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Chapter

Enteropathogenic *Escherichia coli*

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Abstract

The term enteropathogenic *Escherichia coli* (EPEC) was first used in 1955 to describe a number of serogroup-defined *E. coli* strains associated with infantile diarrhea. EPEC are now defined as those that produce a characteristic intestinal histopathology known as attaching and effacing (A/E) and do not produce Shiga toxins. EPEC carry the locus for enterocyte effacement (LEE) pathogenicity island, which contains the *eae* gene that encodes an outer membrane protein called intimin. Typical EPEC (tEPEC) carry a virulence plasmid known as the pEAF (EPEC adhesion factor plasmid) which encodes the bundle-forming pilus (BFP) that mediate localized adherence to epithelial cells, whereas atypical EPEC (aEPEC) do not possess this plasmid. Typical EPEC strains have been associated with severe outbreaks of infant diarrhea in developing countries. Atypical EPEC strains have been linked to diarrhea outbreaks at all ages worldwide. Diarrhea due to aEPEC in children is not as severe as that caused by tEPEC.

Keywords: *Escherichia coli*, EPEC, diarrhea, children, infantile diarrhea

1. History and definition of EPEC

Escherichia coli were first recognized as diarrheal pathogens in 1898, when Lesage demonstrated that serum from diarrhea patients agglutinated strains of *E. coli* isolated from other patients in the same outbreak but not those of control [1]. In 1945, Bray discovered that *E. coli* strains of certain serogroups were the predominant cause of summer diarrhea in infants in the United Kingdom [2]. In 1947, Kauffman published a serotyping scheme based on somatic (O), flagellar (H), and capsular (K) antigens, providing a reliable method of typing diarrheagenic *E. coli* [3].

The term enteropathogenic *Escherichia coli* (EPEC) was introduced in 1955 to describe strains of *E. coli* implicated epidemiologically with infant diarrhea in the 1940s and 1950s [4]. This definition changed as additional serotypes were associated with infantile diarrhea. EPEC were recognized as important causes of infant diarrhea in the 1950s and 1960s in the developed world and subsequently have been shown to be common agents of gastroenteritis in the developing world. The confirmation that EPEC strains were pathogenic came from human volunteer studies carried out by Levine et al. [5].

The definition of EPEC has changed as various mechanisms of pathogenesis have been discovered. During the late 1960s and early 1970s, two other diarrheagenic *E. coli* strains were discovered, strains producing the heat-stable enterotoxin (ST) and the heat-labile enterotoxin (LT) were designated enterotoxigenic *E. coli* (ETEC), and strains demonstrating *Shigella*-like invasiveness were designated enteroinvasive *E. coli* (EIEC). At this time, the original definition of EPEC by Neter [4] has

undergone modification considerably. EPEC were then defined as "diarrheagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either heat-labile (LT) or heat-stable enterotoxins (ST) or to *Shigella*-like invasiveness" [6].

In 1979, the first phenotype characteristic other than serotyping associated with EPEC was the observation of Cravioto et al. [7] that 80% of EPEC strains as defined by serotype could adhere to HEp-2 cells in cell culture, while most non-EPEC strains could not. Later, the adherence pattern of EPEC was described as "localized adherence" (LA), based on the presence of clusters or microcolonies on the surface of HEp-2 cells [8]. Baldini et al. [9] subsequently showed that the ability of EPEC E2348/69 strain (O127:H6) to adhere in a localized adherence pattern was associated with the presence of a 60-MDa plasmid. EPEC strains representing a variety of serotypes were found to possess highly conserved high molecular weight plasmids associated with localized adherence, the so-called EPEC adherence factor (EAF) plasmids [10, 11]. Subsequently, differences in adherence patterns were discerned by Scaletsky et al. [8] and Nataro et al. [12], giving rise to two other categories of diarrheagenic *E. coli* and diffusely adherent *E. coli* and enteroaggregative *E. coli*. Also in the 1980s, a newly recognized clinical syndrome caused by *E. coli* led to the discovery that some diarrheagenic *E. coli* produce a potent cytotoxin known as Shiga toxin (Stx).

In 1983, Moon et al. [13] published electron micrographs of pigs and rabbits infected with EPEC and coined the term "attaching and effacing" (A/E) to describe the loss of microvilli, intimate attachment of the bacteria to the host, and formation of pedestals at the sits of bacteria attachment. In 1987, a number of studies clarified the relationship between LA phenotype and A/E lesion, which confirmed earlier reports that LA is associated with the EAF plasmid, and demonstrated for the first time that A/E is encoded on the chromosome [14].

Originally defined by serotype, EPEC are now defined as those having the ability to cause diarrhea, the ability to produce A/E histopathology on the intestinal epithelium, and the inability to produce Shiga toxins based on pathogenic characteristics [15]. Improvements in techniques allowing a better understanding of the genome and virulence mechanisms among EPEC strains over the years have led to the classification into "typical" and "atypical" subtypes based on the presence or absence of the pEAF plasmid [15].

2. Atypical versus typical EPEC

Most of the typical EPEC strains belong to the traditional EPEC serogroups O55, O86, O111, O114, O119, O127, and O142, and the most common flagellar antigens are H6 and H2 [16, 17]. A less common EPEC type is H34, and a number of typical EPEC strains are nonmotile in conventional testes and classified as H-. Typical EPEC strains belonging to nonclassic serotypes have also been reported [18, 19]. Currently, more than 180 different O serogroups and more than 60 H antigens are recognized. Atypical EPEC belong to a large diversity of classical and nonclassical serotypes [18, 20]. Based on multilocus enzyme electrophoresis analysis of allelic differences among housekeeping genes, typical EPEC strains have been subtyped into two major lineages, previously designated EPEC1 and EPEC2 [16, 17]. EPEC1 includes serotypes O55:H6 and O119:H6, whereas EPEC2 consists of serotypes O111:H2 and O114:H2. Recently, EPEC strains have been demonstrated to cluster in three main lineages, designated EPEC1, EPEC2, and EPEC4, which probably acquired the locus of enterocyte effacement region (LEE) and pEAF independently [21].

Interestingly, it has been found that 35% of the atypical EPEC strains also belong to the typical EPEC lineages [21]. Thus, it has been hypothesized that at least some atypical EPEC may have originated from typical EPEC strains that lost pEAF in the host or in the environment [21].

3. Epidemiology of EPEC

3.1 Incidence

The prevalence of EPEC infection varies between epidemiological studies based on differences in study populations, age, distributions, and methods used for detection and diagnosis [22]. Also, geographic region and socioeconomic class may contribute to the epidemiology of EPEC-induced diarrheal disease [23]. Adults and older children with typical EPEC infections are rarely reported; this has been attributed to the loss of specific receptors with age or development of immunity [24].

