EPEC is highly prevalent during both the first (January 2014) and the second (April 2014) rounds of the survey ([41]). However, there are important regional and temporal variations. In Asia, a separate study found that EPEC (no information about whether the isolates were tEPEC or aEPEC) was responsible for 3.2% of 648 diarrhoea samples in children <5 years old in an Indian hospital [42].

Asymptomatic hosts carrying EPEC

Although there is a significant strong association between EPEC and infant diarrhoea, many studies have found EPEC, especially aEPEC, in asymptomatic controls [5]. The recent EUCODI study shows that EPEC was the most frequent pathogen detected in mixed infections [41]. A study in the Netherlands collected 5197 samples from 29 child-care centres, with 95.4% of samples from children who had no gastroenteritis symptoms at time of sampling and EPEC isolates were most prevalent in asymptomatic samples (19.9%) [43]. Another survey in

Peruvian children isolated EPEC with a similar frequency from children with diarrhoea (7.6%) and those from asymptomatic controls (9.9%) [44]. A study in Mexico found that although aEPEC is most prevalent among diarrhoeagenic *E. coli*, most isolates are from asymptomatic carriers [25]. A recent survey in Germany demonstrated that EPEC has a similar high prevalence (17.4%) in both control and diarrhoeal patients [41].

It has been proposed that at least three reasons exist explaining the frequency of EPEC in symptomatic and control patients. The first one is host susceptibility. The precise mechanism leading to the diarrhoea is not fully understood [45]. Enteropathogens may initiate pathogenesis by binding to the host surface specific receptors, including sugar moieties as well as proteins. The susceptibility to infection may be associated with presence or lack of receptors [46]. Non-specific host barriers may also prevent bacterial pathogenesis. The intact barriers, such as the intestinal microbiota, mucus layer and epithelial cell layer, may prevent diarrhoeal episodes [46]. The immune status of the host prevents clinical illness but does not prevent intestinal colonization. Experiments carried out with enterotoxigenic *E. coli* and *Shigella* have shown that in endemic areas, where individuals are repetitively exposed to enteropathogens, individuals might carry pathogens without suffering from diarrhoeal episodes [47].

It is well known that secretory immunoglobulin A (sIgA) antibodies from intestine and breast milk as well as human breast milk oligosaccharides can prevent enterocyte colonization or mucosal invasion by enteropathogens without killing the bacteria [48]. Breastfeeding can prevent diarrhoea in infants and toddlers and protection is due to the presence of sIgA or non-specific factors, such as lactoferrin and enterotoxin-binding oligosaccharides. In endemic areas, the colostrum of puerperal women is rich in sIgA against EPEC [49–51]. A study in Peru showed that EPEC prevalence increased with age within the first 2 years of life [52]. EPEC was found in 3% of diarrhoea samples in children <6 months old, in 11% of children 6–12 months old, and in 16% of children 13–24 months old. Small infants may be protected from symptomatic EPEC infection due to breastfeeding. In addition, children may acquire natural immunity in some developing countries where EPEC is highly endemic [5]. Host age also plays a role for carrying bacteria asymptomatically. Neter et al. showed that almost 100% of children developed LPS-specific antibodies against three of the most common O serogroups by the age of 12 years [3]. Opintan et al. found that although EPEC is one of the most common pathogens recovered from healthy individuals aged ≥3 years, it has not been detected in healthy infants <2 years old [53].

The second reason is linked to bacterial factors. EPEC strains are heterogeneous serotypes that include different clones or genetic lineages [8]. Some strains cause diarrhoea more frequently than others at the same challenge inoculum. EPEC strain E2348/69 causes more severe diarrhoea than strain E74/68 [46]. Several studies have identified certain virulence genes significantly associated with diarrhoea. Afset et al. used DNA microarray to analyse aEPEC strains isolated from children with and without diarrhoea. Genomic DNA was hybridized against 242 different oligonucleotide probes. They found O-island 122 (OI-122), carrying *efa1/lifA* and several other genes, significantly associated with diarrhoea. In contrast, the phylogenetic marker gene *yhaA* was negatively associated with diarrhoea. Children with diarrhoea were infected with OI-122 *efa1/lifA*-positive, *yhaA*-negative strains, whereas children without diarrhoea had aEPEC strains that were OI-122 *efa1/lifA*-negative, *yhaA*-positive [54]. Afset et al. further compared the phylogenetic ancestry and diarrhoea association of aEPEC strains [55]. Fifty-six aEPEC strains were divided into four phylogenetic groups (B1, A, D, B2) and they found a borderline significant association with diarrhoea for the phylogenetic groups B1 and D. Wang et al. also tried to distinguish aEPEC from diarrhoeic patients and healthy controls. Multiplex real-time PCR was used to examine the intimin gene typing, phylogenetic grouping and virulence profile of isolated aEPEC strains. After examining 159 strains from 679 samples, their results indicated that aEPEC, particularly those from phylogenetic groups B1 or D, virulence group Ia, or intimin typing β1 and γ1, induce diarrhoea in humans [56]. Contreras et al. also characterized a collection of EPEC strains obtained from a study in Peru using PCR-restriction fragment length polymorphism analysis. They found that the κ-intimin allele had the highest clinical severity score compared with other alleles [57].

The third possibility is the variability of diagnostic tests. Barletta et al. hypothesized that presence of symptoms in EPEC infections is related to the bacterial load [58]. They analysed stool samples from a passive surveillance diarrhoeal cohort study, including 1034 Peruvian children. They isolated EPEC with a similar frequency from children with diarrhoea and asymptomatic controls. However, a quantitative real-time PCR assay was applied to determine whether bacterial loads were related to diarrhoea and it was found that bacterial load was significantly higher in the diarrhoea group than in the control group among children with EPEC as the sole pathogen and among children <1 year old. However, it is evident that this detection method had some limitations due to the complexity in faecal samples.

Several other factors may also affect the results reported in different studies. For example, the control samples may be collected from pre- or post-symptomatic patients. Other issues include the sample size, which may not be large enough, or the fact that asymptomatic controls may transmit EPEC to other patients, which cannot be excluded [43]. Finally, environmental

factors, such as poor hygiene and high levels of faecal contamination, may also lead to the bacteria load in control groups [35].

In summary, cumulative data in recent years has indicated that aEPEC are more prevalent than tEPEC in both developing and developed countries. However, tEPEC is still considered *bona fide* pathogens because of their arsenal of virulence factors and association with severe, lethal disease. The recent emergence of aEPEC requires further epidemiological studies that can help to elucidate whether certain serotypes are specifically linked to disease in humans. Further, more investigation is required to identify virulence/fitness factors of aEPEC that mediate the disease process or the ability to be maintained in patients and in healthy individuals. Future studies will answer whether aEPEC is a foe or an innocent bystander in human disease.

Transparency declaration

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was partially supported by NIH/NIAID grant AI079154 to AGT. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIAID or NIH.

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