

Clinical Implications of Enteroadherent *Escherichia coli*

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Abstract Pathogenic *Escherichia coli* that colonize the small intestine primarily cause gastrointestinal illness in infants and travelers. The main categories of pathogenic *E. coli* that colonize the epithelial lining of the small intestine are enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroaggregative *E. coli*. These organisms accomplish their pathogenic process by a complex, coordinated multistage strategy, including non-intimate adherence mediated by various adhesins. These so called “enteroadherent *E. coli*” categories subsequently produce toxins or effector proteins that are either secreted to the milieu or injected to the host cell. Finally, destruction of the intestinal microvilli results from the intimate adherence or the toxic effect exerted over the epithelia, resulting in water secretion and diarrhea. In this review, we summarize the current state of knowledge regarding these enteroadherent *E. coli* strains and the present clinical understanding of how these organisms colonize the human intestine and cause disease.

Keywords Enteropathogenic *E. coli* · Enterotoxigenic *E. coli* · Enteroaggregative *E. coli* · Diarrhea · Pathogenic *Escherichia coli* · Virulence · Adherence

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Introduction

At least six different categories of pathogenic *Escherichia coli* have been associated with gastrointestinal illness and diarrhea in humans (reviewed in [1]). However, this review focuses on three of these categories of *E. coli* and the effects they exert on the villous architecture of the small intestine, as well as the impact of their diverse pathogenic properties on diarrheal disease. Enterotoxigenic *E. coli* (ETEC) cause acute and severe diarrhea as a result of the production of potent toxins altering the biological activity of the intestinal epithelia. Enteropathogenic *E. coli* (EPEC) employ a type III protein secretion system to orchestrate internal changes in target cells. The intimate contact with the host apical enterocyte membrane results in dysregulation of water and solute transport and the disruption of epithelial barrier structure and function. Finally, enteroaggregative *E. coli* (EAEC) displays abundant adherence to the intestinal mucosa by the production of different enterotoxins and cytotoxins, resulting in induction of mucosal inflammation (reviewed in [2]). The following sections summarize recent progress in understanding the clinical outcome of these types of *E. coli* infections with respect to their unique pathogenic properties.

Enterotoxigenic *Escherichia coli*

ETEC are a very diverse group of pathogenic *E. coli* that colonize the small intestine and are a major cause of acute secretory diarrhea in developing countries [3]. Every year, ETEC accounts for 280–400 million cases in children under 5 years of age and an additional 100–400 million cases in children over 5 years of age and in adults [3]. This organism remains a major cause of diarrhea in travelers, including military personnel, and it substantially contributes to both delayed growth and malnutrition due to repeated bouts of infectious

diarrhea. Moreover, malnourished children appear to be at a higher risk of acquiring ETEC infections [4, 5].

The Pathogen and Clinical Implications of the Disease

As a heterogeneous group of pathogens, ETEC strains have in common the ability to colonize the small intestine, with the subsequent production and translocation of the plasmid-encoded heat-labile (LT) and/or heat-stable (ST) enterotoxins [3, 6]. The relative proportion of strains producing LT alone, ST alone, or LT/ST varies from one geographic area to another; but overall, 30–50 % of the clinical ETEC isolates seem to produce STs only [7, 8]. In the classic mechanism of ETEC pathogenesis, small intestinal colonization requires plasmid-encoded colonization factors (CFs), thereby enabling the organism to adhere to the mucosa [6]. Until now, more than 25 CFs have been recognized on human ETEC isolates [9]. The types of CF are subdivided by their antigenicity, molecular weight, N-terminal amino acid sequence of the major subunit, and structural morphology—fimbrial, fibrillar, helical, or nonfimbrial [1, 9]. In the case of the toxins, the STs can be further classified as STa or STb on the basis of structure and function [10]. STa, which is associated with human disease, binds to guanylyl cyclase receptors on the brush border of the intestine, stimulating their activity and increasing intracellular levels of cyclic GMP and activating the cystic fibrosis transmembrane conductance regulator (CFTR), resulting in impaired absorption of Na⁺ and H₂O efflux into the lumen. STb is mostly associated with porcine ETEC strains but can also be found in human isolates (Fig. 1) [10]. In contrast to STa, the STb toxin binds to sulphatide, a widely distributed acidic glycosphingolipid. The LT toxin is secreted [11], and it has been associated with lipopolysaccharide on the bacterial surface, acting as an adhesion and facilitating attachment to host cells [12]. LT toxins encompass at least 16 natural polymorphic toxin variants expressed by ETEC human isolates, but only LT1, produced by the reference ETEC H10407 strain, has been intensively studied as a virulence-associated factor and as a mucosal/transcutaneous adjuvant [13].