For the two last decades, studies conducted worldwide have shown the association of typical EPEC serotypes with diarrhea in children <1 year of age, mainly in poor children of urban centers [24]. This association was particularly strong in infants less than 6 months of age. Between 1977 and 1982, epidemiologic studies in Brazil, Chile, Mexico, and South Africa have shown that 30–40% of infantile diarrhea was caused by typical EPEC serotypes [22]. However, recent studies in these countries have not identified a significant association between typical EPEC and infantile diarrhea. At this time, a change in the epidemiology of EPEC occurred in both developing and developed countries. The proportion of atypical EPEC strains has increased and outnumbered typical EPEC strains, and atypical EPEC strains have also been associated with childhood diarrhea in both developing and developed countries [19, 22, 25]. In Brazil, 92% of EPEC isolates collected from children between 2001 and 2002 were atypical [26], compared to 38% in a 1998–1999 study [27–29]. However, other studies still report typical being more prevalent than atypical EPEC as a cause of diarrhea [30]. Recently, a prospective, population-based case-control study involving seven sites in Africa and Asia showed that typical EPEC was significantly associated with moderate to severe diarrhea in children under 2 years of age in Kenya, whereas atypical EPEC was not associated with this type of diarrhea [31].

3.2 Transmission and reservoirs

Typical EPEC transmission follows a fecal-oral process through contaminated surfaces, weaning fluids, and human carriers [32]. EPEC outbreaks among adults, although rare, seem to occur through ingestion of contaminated food and water; however, no specific environmental reservoir has been identified [24]. EPEC outbreaks have been reported to show a seasonal distribution with peaks during the warm months [33]. Humans are the only known reservoir for typical EPEC, with symptomatic and asymptomatic children and asymptomatic adults being the most likely source [24].

In contrast to the tEPEC, many aEPEC strains have been found in diarrheic as well as in healthy animals and from the environment. Interestingly, animal aEPEC serogroups associated with human diarrhea have been identified (e.g., O26, O103, O119, O128, O142, and O157); however, so far a direct transmission from animals to humans has not been confirmed. In addition, foods including raw meats, pasteurized milk, meat samples, vegetables, and water have been also implicated as vehicles of aEPEC to human infections (reviewed in [34, 35]).

4. EPEC virulence factors and genetics

4.1 Localized adherence

Typical EPEC strains adhere to HeLa, HEp-2, and other cell lines and to organ cultures in vitro in a distinctive pattern of three-dimensional microcolonies so-called localized adherence (LA) pattern within 3 h of infection (**Figure 1A**) [8, 24]. A similar adherence pattern has been seen in tissue biopsies of EPEC-infected humans [37]. The LA phenotype is mediated by a type IV fimbriae bundle-forming pilus (BFP) associated with the EAF plasmid, which mediates bacterium-to-bacterium adherence, resulting in formation of compact microcolonies [38].

Atypical EPEC strains may display a variant LA pattern designated LA-like (LAL) pattern, which is characterized by the presence of loose compact microcolonies or clusters of bacteria in few cells observed in tests using prolonged incubation periods (6 h) (**Figure 1B**) [39, 40]. Interestingly, the LAL pattern is determined in prolonged assays (6 h) of bacteria-cell interaction [39]. LAL is the most common pattern seen among EPEC strains; however, some strains display alternate adherence phenotypes such as diffuse adherence (DA) and aggregative adherence (AA) [19].

4.2 Attaching and effacing (A/E) lesion

The hallmark of EPEC infection is the ability of the organism to attach intimately to epithelial cells and efface microvilli (**Figure 1C**). This effect was first described by Staley et al. [41], although the term attaching and effacing (A/E)

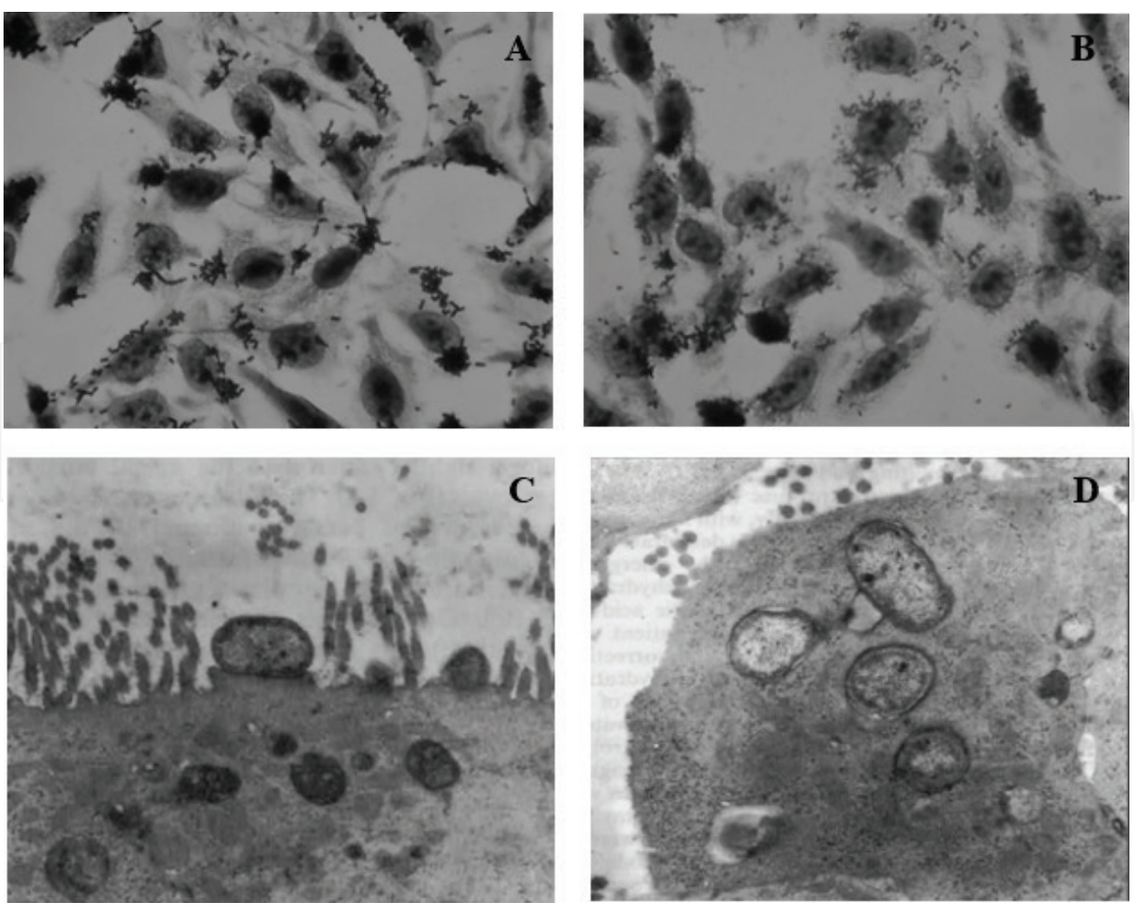


Figure 1.
EPEC adhesion to epithelial cells: (A) localized adherence pattern (LA) of typical EPEC on HeLa cells; (B) localized adherence-like (LAL) pattern of atypical EPEC on HeLa cells; (C) attaching and effacing (A/E) of enterocytes by EPEC; and (D) small bowel biopsy of infant infected with typical EPEC O111ab:H2 [36].

was coined by Moon et al. [13]. The A/E characteristic can be observed by electron microscopic examination of cultured epithelial cells exposed to EPEC or of intestinal biopsies from infants or animals infected with EPEC [24].

