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Enteropathogenic *E. coli* (EPEC) infection in children

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Abstract

Purpose of review—Enteropathogenic *Escherichia coli* (EPEC) are important diarrheal pathogens of young children. Since the diagnosis of EPEC is now based mainly on molecular criteria, there has been an important change in their prevalence. The purpose of this paper is to review the current epidemiology of EPEC infection and the new insights into its physiopathology.

Recent findings—Recent epidemiological studies indicate that atypical-EPEC (aEPEC) are more prevalent than typical-EPEC (tEPEC) in both developed and developing countries, and that aEPEC are important in both pediatric endemic diarrhea and diarrhea outbreaks. Therefore, it is important to further characterize the pathogenicity of these emerging strains. The virulence mechanisms and physiopathology of the attaching and effacing lesion (A/E) and the type-three-secretion-system (T3SS) are complex but well studied. A/E strains use their pool of locus of enterocyte effacement (LEE)-encoded and non-LEE-encoded effector proteins to subvert and modulate cellular and barrier properties of the host. However, the exact mechanisms of diarrhea in EPEC infection are not completely understood.

Summary—Remarkable progress has been made to identify virulence determinants required to mediate the pathogenesis of EPEC. However, fast, easy and inexpensive diagnostic methods are needed in order to define optimal treatment and prevention for children in endemic areas.

Keywords

Enteropathogenic *Escherichia coli*; EPEC; diarrhea; children; epidemiology; physiopathology

Introduction

Diarrhea remains the second leading cause of death in children younger than 5 years globally, accounting for 1.3 million deaths annually (1). Enteropathogenic *Escherichia coli* (EPEC), one of the diarrheagenic *E. coli* pathotypes, are among the most important pathogens infecting children worldwide because of their high prevalence in both the community and hospital setting (2), and because they are one of the main causes of

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persistent diarrhea (3). Since the diagnosis of these pathogens is now based mainly on molecular diagnosis, there has been an important change in the prevalence and distribution of these pathogens. The purpose of this paper is to review the current epidemiology of EPEC infection in children and the new insights into its pathophysiology.

EPEC definition and classification

EPEC were originally serogroup-defined *E. coli* associated with infantile diarrhea. However, it became clear that serogroup/serotype designation over-diagnosed EPEC. Subsequently, they were defined by their characteristic localized adherence pattern in tissue cultured cells. Currently, they are identified mainly based on the presence of specific virulence genes. A hallmark phenotype of EPEC is the ability to produce attaching and effacing (A/E) lesions (4). In the A/E lesion the bacteria attach tightly to the host cell membrane causing a disruption of the cell surface leading to effacement of microvilli. Intestinal cell attachment is mediated by an outer membrane protein called intimin, encoded by *eae*, which is currently used for the molecular diagnosis of EPEC. All of the genetic elements required for the A/E lesion are encoded on a large genomic pathogenicity island called the locus of enterocyte effacement (LEE). Furthermore, EPEC are classified into typical and atypical strains based on the presence of the plasmid *E. coli* adherence factor (EAF). There are two important operons on this plasmid, *bfp* and *per*, the first encoding the type IV bundle-forming pilus (BFP), and the second encoding a transcriptional activator called plasmid encoded regulator (Per). By definition all EPEC lack genes to produce shiga toxin (*stx*). *E. coli* strains that are *eae+bfpA+stx-* are classified as typical EPEC (tEPEC), most of these strains belong to classic O:H serotypes, and produce the localized adherence (LA) phenotype associated with the production of BFP (5). On the other hand, *E. coli* strains that are *eae+bfpA-stx-* are classified as atypical EPEC (aEPEC). These strains display localized-like (LAL), diffuse (DA), or aggregative adherence (AA) patterns. The LAL pattern in aEPEC is associated with the *E. coli* common pilus and other known adhesins (6). More than 200 O-serogroups have been identified among aEPEC strains; most do not belong to the classic EPEC O-serogroups and many have been designated non-typeable (7) [7*]. In summary, some *E. coli* strains that belong to classical EPEC serogroups do not harbor the EPEC virulence genes, and conversely some genetically-defined EPEC strains do not belong to the classical EPEC serogroups.