The diarrhea produced by ETEC is secretory. The disease begins with a sudden onset of watery stool (without blood or inflammatory cells) (Table 1). Other signs and symptoms include headache, fever, nausea, and vomiting, leading to dehydration (reviewed in [7]). The LT and ST enterotoxins are responsible for the electrolyte and fluid lost into the intestinal lumen and the onset of diarrhea after a short incubation period of 1–2 days. The patients are afebrile, and usually the self-limited diarrhea lasts only 3–4 days; however, some patients have prolonged diarrheal illness lasting a week or more [14]. In travelers, the diarrhea in the majority of the cases resolves without the need for medical care and might include other symptoms, such as cramping, nausea, and low-grade fever [15]. With adequate treatment, the mortality is very low (<1 %) [10].

Current Progress in Prevention and Treatment

Natural immunity to ETEC infection has been described in people repeatedly challenged with this bacterium [16]. However, ETEC remains the most common cause of diarrhea; therefore, there is intense work trying to obtain an ETEC vaccine. The design of such a vaccine is based on the knowledge of the mechanisms of immune protection during ETEC infections, and, as such, studies suggest that immune responses against CFs may protect against ETEC [8, 17]. Strains expressing CFs within specific isolates have been shown to induce substantial immune responses not only against homologous CFs, but also against other CFs [7]. On the basis of the identification of CFs as key protective ETEC antigens, the approach to developing a vaccine included preparing killed ETEC that express the most important immunogenic CFs [8]. Thus, CFs on ETEC inactivated by mild formalin-treatment have been shown to be more stable than purified CFs in the gastrointestinal milieu, as well as to retain immunogenicity, fimbrial structure, and capacity to bind to eukaryotic cells. The vaccine, which is not clinically available; yet, it is recommended to be delivered by the oral or gastrointestinal route to induce optimal local intestinal immune responses [8].

It has long been demonstrated that second-time travelers develop anti-LT antibodies and have lower rates of LT-ETEC travelers' diarrhea as compared with newly arrived visitors [18]. In such cases, antibodies against ETEC LT and major CFs cooperated synergistically for protection against LT-producing ETEC expressing homologous CFs [19]. Other studies in humans revealed that repeated oral antigen administration was optimal in inducing intestinal immune responses; therefore, oral inactivated vaccines, which consist of toxin antigen and whole cells—that is, the licensed recombinant cholera B subunit (rCTB)-WC cholera vaccine Dukoral—have been developed. Another oral ETEC vaccine consisting of rCTB and formalin-inactivated *E. coli* bacteria expressing major CFs has been shown to be safe and immunogenic in adults and children in different countries. The status of this vaccine remains at clinical trials [19].

However, phase I safety and immunogenicity study in healthy adult volunteers was completed as a three-strain combination, live attenuated vaccine known as ACE527. This vaccine proved to be well tolerated and immunogenic at dose levels of 10¹⁰ and 10¹¹ total CFU [20]. Strong immune responses to LTb and to CFs expressed on all three constituent strains were induced, with at least 50 % of subjects in the high-dose group responding to LTb, CFA/I, CS3, and CS6. This is the first study showing that an oral cellular ETEC vaccine induces both mucosal and serum responses [20].