4.3 Invasiveness

Intracellular typical EPEC have been observed both in tissue culture and in small intestinal biopsies from an EPEC-infected infant (**Figure 1D**) [36]. Fletcher et al. [42] and Scaletsky et al. [43] have reported that EPEC O111:NM strains contain plasmid sequences that confer invasiveness upon *E. coli* K12 strains. However, despite their invasive potential in vitro, most EPEC are considered as noninvasive pathogens [44].

4.4 Biofilm formation

Typical EPEC have the ability to form biofilms on abiotic surfaces under static conditions, or on a flow through continuous culture system, and a model of EPEC biofilm formation has been proposed [45]. Biofilm formation requires adhesive structures as type 1 pili, antigen 43, BFP, and the EspA filament (see below) as participants in bacterial aggregation during biofilm formation on abiotic surfaces [45]. Atypical EPEC strains have also been shown to adhere to abiotic surfaces (polystyrene and glass) [46, 47]. The non-fimbrial adhesin curli and the T1P were shown to mediate binding to these surfaces in some atypical EPEC at different temperatures [48, 49].

4.5 The EAF plasmid

Typical EPEC strains possess a large virulence plasmid called the EPEC adherence factor (EAF) plasmid [9], which varies in sequence among different EPEC strains but is somewhat conserved [12]. The EAF plasmid pMAR2 is found among strains of the EPEC1 lineage, whereas pB171 is more common among EPEC2 strains [50, 51]. Two sets of genes located on the EAF plasmid are important for pathogenicity: the *bfp* gene cluster encoding BFP [38] and the *per* locus encoding a transcriptional activator called plasmid-encoded regulator (Per) [51]. Both BFP and PerA have been shown to contribute to virulence in human volunteers [52]. Between pMAR2 and pB171, the *bfp* and *per* loci share 99% sequence similarity [50]. Studies of comparison genomics of the EAF plasmids from varied EPEC phylogenomic lineages demonstrated significant plasmid diversity among isolates within the same phylogenomic lineage [53].

4.6 Bundle-forming pilus (BFP)

Typical EPEC strains produce a type IV pilus, the bundle-forming pili (BFP), which interconnects bacteria within microcolonies, promoting their stabilization and producing the LA phenotype [38]. The BFP is encoded by an operon of 14 genes contained on the EAF plasmid, with *bfpA* encoding the major structural subunit (bundlin) [54]. These 14 *bfp* genes are highly conserved among EPEC1 and EPEC2 strains. Some O128:H2 and O119:H2 EPEC strains that contain part of the *bfpA* gene have the rest of the *bfp* gene cluster deleted and replaced with an IS*66* element [55, 56].

4.7 The locus of enterocyte effacement (LEE) and the type III secretion system (TTSS)

The locus of enterocyte effacement (LEE) is a 35.6-kb pathogenicity island of EPEC containing genes necessary for the formation of the A/E lesion [57]. The EPEC LEE contains at least 41 open reading frames that are organized into five operons

(*LEE1* to *LEE5*) [58–60]. *LEE1*, *LEE2*, and *LEE3* encode a type III protein secretion system (T3SS) and Ler (LEE-encoded regulator) regulators, such as GrlA (global regulator of LEE activator, formerly called Orf11) and GrlR (Grl repressor, formerly called Orf10) [61]. *LEE4* encodes the EPEC-secreted proteins EspA, EspB, and EspD via the type III system. LEE5 encodes intimin and its translocated receptor, Tir [62]. Besides Tir, the EPEC genome contains other six LEE-encoded effector proteins translocated into the cell (Map, EspF, EspG, EspZ, EspH, and EspB), which interfere with different aspects of the cell physiology ([58, 59]; reviewed in [44]) [63].

In addition to the LEE effectors, various non-LEE (Nle)-encoded effector genes (*cif*, *espI/nleA*, *nleB*, *nleC*, *nleD*, *nleE*, and *nleH*) [59, 63] were described, which are located outside the LEE region of EPEC, in at least six chromosomal PAIs, or in prophage elements (reviewed in [64] and [65]). Although they are not required for AE lesion formation, it is understood that they contribute to increased bacterial virulence [66].

The LEE region of some atypical EPEC strains display a genetic organization similar to that found in the typical EPEC prototype E2348/69 strain [66]. Although the T3SS-encoding genes are considerably conserved [66, 67], the effector protein-encoding genes display important differences, and remarkable differences can be detected at the 5′ and 3′ flanking regions of atypical EPEC, suggesting the occurrence of different evolution events [68].

The expression of LEE genes is controlled by Per, which is encoded on the EAF plasmid present in typical EPEC strains. Per activates Ler, which in turn activates the *LEE2*, *LEE3*, *LEE4*, and *LEE5* operons, and the genes *espF*, *espG*, and *map* [58, 59]. The Ler protein is a histone-like nucleoid-structuring protein (H-NS) that responds to an environmental stimulus (temperature). Ler also controls genes located outside the LEE, such as *espC* and *nleA* [60]. Additional regulatory system has been shown to control expression of the LEE [69]. The AI-2 (autoinducer-2) quorum-sensing system regulates LEE1 operon, which increases expression of the *LEE3* and *LEE4* operons via the *ler* gene product. Two novel LEE-encoded regulators that have roles in *ler* expression were reported, GrlA (global regulator of LEE activator) and GrlR (Grl repressor) [61]. GrlR and GrlA are positive and negative regulators, respectively, required for the expression of several LEE-encoded genes [61]. Other LEE regulators include the integration host factor (IHF); Bip, a tyrosine-phosphorylated GTPase; Fis (factor for inversion stimulation); and GadX, which is a member of the AraC transcription factor family [58].

4.8 Intimin and Tir

Intimin is a 94-KDa outer membrane adhesin encoded by the *eae* gene and required for intimate adherence of EPEC to epithelial cells at the sites of A/E lesions [24]. N-Terminal portions are highly conserved, whereas C-terminus portions are highly variable [70]. C-Terminal intimin differences have been used as a basis for classification into several distinct subtypes (represented by the Greek letters to α (alpha) through ζ (zeta) [71, 72]); human EPEC1 strains express subtype α, while EPEC2 strains express subtype β. The N-terminus portion binds intimin in the bacterial outer membrane, whereas the C-terminus portion binds intimin to Tir. The binding of intimin to Tir leads to intimate adherence of the bacterium to the epithelium and pedestal formation beneath adherent bacteria. In addition, Tir inhibits NF-κB activity by targeting tumor necrosis factor alpha (TNF-α) receptor-associated factors [73].