EPEC epidemiology

Overall, the significance of EPEC as pathogens has declined in published literature of the last several decades (8, 9) [8**]. It is unclear whether EPEC infections have declined due to interventions, particularly breastfeeding promotion, or whether earlier studies that based diagnosis on O- or O:H-typing overestimated the relative contribution of these organisms compared to recent studies in which EPEC identification was based on molecular methods and/or adherence assays. In a systematic review of pediatric diarrhea etiology, using 266 studies published between 1990 and 2002, EPEC were still identified as being among the most important pathogens, with a median prevalence of 8.8% (IQR, inter-quartile range, 6.6–13.2) in the community setting, 9.1% (IQR 4.5–19.4) in the outpatient setting and 15.6% (IQR 8.3–27.5) in the inpatient setting (2). The distribution of pathogens in the inpatient setting represents the most severe cases and can be used as a proxy for cases associated with mortality. In this context, EPEC was the second most common cause of inpatient diarrhea after rotavirus (25.4%). However, there are important regional and temporal variations. In a recent study of hospitalized diarrheal patients in India, EPEC was responsible for 3.2% of 648 diarrhea samples in children younger than 5 years of age (10).

We have recently studied diarrheagenic *E. coli* prevalence in Peru using 8,000 *E. coli* strains previously isolated from 8 different studies in children mainly younger than 36 months of age and primarily from cohort studies in peri-urban Lima (11). Diarrheagenic *E. coli* were detected by a multiplex real-time PCR using specific primers for each of the six diarrheagenic *E. coli* (12, 13). The same methodology was used in all samples. Overall, the average EPEC prevalence in diarrhea samples (n=4,243) was 8.5% (95% CI: 7.6–9.3), second only to enteroaggregative *E. coli* (EAEC, 9.9%). EPEC prevalence increased with age. EPEC was found in 3% of diarrheal samples in children <6mo, in 11% of children 6–12mo, and in 16% of children 13–24mo. However, in this setting exclusive breastfeeding is very prevalent (>80% for infants younger than 6mo), and therefore, small infants may be protected from symptomatic EPEC infection. Among control samples collected from asymptomatic children from the same study sites (n=3,760), EPEC was the most prevalent pathotype with an average prevalence of 10.9% (95% CI: 9.4–11.4), followed by EAEC (10.4%).

Although many studies have found a significant association of EPEC with infant diarrhea compared to control samples (14–16), several studies have found EPEC with similar frequency among diarrheal and control samples (11, 17–19). Healthy carriage of enteric pathogens in general is very common in developing countries. Colonization rather than illness may result from an interplay of multiple factors including host susceptibility (related to the child's age, breastfeeding, nutritional and immunological status), bacterial factors (different virulence genes), and environmental factors (poor hygiene and high fecal contamination). Recently, we have developed a quantitative real time PCR (qRT-PCR) to determine EPEC bacterial load in stool samples. EPEC bacterial load measured by qRT-PCR was significantly higher in EPEC associated diarrheal samples than in EPEC associated control samples (20). Therefore, we proposed that in addition to the factors mentioned above, the bacterial load should be considered in the interpretation of illness and colonization of EPEC in the gut.

The pathogenic potential of aEPEC it has long been controversial. However, epidemiological studies clearly have demonstrated that aEPEC are important in endemic diarrhea in children as well as diarrhea outbreaks (21–26). Recent data suggests that aEPEC are more prevalent than tEPEC in both developing and developed countries (9, 10, 16, 17, 27–31) [31*].

Prolonged and persistent episodes of illness in children constitute a significant portion of the global burden of diarrheal disease (32). Persistent diarrhea is commonly associated with parasitic infections such as *Cryptosporidium* and *Giardia*. A recent systematic review of pathogens associated with persistent diarrhea (diarrhea >14 days) in developing countries has found the diarrheagenic *E. coli*, particularly EPEC, ETEC and EAEC, are the main pathogens associated with this complication, responsible for 30–40% of all persistent episodes in children (3). Among children with persistent diarrhea from developed countries, aEPEC are the most common pathogens isolated (27, 33). Persistent diarrhea is the most common clinical presentation in aEPEC cases, accounting for more than half of the episodes (21, 27, 33). These findings indicate that aEPEC may have an innate propensity to persist longer in the intestine than other diarrheagenic *E. coli*.