Epidemiological studies indicate that immune responses to uncharacterized, chromosomally encoded antigens could contribute to protection resulting from repeated ETEC infections. As such, studies of immune responses to ETEC

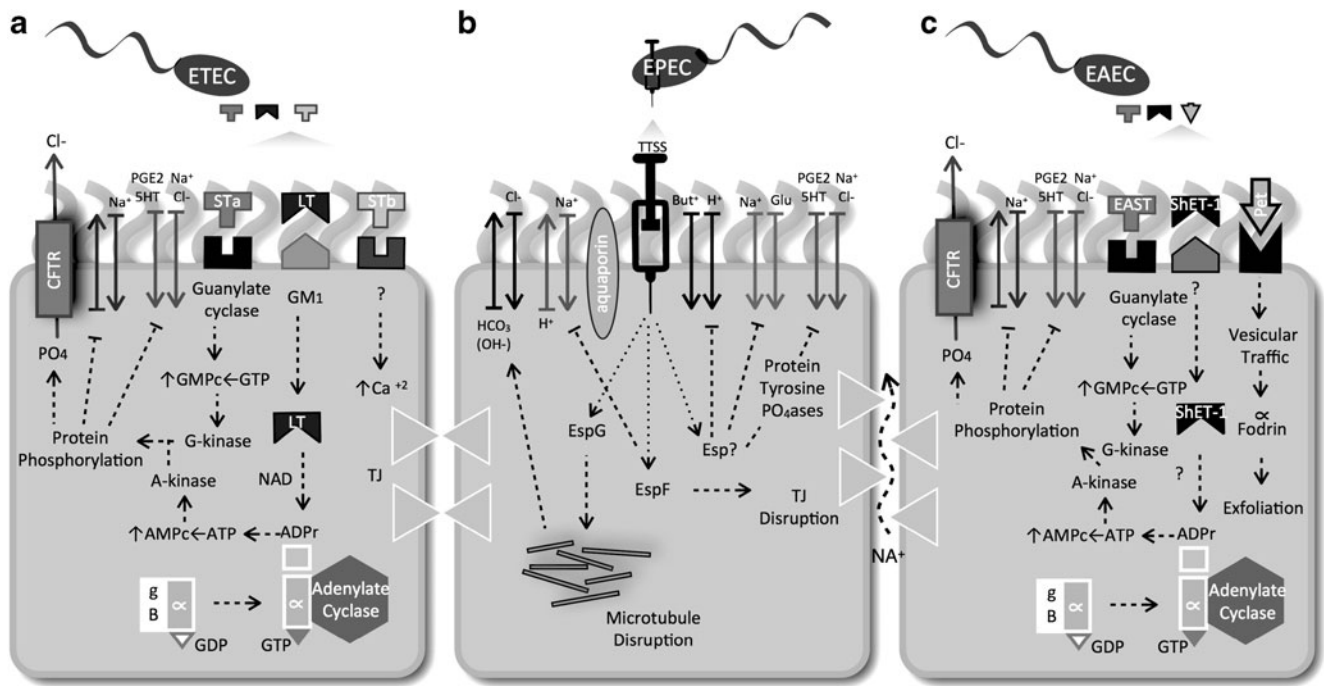


Fig. 1 ETEC-, EPEC-, and EAEC-induced diarrhea is a multifactorial event leading to disruption of ion/H₂O balance. **a** In an ETEC infection, the LT toxin (A₁ subunit) catalyzes the ADP-ribosylation of the α -subunit of G_s-protein, resulting in activation of adenylate cyclase and increased levels of intracellular cAMP. The activation of the cAMP-dependent A kinase results in phosphorylation of apical membrane transporters (i.e., the cystic fibrosis transmembrane conductance regulator, CFTR), resulting in secretion of Cl⁻ and HCO₃⁻ and decreased absorption of Na⁺ and Cl⁻. STa acts by binding to the GC-C membrane receptor. Activation of GC-C results in increased levels of intracellular cGMP, elevated Cl⁻ secretion, and decreased Na⁺ absorption, due to activation of cGMP-dependent kinase (G-kinase) and/or the cAMP-dependent kinase (A-kinase). **b** EPEC infection decreases epithelial ion absorption, resulting in diarrhea due to bacterial protein effectors secreted by the type III secretion system (TTSS) having an effect on Cl⁻ and Na⁺ ion activity. Some TTSS secreted proteins (Esp)