4.9 Other potential adhesins

In addition to BFP, two other EPEC surface structures, rodlike fimbriae and fibrillae, have been characterized and have been suggested to be involved in the

interaction of EPEC with host cells [74, 75]. Additionally, EPEC strains encode a large surface protein, lymphocyte inhibitory factor (LifA), that contributes to epithelial cell adherence in vitro [76, 77] and is required for intestinal colonization of mice by the related A/E pathogen *C. rodentium* [78]. The *lifA* gene is more commonly found among typical rather than atypical EPEC strains [79]; however, atypical EPEC strains harboring *lifA* have a significant association with diarrhea in children under 5 years of age [80]. A novel gene cluster, designated the locus for diffuse adherence (*lda*), was found in an atypical EPEC O26 strain that is responsible for mediating DA adherence; its expression is induced by bile salts [81]. The *E. coli* common pilus (ECP) has also been shown to act as an accessory adherence factor in EPEC, playing a role during cell adherence and/or in bacterium-bacterium interactions [82].

4.10 Flagella

Flagella has been suggested to be involved in EPEC adherence to epithelial cells [83]. EPEC mutants with transposon insertion in the flagellar gene *fliC* were deficient in localized adherence, and anti-flagellum antibodies were effective in blocking the adherence of several EPEC serotypes [83]. However, a subsequent study has not confirmed a role of flagella in EPEC adherence [84].

5. EspC

EspC is a high-molecular-weight secreted protein of EPEC that induces cytopathic effects on epithelial cells, including cytoskeletal damage [24, 85]. EspC is a member of the serine protease autotransporters of the *Enterobacteriaceae* (SPATE) family of autotransporter proteins that encodes its own transport mechanism. Moreover, espC has been shown to interact with and degrade hemoglobin [86] and to hydrolyze other proteins such as pepsin, factor V, and spectrin [87]. In addition, EspC confers enhanced lysozyme resistance to EPEC [87] and serves as a substratum for adherence and biofilm formation as well as to protect bacteria from antimicrobial compounds [88]. EspC is encoded in a 15-kb chromosomal island specific to EPEC1 strains [24].

6. Other toxins

Scott and Kaper [89] reported a cytolethal distending toxin (CDT) in an EPEC strain that induces chromatin disruption, which leads to G2/M-phase growth arrest of the target cell and ultimately cell death [90]. A study has suggested that most EPEC strains from diarrhea harbor the CDT gene [91]. Another toxin is the enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) also present in EPEC strains [92]. The use of an EAST1 DNA probe suggests that this toxin is expressed by a number of clinical EPEC isolates [18, 93]. The role of CDT and EAST1 in EPEC pathogenesis remains to be elucidated.

7. Model of EPEC pathogenesis

A three-stage model of EPEC pathogenesis was first described in the early 1990s [94], Clarke et al. [95], including localized adherence to the host cell, signal transduction, and intimate attachment with pedestal formation (**Figure 2**).

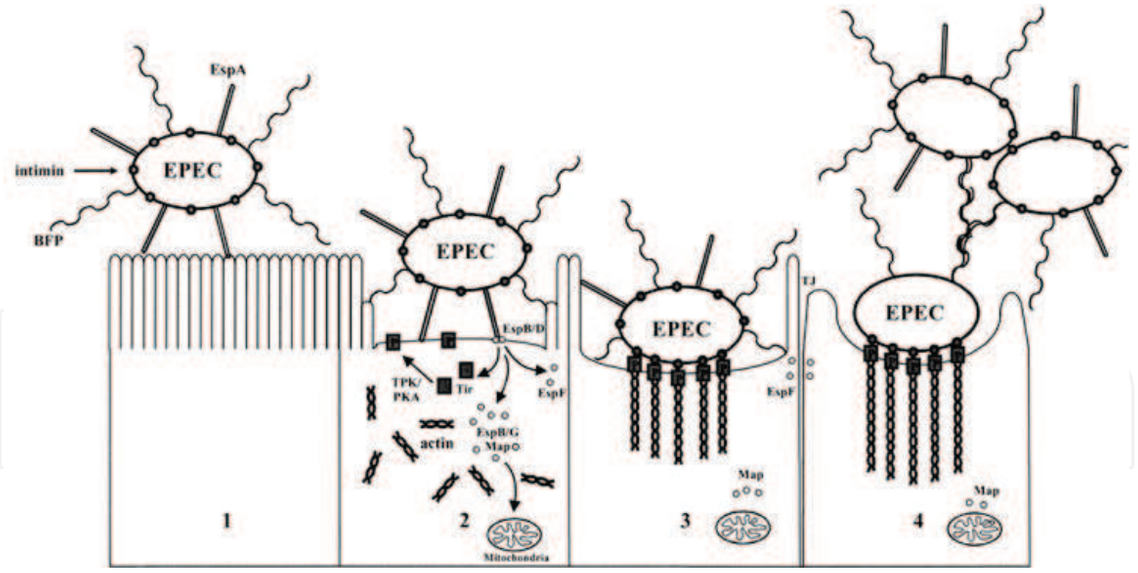


Figure 2.
Four-stage model of EPEC pathogenesis. Reprinted from Clarke et al. [95].

In the first stage, an attachment of typical EPEC to the surface of the host intestinal epithelium is mediated by the bundle-forming pili (BFP). The filament EspA also promotes attachment, albeit in a less efficient manner, and could mediate adherence of atypical EPEC strains. In the second stage, Tir and effector proteins (EspB, EspD, EspF, EspG, and Map), translocated into the host cells via type III system apparatus, activate cell-signaling pathways, causing alterations in the host cell cytoskeleton and resulting in actin accumulation and loss of microvilli [58]. In the third stage, bacteria intimately adhered to host cell by intimin-Tir interactions amplifies the accumulation of filaments of actin and other cytoskeletal proteins that result in pedestal-like structures [62, 96, 97]. Finally, the translocated effectors disrupt host cell processes, causing loss of tight-junction integrity and mitochondrial function and leading to both electrolyte loss and eventual cell death.

8. Diagnosis

Traditionally, the identification of EPEC was based on the O:H serotyping, but serotype designation is no longer precise. The identification of EPEC was based on the characteristic of EPEC's attachment to epithelial cells and may include phenotypic or genotypic tests. The HeLa adherence assay distinguishes EPEC from other *E. coli* by their ability to adhere in a localized pattern (LA) on the surface of cells [8]. The fluorescent actin-staining (FAS) assay, originally described by Knutton et al. [98], leads to the identification of the A/E lesion, by detecting actin condensation under EPEC adhesion pedestals. DNA probes and PCR targeting genes responsible for these characteristics were developed. A 1-kb EAF fragment probe was initially developed as a diagnostic DNA probe (the EAF probe) and subsequently refined as an oligonucleotide probe as well as PCR primers [10, 11, 99]. The identification of *bfpA*, the structural gene encoding BFP, led to the development of more specific and sensitive probe or PCR tests to detect typical EPEC strains [74, 75, 100]. However, some PCR primers may fail to identify all typical EPEC strains since multiple alleles of *bfpA* have been identified [101]. The *eae* sequences by DNA probes and PCR primers have been used to detect the presence of LEE encoding A/E lesion [24].