Virulence genes

EPEC is considered a non-invasive pathogen and relies upon a T3SS to deliver effector proteins directly into host cells to subvert numerous host cell functions ultimately leading to disease. The first EPEC effectors discovered are all encoded on the LEE pathogenicity island. More recently, effectors encoded outside the LEE region have been found in all A/E

pathogens. EPEC strain E2348/69 (serotype O127:H6) has been used worldwide as a prototype strain to study EPEC biology, genetics, and virulence. The recent completion of the EPEC genome sequence of strain E2348/69 (34) [34*] enabled analysis of over 400 known/predicted effector sequences and identified only 21 putative effectors, providing a clear picture of the core LEE and non-LEE effector genes.

LEE contains genes encoding the outer membrane adhesin (intimin), T3SS machinery (Esc and Sep proteins), chaperones (Ces proteins), translocators (EspA, EspB, and EspD) and effector proteins (EspF, EspG, EspH, Map and EspZ), as well as the translocated intimin receptor (Tir), and the regulatory proteins Ler (LEE-encoded regulator), GrlR (global regulator of LEE proteins, repressor), and GrlA (global regulator of LEE proteins, activator). EspA forms a needle-shaped structure projecting from the bacterial surface to the plasma membrane of the host cell. Following bacterial attachment, EspB and EspD interact and form a pore in the host membrane. EspB is a translocator and a translocated effector (35). In general, many translocated effectors show overlapping functions and have cooperative activities, a theme that has been summarized as “multifunctional, cooperative and redundant” EPEC effector behavior (36) [36**].

The non-LEE-encode (Nle) (37) effectors genes are clustered in six pathogenicity islands scattered throughout the genome (36) [36**]. The Nle effectors known so far are: NleA-H, EspG2/ Orf3, Cif, EspJ and EspL. NleA (also called EspI) is reported to inhibit protein secretion; EspJ inhibits phagocytosis; NleE and NleH activate innate immune responses. Studies in animal models suggest that EspJ, NleB, NleE, NleF and NleH play a role in colonization and full virulence (36, 38) [36**].

A/E *E. coli* pathogens use their collection of LEE-encoded and non-LEE-encoded effector proteins to subvert and modulate cellular and barrier properties of the host in a well-controlled manner. LEE regulation is a very complex process governed by diverse types of regulatory influences such as environmental factors, a hierarchy of LEE-encoded regulators (Ler, GrlR, and GrlA) and a system of *E. coli* global regulators (H+NS, IHF, FIS, Quorum sensing, SOS-response) (39, 40).

Physiopathology

A/E lesions are characterized by intimate bacterial adherence to enterocytes, effacement of surface-absorptive microvilli, F-actin rearrangement, and the outgrowth of cuplike pedestals beneath the adherence site. The T3SS, Intimin, and Tir are all essential virulence determinants of the intimate adherence, a process that requires the T3SS-dependent insertion of Tir into the plasma membrane to act as a receptor for bacterial binding via Tir-Intimin interaction (41, 42). However, Tir-Intimin interaction also triggers signaling cascades leading to responses, including phosphorylation of a host phospholipase and recruitment of cytoskeletal proteins beneath the adherent bacteria (43–45) [44*]. Intimin can also subvert cellular processes independently of Tir (45). Map (mitochondrial-associated protein), not only targets host cell mitochondria (43), but also contributes to disruption of epithelial barrier function (46), a process linked with diarrheal disease.

Microvilli effacement is developed in two-step process that requires the cooperative action of three injected effectors (Map, EspF, and Tir) as well as Intimin, and leads to the retention of the detached microvillar material. Additionally EPEC rapidly inactivate the sodium-D-glucose cotransporter (SGLT-1) by multiple mechanisms. SGLT-1 play a crucial role of in the daily uptake of fluids from the normal intestine (47).