affecting ion balances are EspF, inhibiting function of Na⁺/H⁺ exchange isoform 3 (NH3), and EspG, disrupting microtubules and leading to decrease in apical Cl⁻/HCO₃⁻ exchange activity. EPEC, via unknown effector molecules, decrease butyrate (But) absorption and induce activation of protein tyrosine phosphatases (PTPases). This results in decreasing function of serotonin transporter (SERT) and increasing prostaglandin E-2 (PGE-2) and 5-hydroxytryptamine (5-HT) availability, further affecting ion absorption and motility, resulting in diarrhea. **c** EAEC infection produces two plasmid-encoded toxins: the *E. coli* heat stable enterotoxin (EAST) and the plasmid encoded-toxin (Pet). Furthermore, the chromosomally encoded protein involved in colonization (Pic) (not shown), the *Shigella* enterotoxin 1 (ShET1), and hemolysin E (HlyE) are also produced. Although several toxins are secreted by EAEC, the mechanism of action associated with the EAEC-mediated diarrheal disease has not been fully established but involves G-kinase- and A-kinase-dependent mechanisms

infection had identified a class of surface-expressed molecules known as autotransporters (ATs), which have been fully identified with the completion of the genome sequence of the prototype ETEC strain H10407 [21•]. Two chromosomally encoded ATs identified in ETEC were found immunogenic and protective in an animal model, possibly meaning that conserved ATs might contribute to the protective immune responses that follow natural ETEC infection and, therefore, offering new potential targets for vaccine development [22]. In addition, other ETEC vaccines include one more candidate that is active against cholera toxin and confers cross-protection against ETEC [23]. Finally, a recombinant, transdermal LT vaccine has also been recently evaluated [24]; however, this and other vaccines are available for clinical trials but are not commercially available.

In the case of antibiotic treatment, prophylaxis can be considered for individuals in whom a single episode of travelers' diarrhea would have costly adverse consequences—for

example, during critically important trips or when travelers' diarrhea might adversely affect an underlying illness. Several antimicrobials have demonstrated efficacy in the treatment of ETEC-associated diarrhea, including rifaximin, fluoroquinolones, and azithromycin (reviewed in [25]). Rifaximin, a rifamycin derivative that is generally well tolerated, has received significant attention for its use in prophylaxis [26•]. Rifaximin has been approved only for the treatment of travelers' diarrhea caused by noninvasive types of *E. coli*, since it is not effective in treating invasive enteric pathogens [26•]. Therefore, rifaximin remains an excellent choice for treatment of travelers' diarrhea due to its favorable pharmacokinetics, in vitro susceptibility profile, and efficacy and safety data from clinical trials [27]. Furthermore, rifaximin is currently approved in the United States by the FDA for the treatment of travelers' diarrhea caused by noninvasive diarrheagenic *E. coli* and is approved in more than 30 other countries for a variety of gastrointestinal disorders [26•].

Table 1 Characteristics of enteroadherent *E. coli* affecting the human small intestine

Category	Clinical manifestations	Susceptible population	Virulence factors	Diagnostic	Treatment
ETEC	Watery stool (without blood or inflammatory cells) leading to dehydration, headache, fever, nausea, and vomiting	Children 0–5 years of age and adults traveling to developing countries	ST, LT, CFs	Culture, detection of ST (STa, STb) and LT, CFAs using ELISA ^a and PCR-based methods ^a	Self-limited, responsive to oral rehydration therapy (low response in children <2 years); antimicrobial therapy on individual cases
EPEC	Secretory and persistent diarrhea, anorexia, low fever, and rapid wasting	Children 0–2 years of age, occasionally adults	pEAF, BFP, LEE, and Nle effectors	Culture, adherence patterns (LA LAL, etc.), serotyping, PCR-based methods ^a	Self-limited, responsive to oral rehydration therapy; antimicrobial therapy on individually cases
EAEC	Persistent and acute diarrhea, mucoid stools, abdominal pain, nausea, vomiting, occasionally fever	People of all ages in developing and industrialized countries, HIV-infected adults	EAST Pet PicShET-1 Aap AAF/II	Culture, adherence pattern (stacked-brick pattern), pAA DNA probe, multiplex and real-time PCR assays ^a	Self-limited, responsive to oral rehydration therapy; antimicrobial therapy on individual cases