9. Clinical features of EPEC infection

9.1 Symptoms

The most common symptoms reported in EPEC infection are watery diarrhea, dehydration, vomiting, food intolerance, and low-grade fever [24, 97]. In addition, EPEC infection may lead to severe malabsorption of nutrients resulting in nutritional aggravation and persistence of diarrhea [102]. Edema, neutrophil infiltrate, and reduced enzymatic activity in the intestinal mucosa have been also found in EPEC infection [103]. EPEC diarrhea often lasts 1–2 weeks but can become persistent, lasting more than 2 weeks, and may result in severe infection [24, 25, 32, 102]. In a recent case-control study, EPEC infection was associated with a 2.8-fold elevated risk of death among infants in Kenya [24, 31, 97].

9.2 Treatment

Treatment of EPEC diarrhea includes oral rehydration therapy to prevent dehydration by correcting fluid and electrolyte losses. Oral rehydration may be sufficient for cases of self-limited acute diarrhea, but persistent cases of diarrhea may include parenteral rehydration, and more severe cases may require total parental nutrition and use of antimicrobials [102]. Multidrug resistance has been reported in EPEC strains from diverse parts of the world [27–29, 44, 104, 105]. Alternative therapies, employing the use of bismuth subsalicylate, specific bovine anti-EPEC milk immunoglobulins, and also zinc, have been proven useful for treatment and prevention of EPEC diarrhea [106].

9.3 Vaccines

There are no currently available vaccines to prevent EPEC infection. However, a recent study has used bacterial ghosts devoid of cytoplasmic contents but expressing all EPEC surface components in vaccination challenge experiments with mice, and the results showed 84–90% protection in control mice [107]. Interestingly, protective effect of breast-feeding was shown to provide excellent protection against EPEC infection. Several investigators have shown that breast milk provides protection against EPEC O antigens and outer membrane proteins [108, 109]. Furthermore, IgA antibodies against BFP, intimin, EspA, and EspB proteins were identified in maternal colostrum and serum samples [110–118].

10. Conclusion

Much progress has been made in the last 20 years toward understanding the pathogenesis of EPEC. It has been shown that typical EPEC are still important pathogens associated with severe outbreaks of infant diarrhea, and atypical EPEC are emerging pathogens associated with sporadic outbreaks at all ages worldwide.

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References

[1] Lesage AA. Contribution á l´étude des enteritis infantiles-serodiagnostic des races de *Bacterium coli*. Comptes Rendues des Societe Biologique (Paris). 1897;**49**:900-901

[2] Bray J. Isolation of antigenically homogeneous strains of *Bacterium coli neopolitanum* from summer diarrhoea of infants. The Journal of Pathology and Bacteriology. 1945;**57**:239-247

[3] Kauffmann F, Dupon A. *Escherichia coli* strains from infantile epidemic gastroenteritis diarrhea. Acta Neuropathologica. 1950;**27**:221-226

[4] Neter E, Westphal O, Luderitz O, et al. Demonstration of antibodies against enteropathogenic *Escherichia coli* in sera of children of various ages. Pediatrics. 1955;**16**:801-807

[5] Levine MM, Bergquist EJ, Nalin DR, et al. *Escherichia coli* strains that cause diarrhea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet. 1978;**1**(8074):1119-1122

[6] Edelman R, Levine MM. From the National institute of allergy and infectious diseases. Summary of a workshop on enteropathogenic *Escherichia coli*. The Journal of Infectious Diseases. 1983;**147**:1108-1118

[7] Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of *Escherichia coli* belonging to traditional infantile enteropathogenic serotypes. Current Microbiology. 1979;**3**:95-99

[8] Scaletsky IC, Silva ML, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infection and Immunity. 1984;**45**:534-536

[9] Baldini MM, Kaper JB, Levine MM, et al. Plasmid mediated adhesion of enteropathogenic *Escherichia coli*. Journal of Pediatric Gastroenterology and Nutrition. 1983;**2**:534-538

[10] Nataro JP, Baldini MM, Kaper JB, Black RE, Bravo N, Levine MM. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. The Journal of Infectious Diseases. 1985a;**152**:560-565

[11] Nataro JP, Scaletsky ICA, Kaper JB, Levine MM, Trabulsi LR. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infection and Immunity. 1985b;**48**:378-383

[12] Nataro JP, Maher KO, Mackie P, Kaper JB. Characterization of plasmids encoding the adherence factor of enteropathogenic *Escherichia coli*. Infection and Immunity. 1987;**55**:2370-2377

[13] Moon HW, Whipp SC, Argenzio RA, et al. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infection and Immunity. 1983;**41**:1340-1351

[14] Knutton S, Baldini, MM, Kaper JB, McNeish AS. Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells. Infection and Immunity. 1987;**55**:78-85

[15] Kaper JB. Defining EPEC. Revista de Microbiologia, Sao Paulo. 1996;**27**:130-133

[16] Ørskov F, Whittam TS, Cravioto A, Ørskov I. Clonal relationships among classic enteropathogenic *Escherichia coli* (EPEC) belong to different O groups. The Journal of Infectious Diseases. 1990;**162**:76-81

[17] Whittam TS, McGraw EA. Clonal analysis of EPEC serogroups. Revista de Microbiologia. 1996;**27**:7-16

[18] Dulguer MV, Fabricotti SH, Bando SY, et al. Atypical enteropathogenic *Escherichia coli* strains: Phenotypic and genetic profiling reveals a strong association between enteroaggregative *E. coli* heat-stable enterotoxin and diarrhea. The Journal of Infectious Diseases. 2003;**188**:1685-1694

[19] Trabulsi LR, Keller R, Gomes TAT. Typical and atypical enteropathogenic *Escherichia coli*. Emerging Infectious Diseases. 2002;**8**:508-513

[20] Vieira MA, Andrade JR, Trabulsi LR. Phenotypic and genotypic characteristics of *Escherichia coli* strains of non-enteropathogenic *E. coli* (EPEC) serogroups that carry EAE and lack the EPEC adherence factor and Shiga toxin DNA probe sequences. The Journal of Infectious Diseases. 2001;**183**:762-772

[21] Hazen TH, Sahl JW, Fraser CM, et al. Refining the pathovar paradigm via phylogenomics of the attaching and effacing *Escherichia coli*. PNAS. 2013;**110**:12810

[22] Ochoa TJ, Barletta F, Contreras C, et al. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;**102**:852-856

[23] Maranhão HS, Medeiros MCC, Scaletsky ICA, et al. The epidemiological and clinical characteristics and nutritional development of infants with acute diarrhea, in northeastern Brazil. Annals of Tropical Medicine and Parasitology. 2008;**102**:357-365

[24] Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews. 1998;**11**:142-201

[25] Hu J, Torres AG. Enteropathogenic *Escherichia coli*: Foe or innocent bystander? Clinical Microbiology and Infection. 2015:1-6

[26] Franzolin MR, Alves RCB, Keller R, Gomes TAT, Beutin L, Barreto ML, et al. Prevalence of diarrheagenic *Escherichia coli* in children with diarrhea in Salvador, Bahia, Brazil. Memórias do Instituto Oswaldo Cruz. 2005;**100**:359-363