Actin polymerization is thought to enhanced pathogenicity by disturbing the cytoskeleton of the target cells leading to changes in cell shape, motility and signaling. However, some

studies have shown that there is no direct correlation between actin polymerization, cell attachment, pedestal formation, effacement of the brush border microvilli, and bacterial colonization *in vivo* (48) [48*]. Not all clinical isolates polymerized actin, measured *in vitro* by the fluorescent actin stain (FAS test) (17, 49). While FAS-positive strains are likely to be pathogenic, LEE-positive strains that fail to trigger actin polymerization *in vitro* cannot be classified as nonpathogenic, and may still be able to induce A/E *in vivo* (50). These results show that relying on the FAS test (*in vitro*) alone may not be sufficient for determining the virulence of an EPEC strain.

EPEC heterogeneity

Clinical isolates of EPEC, especially aEPEC, have a great diversity of virulence factors (22, 23, 31, 51, 52). Among aEPEC strains the *efa1/lifA* gene, which encodes lymphostatin, a protein that inhibits lymphocyte proliferation, was found to be significantly associated with diarrhea (52). Recent studies have demonstrated that tEPEC and aEPEC strains differ in other phenotypic and genotypic characteristics (5, 6, 22, 23, 28, 29, 53), as well as on antimicrobial resistance patterns and mechanisms (54). Some aEPEC strains may invade intestinal cells *in vitro* with varying efficiencies (55).

Various subgroups based on evolutionary considerations and presence of certain isotypes particularly of the intimin gene have been proposed (56). Currently, 27 *eae* variants encoding distinct intimin types and subtypes have been established (55, 57). However, little is known about whether specific alleles are related to specific clinical characteristics. Most studies have only described the allele distribution as related to diarrhea and control samples (17, 22, 58, 59); few have described the allele distribution in relation to duration and severity of the diarrheal episodes (60). Further studies are needed to evaluate additional virulence markers to determine whether relationships exist between specific variants and clinical features of disease.

Clinical aspects of EPEC infection in children

EPEC most commonly causes acute diarrhea and may also cause persistent diarrhea. The clinical characteristics of EPEC infection have not been optimally described, since few studies have searched for all common pathogens in order to rule out mixed infections, which are very common in endemic areas (8, 10, 61). Among single pathogen infections in the community setting we found that EPEC had the second highest severity score and ORS usage, only after rotavirus, and followed by ETEC (61). For diagnosis, PCR should be used for the proper identification of EPEC (12, 62). However, molecular methods are still not easily available in clinical laboratories worldwide. It is unrealistic and unnecessary for every diarrheal specimen to be screened by molecular methods, since most infection resolve spontaneously. Nevertheless, for clinical purposes it would be ideal if severe cases, bloody diarrhea cases, hospital-acquired or suspected outbreaks were investigated for all the diarrheagenic *E. coli*, including EPEC. O-serogroup identification should not be used in clinical laboratories, except as part of outbreak investigations. In relation to treatment, few studies have evaluated in a systematic manner the value on antimicrobials for the management of EPEC infection in children. In order to design proper clinical trials, fast, easy and inexpensive diagnostic methods are needed. Finally, with the introduction of the rotavirus vaccines into pediatric immunization programs, and the push for development of ETEC and *Shigella* vaccines for children, EPEC should be the next priority for vaccine development for pediatric diarrheal disease, due to its high morbidity and mortality rates.

Conclusions

Recent epidemiological studies indicate that aEPEC are more prevalent than tEPEC, in both developed and developing countries, and that aEPEC are important in both endemic diarrhea in children and diarrhea outbreaks. Therefore, it is important to further characterize the pathogenicity of these strains and to gain additional insight into the evolutionary aspects of this emerging pathotype. The virulence mechanisms and physiopathology of the A/E lesion and the T3SS are complex and well studied. However, the exact mechanisms of diarrhea in EPEC infection are not completely understood. From the clinical perspective, fast, easy and inexpensive diagnostic methods are needed in order to define optimal treatment and prevention for children in endemic areas.

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*Of special interests

**Of outstanding interest

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