Table is adapted from references [1, 2, 7, 25, 33, 43, 51–53]. HIV, human immunodeficiency virus; ST, heat-stable toxin; LT, heat-labile toxin; CFs, Colonization factors; pEAF, plasmid enteroadherent factor of EPEC; BFP, bundle forming pilus; LEE, locus of enterocyte effacement; EAST, enteroaggregative heat-stable toxin; Pet, plasmid encoded-toxin; Pic, protein involved in colonization; ShET-1, Shigella enterotoxin-I; Aap, dispersin; AAF/II, aggregative adherence factor II

^a Commercial kits available for clinical use

Enteropathogenic *Escherichia coli*

EPEC isolates are a significant cause of infantile diarrhea worldwide and particularly in developing countries. The populations more susceptible to EPEC gastroenteritis are infants less than 2 years of age, with significant mortality rates (10–40 %) [28, 29].

The Pathogen and Clinical Implications of the Disease

EPEC is characterized for the production of a characteristic histopathological lesion on the apical surface of the enterocytes, known as Attaching and Effacing (A/E). EPEC strains are known to possess specific determinants of virulence—that is, an EPEC adherence factor plasmid (pEAF) and the chromosomal-encoded pathogenicity island known as the *locus of enterocyte effacement* (LEE) [30]. Adherence, as the first step in the colonization process of EPEC, is mediated by several surface factors that promote the localized adherence (LA) phenotype on intestinal epithelial cells [31]. The bundle-forming pilus (BFP), encoded in pEAF, is responsible for the formation of microcolonies in association with the LA phenotype, followed by a type III secretion (LEE encoded) mediated injection of effector proteins, effacement of microvilli, and, finally, intimate adherence on intestinal epithelial cells (reviewed in [1, 31, 32])

EPEC is divided into two groups, typical EPEC (tEPEC) and atypical EPEC (aEPEC). tEPEC is characterized by the presence of the pEAF, while this plasmid is absent in aEPEC [33]. aEPEC strains are generally defined as *E. coli* strains that may or may not belong to the classical EPEC O-serogroups that can produce A/E lesions, do not express BFP, and lack Shiga-toxin-encoding genes. Because more than 200 O-serogroups have been identified among aEPEC strains and many are non-typeable [30, 34, 35], investigation regarding the clinical significance of this group of pathogens has been complicated.

The LEE pathogenicity island, as a key virulence determinant, encodes the type III secretion system involved in translocation of bacterial effector proteins to the host cell, which are encoded either within the LEE or elsewhere in the EPEC genome (non-LEE or Nle). The functions of the large repertoire of EPEC effectors has been grouped depending on the cellular pathway affected as cytoskeletal rearrangers, immune modulators, or those causing electrolyte disturbances and destruction of microvilli (Fig. 1) [36, 37].

Upon colonization and initiation of intestinal disturbances, several manifestations of the EPEC-associated disease in humans are evident, including diarrhea, anorexia, rapid wasting, and sometimes death within several days (Table 1). EPEC diarrhea is a self-limiting disease due to a direct relation with the status of host immune response, and EPEC infection has been found to induce activation of innate immune cells, leading to both stimulation of a protective antibody response and

deleterious inflammation [38•]. However, EPEC infections remain one of the main causes of persistent secretory diarrhea. “Secretory” diarrhea results from increased chloride secretion and decreased sodium absorption, resulting in increased mucosal permeability. Persistent diarrhea is defined as the passage of loose stools for more than 2 weeks, with progression to chronic diarrhea at the 4-week mark [39, 40]. Overall, the damage caused by EPEC to the mucosa of the small intestine can be summarized as the deregulation of water and solute transport and the disruption of the epithelial barrier structure and function [28, 39–41].