[27] Scaletsky ICA, Souza TB, Aranda KRS, Okeke IN. Genetic elements associated with antimicrobial resistance in enteropathogenic *Escherichia coli* (EPEC) from Brazil. BMC Microbiology. 2010;**10**:25

[28] Scaletsky ICA, Aranda KR, Souza TB, Silva NP, Morais MB. Evidence of pathogenic subgroups among atypical enteropathogenic *Escherichia coli* strains. Journal of Clinical Microbiology. 2010;**47**:3756-3759

[29] Scaletsky ICA, Aranda KRS, Souza TB, Silva NP. Adherence factors in atypical enteropathogenic *Escherichia coli* strains expressing the localized adherence-like pattern in HEp-2 cells. Journal of Clinical Microbiology. 2010;**48**:302-306

[30] Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhea. Journal of Medical Microbiology. 2006;**55**:1159-1163

[31] Kotloff KL, Nataro JP, Blackwelder W, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (The Global Enteric Multicentre Study, GEMS): A prospective, case-control study. Lancet. 2013;**382**(9888):209-222

[32] Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and

pathogenesis. Epidemiology Review. 1984;**6**:31-51

[33] Behiry IK, Abada EA, Ahmed EA, Labeed RS. Enteropathogenic *Escherichia coli* associated with diarrhea in children in Cairo, Egypt. Scientific World Journal. 2011;**11**:2613-2619

[34] Hernandes RT, Elias WP, Vieira AM, Gomes TAT. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiology Letters. 2009;**297**:137-149

[35] Kolenda R, Burdukiewicz M, Schierck P. A systematic review and meta-analysis of the epidemiology of pathogenic *Escherichia coli* of calves and the role of calves as reservoirs for human pathogenic *E. coli*. Frontiers in Cellular and Infection Microbiology. 2015;**5**:23

[36] Fagundes-Neto U, Freymuller E, Gatti MSV, et al. Enteropathogenic *Escherichia coli* O111ab:H2 penetrates the small bowel epithelium in an infant with acute diarrhea. Acta Paediatrica. 1995;**84**:453-455

[37] Rothbaum R, McAdams AJ, Giannella R, Partin JC. A clinicopathological study of enterocyte-adherent *Escherichia coli*: A cause of protracted diarrhea in infants. Gastroenterologia. 1982;**83**:441-454

[38] Girón JA, Ho AS, Schoolnik GK. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. Science. 1991;**254**:710-713

[39] ICA S, Pelayo JS, Giraldi R, et al. EPEC adherence to HEp-2 cells. Proceedings of the International Symposium on Enteropathogenic *Escherichia coli* (EPEC). Revista de Microbiologia. 1996;**27**:58-62

[40] Scaletsky ICA, Pedroso MZ, Oliva CAG, et al. A localized adherence-like pattern as a second pattern of adherence of classic enteropathogenic *Escherichia coli* to HEp-2 cells that is associated with infantile diarrhea. Infection and Immunity. 1999;**67**:3410-3415

[41] Staley TE, Jones EW, Corley LD. Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. The American Journal of Pathology. 1969;**56**:371-392

[42] Fletcher JN, Embaye HE, Getty B, et al. Novel invasion determinant of enteropathogenic *Escherichia coli* plasmid pLV501 encodes the ability to invade intestinal epithelial cells and HEp-2 cells. Infection and Immunity. 1992;**60**:2229-2236

[43] Scaletsky ICA, Gatti MSV, Da Silveira, et al. Plasmid encoding for drug resistance and invasion of epithelial cells in enteropathogenic *Escherichia coli* O111:H. Microbial Pathogenesis. 1995;**18**:387-399

[44] Croxen MA, Law RJ, Scholz R, et al. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews. 2013;**26**:822-880

[45] Moreira CG, Palmer K, Whiteley M, et al. Bundle-forming pili and EspA are involved in biofilm formation by enteropathogenic *Escherichia coli*. Journal of Bacteriology. 2006;**188**:3952-3961

[46] Culler HF, Mota CM, Abe CM, et al. Atypical enteropathogenic *Escherichia coli* strains form biofilm on abiotic surfaces regardless of their adherence pattern on cultured epithelail cells. BioMed Research International. 2014;**26**:822-880

[47] Nascimento HH, Silva LEP, Souza RT, et al. Phenotypic and genotypic characteristics associated with biofilm formation in clinical isolates of atypical enteropathogenic *Escherichia coli* (aEPEC) strains. BMC Microbiology. 2014;**14**:184

[48] Hernandes RT, Dela Cruz MA, Yamamoto D, et al. Dissection of the role of pili and type 2 and 3 secretion systems in adherence and biofilm formation of an atypical enteropathogenic *Escherichia coli* strain. Infection and Immunity. 2013;**81**:3793-3802

[49] Weiss-Muzkat M, Shakh D, Zhou Y, et al. Biofilm formation and multicellular behavior in *Escherichia coli* O55:H7, an atypical enteropathogenic strain. Applied and Environmental Microbiology. 2010;**76**:1545-1554

[50] Brinkley C, Burland V, Keller R, et al. Nucleotide sequence analysis of the enteropathogenic *Escherichia coli* adherence factor plasmid pMAR7. Infection and Immunity. 2006;**74**:5408-5413

[51] Tobe T, Hayashi T, Han C, et al. Complete DNA sequence and structural analysis of the enteropathogenic *Escherichia coli* adherence factor plasmid. Infection and Immunity. 1999;**67**:5455-5462

[52] Bieber D, Ramer SW, Wu CY, et al. Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*. Science. 1998;**280**:2114-2118

[53] Hazen TH, Kaper JB, Nataro JP, Rasko DA. Comparative genomics provides insight into the diversity of the attaching and effacing *Escherichia coli* virulence plasmids. Infection and Immunity. 2015;**83**:4103-4117

[54] Stone KD, Zhang H, Carlson LK, Donnenberg MS. A cluster of fourteen genes from enteropathogenic *Escherichia coli* is sufficient for the biogenesis of a type IV pilus. Molecular Microbiology. 1996;**20**:325-337

[55] Bortoloni M, Trabulsi L, Keller R, et al. Lack of expression of bundle-forming pili in some clinical isolates of enteropathogenic *Escherichia coli* (EPEC) is due to a conserved large deletion in the *bfp* operon. FEMS Microbiology Letters. 1999;**179**:169-174

[56] Teixeira NB, Rojas TCG, Silveira WD, et al. Genetic analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor (EAF) plasmid reveals a new deletion within the EAF probe sequence among O119 typical EPEC strains. BMC Microbiology. 2015;**15**:200

[57] McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**:1664-1668

[58] Dean P, Kenny B. The effector repertoire of enteropathogenic *E. coli* ganging up on the host cell. Current Opinion in Microbiology. 2009;**12**:101-109

[59] Deng W, Puente JL, Grunheid S, et al. Dissecting virulence: Systematic and functional analyses of a pathogenicity island. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:3597-3602

[60] Elliott SJ, Sperandio V, Giron JA, et al. The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic *Escherichia coli*. Infection and Immunity. 2000;**68**:6115-6126

[61] Barba J, Bustamante VH, Flores-Valdez MA, et al. A positive regulatory loop controls expression of the locus of enterocyte effacement-encoded regulators Ler and GrlA. Journal of Bacteriology. 2005;**187**:7918-7730

[62] Kenny B, DeVinney R, Stein M, et al. Enteropathogenic *Escherichia coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell. 1997;**91**:511-520

[63] Santos A, Finlay BB. Bringing down the host: Enteropathogenic and enterohaemorrhagic *Escherichia coli* effector-mediated subversion of host innate immune pathways. Cellular Microbiology. 2015;**17**:318-332

[64] Wong ARC, Pearson JS, Bright MD, et al. Enteropathogenic and enterohaemorrhagic *Escherichia coli*: Even more subservive elements. Molecular Microbiology. 2011;**80**:1420-1438

[65] Vossenkämper A, MacDonald TT, Marches O, et al. Always one step ahead: How pathogenic bacteria use the type III secretion system to manipulate the intestinal mucosal immune system. Journal of Inflammation. 2011;**8**:11

[66] Gärtner JF, Schmidt MA, Ga JF, et al. Comparative analysis of locus of enterocyte effacement pathogenicity islands of atypical enteropathogenic *E. coli*. Society. 2004;**72**:6722-6728

[67] Ingle DJ, Tauschek M, Edwards DJ, et al. Evolution of atypical enteropathogenic *E. coli* by repeated acquisition of LEE pathogenicity island variants. Nature Microbiology. 2016;**1**:15010

[68] Müller D, Benz I, Liebchen A, et al. Comparative analysis of the locus of enterocyte effacement and its flanking regions. Infection and Immunity. 2009;**77**:3501-3513

[69] Sperandio V, Li CC, Kaper JB. Quorum-sensing *Escherichia coli* regulator A: A regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. Infection and Immunity. 2002;**70**:3085-3093

[70] Frankel G, Candy DCA, Everest P, Dougan G. Characterization of the C-terminal domains of intimin-like proteins of enteropathogenic and enterohemorrhagic *Escherichia coli*, *Citrobacter freundii*, and *Hafnia alvei*. Infection and Immunity. 1994;**62**:1835-1842

[71] Lacher DW, Steinsland H, Whittam TS. Allellic subtyping of the intimin locus (*eae*) of pathogenic *Escherichia coli* by fluorescent RFLP. FEMS Microbiology Letters. 2006;**261**:80-87

[72] Lacher DW, Steinsland H, Blank TE, et al. Molecular evolution of typical enteropathogenic *Escherichia coli*: Clonal analysis by multilocus sequence typing and virulence gene allelic profiling. Journal of Bacteriology. 2007;**189**:342-350

[73] Ruchaud-Sparagano M-H, Muhlen S, Dean P, Kenny B. The enteropathogenic *Escherichia coli* (EPEC) Tir effector inhibits NF-κB activity by targeting TNFα-receptor-associated factors. PLoS Pathogens. 2011;**7**:e1002414

[74] Girón JA, Ho ASY, Schoolnik GK. Characterization of fimbriae produced by enteropathogenic *Escherichia coli*. Journal of Bacteriology. 1993a;**175**:7391-7403

[75] Girón JA, Donnenberg MS, Martin WC, Jarvis KG, Kaper JB. Distribution of the bundle-forming pilus structural genes (*bfpA*) among enteropathogenic *Escherichia coli*. The Journal of Infectious Diseases. 1993b;**168**:1037-1041

[76] Badea L, Doughty S, Nicholls L, et al. Contribution of Efa/LifA to the adherence of enteropathogenic *Escherichia coli* to epithelial cells. Microbial Pathogenesis. 2003;**34**:205-215

[77] Klapproth JM, Scaletsky ICA, McNamara BP, et al. A large toxin from pathogenic *Escherichia coli* strains that inhibits lymphocyte activation. Infection and Immunity. 2000;**68**:2148-2155

[78] Klapproth JM, Sasaki M, Sherman M, et al. *Citrobacter rodentium lifA/efa1* is essential for colonic colonization and crypt cell hyperplasia *in vivo*. Infection and Immunity. 2005;**73**:1441-1451

[79] Vieira MAM, Salvador FA, Silva RM, et al. Prevalence and characteristics of the O122 pathogenicity island in typical and atypical enteropathogenic *Escherichia coli* strains. Journal of Clinical Microbiology. 2010;**48**:1452-1455

[80] Afset JE, Bruant G, Brousseau R, et al. Identification of virulence genes linked with diarrhea due to atypical enteropathogenic *Escherichia coli* by DNA microarray analysis and PCR. Journal of Clinical Microbiology. 2006;**44**:3703-3711

[81] Torres AG, Tutt CB, Durval L, et al. Bile salts induce expression of the afimbrial LDA adhesion of atypical enteropathogenic *Escherichia coli*. Cellular Microbiology. 2007;**9**:1039-1049

[82] Saldaña Z, Erdem AL, Schuller S, et al. The *Escherichia coli* common pilus and the bundle-forming pilus act in concern during the formation of localized adherence by enteropathogenic *E. coli*. Journal of Bacteriology. 2009;**191**:3451-3461

[83] Girón JA, Torres AG, Freer E, et al. The flagella of enteropathogenic *Escherichia coli* mediate adherence to epithelial cells. Molecular Microbiology. 2002;**44**:361-479

[84] Clearly J, Lai LC, Shaw RK, et al. Enteropathogenic *Escherichia coli* (EPEC) adhesion to intestinal epithelial cells: Role of bundle-forming pili (BFP), EspA filaments and intimin. Microbiology. 2004;**150**:527-538

[85] Vidal JE, Navarro-Garcia F. EspC translocation into epithelial cells by enteropathogenic *Escherichia coli* requires a concerted participation of type V and III systems. Cellular Microbiology. 2008;**10**:1976-1986

[86] Drago-Serrano ME, Parra SG, Manjarrez-Hernández HA. EspC, an autotransporter protein secreted by enteropathogenic *Escherichia coli* (EPEC), displays protease activity on human hemoglobin. FEMS Microbiology Letters. 2006;**265**:35-40

[87] Salinger N, Kokona B, Fairman R, Okeke I. The plasmid encoded regulator activates factors conferring lysozyme resistance on enteropathogenic *Escherichia coli* strains. Applied and Environmental Microbiology. 2009;**75**:275-280

[88] Xicohtencatl-Cortes J, Saldaña Z, Deng W, et al. Bacterial macroscopic ropelike fibers with cytopathic and adhesive properties. The Journal of Biological Chemistry. 2010;**285**:32336-32342

[89] Scott DA, KaperJB. Cloning and sequencing of the genes encoding *Escherichia coli* cytolethal distending toxin. Infection and Immunity. 1994;**62**:244-251