Current Progress in Prevention and Treatment

The estimated prevalence of EPEC as a cause of diarrheal disease is 8.8 % in community-based cohort studies and 9.1 % and 15.6 %, respectively, in the in the outpatient and inpatient settings, making EPEC the second most common cause of inpatient diarrhea after rotavirus (25.4 %) [35•, 41]. Interestingly, the prevalence of EPEC infections, mainly caused by tEPEC, has decreased in the last several decades, possibly due to breast-feeding promotion, improvement of sanitation, or overestimated diagnosis based on serological tests. Furthermore, several studies have found EPEC at similar frequencies among diarrheal and control samples [35•, 41, 42]. In contrast, aEPEC has become important as the cause of endemic diarrhea in children in developing countries, as well as the causative agent of diarrheal outbreaks. Current data indicate that aEPEC is more prevalent and produces a longer duration of diarrhea than does tEPEC in both developing and developed countries [34, 35•, 42]. Although an animal reservoir for aEPEC is unknown, aEPEC isolates have been detected in domestic animals [35•, 42].

Oral rehydration remains the best therapy for EPEC infections, leading to decreased morbidity and mortality [43]. However, children with an acute EPEC infection are more likely to fail to respond to oral rehydration therapy, while those developing persistent diarrhea require hospitalization and may suffer from cow’s milk intolerance [43, 44]. Therefore, antimicrobials are recommended for EPEC persistent infections, in which the choice of effective treatment may be crucial for patient recovery and even survival [34]. An association was recently reported between classical EPEC serotypes and antimicrobial resistance, with the identification of a conjugative and multi-resistant plasmid in classical serogroups of EPEC [34]. The most common antibiotic resistances found in classical EPEC are ampicillin, tetracycline, streptomycin, and sulfonamides; a high percentage of tEPEC strains are multiresistant. aEPEC has shown resistance only to trimethoprim. Therefore, the antibiotics recommended are ceftazidime, ceftriaxone, imipenem, or piperacillin-tazobactam, for which no resistance has been reported [34, 42]. The use of probiotics has been proposed as an alternative therapy for treatment of A/E pathogens,

including EPEC, because they promote tight junction formation and integrity of intestinal barrier function in vitro and in animal models of infection [45, 46].

Current Progress in Diagnostics

Serotyping (O:H) and adherence to tissue cultured cells have been historically used to diagnose patients with EPEC-associated diarrhea. However, fast, easy, and inexpensive diagnostic methods are required to define optimal treatment and prevention for children in endemic areas [35•]. In addition, aEPEC strains include diverse serotypes, coming mainly from nonclassical, nontypeable EPEC serogroups, many of which are nonmotile [33]. Thus, some efforts have been directed toward improving and optimizing phenotypic and genotypic assays used for EPEC identification, and these include determining rapid adherence patterns on HEp-2/HeLa cells and immunological detection of BFP, which allows investigators to distinguishing tEPEC from aEPEC [33, 35•, 39].