[90] Lara-Tejero M, Galan JE. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. Science. 2000;**290**:354-357

[91] Albert MJ, Faruque SM, Faruque ASG, et al. Controlled study of cytolethal distending toxin-producing Escherichia coli infections in Bangladeshi children. Journal of Clinical Microbiology. 1996;**34**:717-719

[92] Yamamoto T, Wakisaka N, Sato F, Kato A. Comparison of the nucleotide

sequence of enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 genes among diarrhea-associated *Escherichia coli*. FEMS Microbiology Letters. 1997;**147**:89-96

[93] Silva LEP, Souza TB, Silva NP, Scaletsky ICA. Detection and genetic analysis of the enteroaggregative *Escherichia coli* heat-stable enterotoxin (EAST1) gene in clinical isolates of enteropathogenic *Escherichia coli* (EPEC) strains. BMC Microbiology. 2014;**14**:135

[94] Donnenberg MS, Kaper JB. Enteropathogenic *Escherichia coli*. Infection and Immunity. 1992;**60**:3953-3961

[95] Clarke SC et al. Virulence of enteropathogenic *Escherichia coli*, a global pathogen. Clinical Microbiology Reviews. 2003;**6**:365-378

[96] Campellone KG, Giese A, Tipper DJ, Leong JM. A tyrosine-phosphorylated 12-aminoacid sequence of enteropathogenic *Escherichia coli* Tir binds the host adaptor protein Nck and is required for Nck localization to actin pedestals. Molecular Microbiology. 2002;**43**:1227-1241

[97] Goosney DL, DeVinney R, Finlay BB. Recruitment of cytoskeletal and signaling proteins to enteropathogenic and enterohemorrhagic *Escherichia coli* pedestals. Infection and Immunity. 2001;**69**:3315-3322

[98] Knutton S, Baldwin T, Williams PH, McNeish AS. Actin accumulation at sites of bacterial adhesion to tissue culture cells: Basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infection and Immunity. 1989;**57**:1290-1298

[99] Franke J, Franke S, Schmidt H, et al. Nucleotide sequence analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. Journal of Clinical Microbiology. 1994;**32**:2460-2463

[100] Gunzburg ST, Tornieporth NG, Riley LW. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. Journal of Clinical Microbiology. 1995;**33**:1375-1377

[101] Blank TE, Zhong H, Bell AL, et al. Molecular variation among type IV pilin (*bfpA*) genes from diverse enteropathogenic *Escherichia coli* strains. Infection and Immunity. 2000;**68**:7028-7038

[102] Fagundes-Neto U, Scaletsky ICA. The gut at war: The consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. São Paulo Medical Journal/Revista Paulista de Medicina. 2000;**118**:21-29

[103] Arenas-Hernandez MM, Martinez-Laguna Y, Torres AG. Clinical implications of enteroadherent *Escherichia coli*. Current Gastroenterology Reports. 2012;**14**:386-394

[104] Mitra M, Ahmad P, Mehdi R, Hosein A, Ahmad K. Multiple drug resistance of enteropathogenic *Escherichia coli* isolated from children with diarrhea in Kashan, Iran. African Journal of Microbiology Research. 2011;**5**:3305-3309

[105] Subramanian K, Selvakkumar C, Vinaykumar KS, et al. Tackling multiple antibiotic resistance in enteropathogenic *Escherichia coli* (EPEC) clinical isolates: A diarylheptanoid from Alpinia officinarum shows promising antibacterial and imunomodulatory activity against EPEC and its lipopolysacharide-induced inflammation. International Journal of Antimicrobial Agents. 2009;**33**:244-250

[106] Patel A, Mamtani M, Dibley MJ, et al. Therapeutic value of zinc supplementation in acute and persistent diarrhea: A systematic review. PLoS One. 2010;**5**:e10386

[107] Liu J, Wang WD, Liu YJ, et al. Mice vaccinated with enteropathogenic *Escherichia coli* ghosts show significant protection against lethal challenges. Letters in Applied Microbiology. 2012;**54**:255-262

[108] Costa-Carvalho BT, Bertipaglia A, Solé D, et al. Detection of immunoglobulin (IgG and IgA) anti-outer membrane proteins of enteropathogenic *Escherichia coli* (EPEC) in saliva, colostrum, breast milk, serum, cord blood and amniotic fluid. Study of inhibition of localized adherence of EPEC to HeLa cells. Acta Paediatrica. 1994;**83**:870-873

[109] Cravioto A, Tello A, Villafan H, et al. Inhibition of localized adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells by immunoglobulin and oligosaccharide fractions of humancolostrum and breast milk. The Journal of Infectious Diseases. 1991;**163**:1247-1255

[110] Fernandes RCSC, Quintana Flores VM, Sousa de Macedo Z, Medina-Acosta E. Coproantibodies to the enteropathogenic *Escherichia coli* vaccine candidates BfpA and EspB in breast fed and artificially fed children. Vaccine. 2003;**21**:1725-1731

[111] Parissi-Crivelli A, Parissi-Crivelli JM, Girón JA. Recognition of enteropathogenic *Escherichia coli* virulence determinants by human colostrum and serum antibodies. Journal of Clinical Microbiology. 2000;**38**:2696-2700

[112] Quintana Flores VM, Fernandes RCCS, Sousa de Macedo Z, Medina-Acosta E. Expression and purification of the recombinant enteropathogenic *Escherichia coli* vaccine candidates BfpA and EspB. Protein Expression and Purification. 2002;**25**:16-22

[113] Sanches MI, Keller R, Hartland EL, et al. Human colostrum and serum contain antibodies reactive to the intimin-binding region of the enteropathogenic *Escherichia coli* translocated intimin receptor. Journal of Pediatric Gastroenterology and Nutrition. 2000;**30**:73-77

[114] Stakenborg T, Vandekerchove D, Mariën J, et al. Protection of rabbits against enteropathogenic *Escherichia coli* (EPEC) using an intimin null mutant. BMC Veterinary Research. 2006;**2**:22

[115] Tacket CO, Sztein MB, Losonsky G, et al. Role of EspB in experimental human enteropathogenic *Escherichia coli* infection. Infection and Immunity. 2000;**68**:3689-3695

[116] Carbonare SB, Silva ML, Palmeira P, Carneiro-Sampaio MM. Human colostrum IgA antibodies reacting to enteropathogenic *Escherichia coli* antigens and their persistence in the faeces of a breastfed infant. Journal of Diarrhoeal Diseases Research. 1997;**15**:53-58

[117] Donnenberg MS, Tacket CO, Losonsky G, et al. Effect of prior experimental human enteropathogenic *Escherichia coli* infection on illness following homologous and heterologous re-challenge. Infection and Immunity. 1998;**66**:52-58

[118] Loureiro I, Frankel G, Adu-Bobie J, et al. Human colostrum contains IgA antibodies reactive to enteropathogenic *Escherichia coli* virulence-associated proteins: Intimin, BfpA, EspA, and EspB. Journal of Pediatric Gastroenterology and Nutrition. 1998;**27**:166-171