A large number of multiplex PCRs and real-time PCRs assays have been designed to detect EPEC genes from stool cultures, such as *eae* and *bfpA*, encoding intimin and BfpA (major Bfp subunit), respectively [47, 48]. The advantage of these methods is that they are more sensitive and specific; however, further phenotypic characterization is required to define an aEPEC strain, and therefore, this type of methodology is still limited to research settings and is used in case of an outbreak, episodes of severe diarrhea, and epidemiologic studies [33, 47, 48]. Two recent studies have shown significant promise for developing rapid reliable diagnostic tests to detect tEPEC and aEPEC isolates. The first reported a comparative analysis of multiplex PCR (mPCR) with serogrouping and PCR-RFLP of the *fliC* gene [49]. This assay allows differentiation of tEPEC and aEPEC strains from the overall EPEC isolates and can be adapted, when required, to allow screening of a large number of isolates [49]. The second study investigated whether Shiga toxin-producing *E. coli* (STEC) can be distinguished from EPEC (tEPEC and aEPEC) strains, by using mPCR methodology with specific biomarkers associated with each strain’s respective virulence genotype. The study demonstrated consistent amplification of genes specific to the prototype STEC O157:H7 and EPEC O127:H6 E2348/69 strains [50•]. Furthermore, the method was used to screen with high sensitivity and specificity those clinical samples from hemolytic uremic syndrome and diarrheal patients [50•]. As a result of these advances, mPCR approaches are currently the leading diagnostic methodology for the differentiation of pathogenic *E. coli* strains.

Enteroaggregative *Escherichia coli*

The EAEC category is heterogeneous and associated with cases of acute or persistent diarrhea in children and adults

worldwide (reviewed in [51, 52]). EAEC has received increasing attention during the past decade as a cause of watery diarrhea, which in a significant proportion of patients becomes persistent. Furthermore, sporadic cases and outbreaks of EAEC-caused diarrhea have been also described (reviewed in [53]).

The Pathogen and Clinical Implications of the Disease

The classical definition of EAEC pathogenesis indicates that the microorganism has the ability to adhere to epithelial cells in a very characteristic “stacked-brick” pattern and is capable of forming biofilms [53]. Although many studies searching for specific virulence determinants of EAEC have been published, the process by which this pathogen causes persistent diarrhea is still unknown (Table 1). However, in vitro organ cultures studies have confirmed that EAEC adheres to the intestinal mucosa and creates a mucoid biofilm on the small bowel surface [54]. Therefore, it has been proposed that host cellular changes during EAEC infection result in digestive-absorptive abnormalities due to these biofilm-forming bacteria, prolonging the diarrhea (Fig. 1) [54]. Furthermore, it is well known that EAEC requires a variety of virulence factors, including the so-called aggregative adherence fimbriae and enterotoxins, to cause damage, but since EAEC strains are usually recovered from healthy as well as diseased subjects, the overall mechanisms by which EAEC exerts its pathogenic process remains poorly characterized (reviewed in [51, 52]). Several EAEC strains carry the *aggR* regulon, which comprises virulence genes defining typical, pathogenic EAEC from *aggR*-negative, atypical strains, which are considered nonpathogenic [51, 55]. Although several studies demonstrated that EAEC strains are able to invade cultured epithelial cells, the contribution of this invasion to the disease remains unknown [56, 57]. Furthermore, in a recent study that attempted to link the distribution of genes encoding putative invasins with the ability of *aggR*-positive and *aggR*-negative EAEC isolates to enter intestinal epithelial cells, there was no correlation between the invasion efficiency of these strains and the presence of any particular gene involved in invasion [58].

Upon adhesion, EAEC produce several toxins, including a heat-stable enterotoxin (EAST-1). Several studies have suggested a role of EAST-1 in diarrhea; however, the correlation between diarrhea and the presence of an *astA* gene (encoding for EAST-1) remains inconclusive [59]. Another toxin characterized in EAEC strains is the plasmid-encoded toxin (Pet) [60], a type V serine protease autotransporter that has been shown to induce increased mucus release, exfoliation of cells, and development of crypt abscesses. The eukaryotic target of Pet is the actin-binding protein alpha-fodrin, and cleavage of this protein results in disruption of the actin cytoskeleton organization [60]. Furthermore, it has been reported that Pet also cleaves spectrin, but recent evidence suggests that

mechanisms other than host spectrin redistribution occur during Pet intoxication [61]. Interestingly, a case-control study of moderate-to-severe acute diarrhea among children, 0–59 months of age, was used to identify potential virulence factors among the EAEC strains. The data showed that only a subset of EAEC strains were pathogenic and identified the gene encoding the autotransporter SepA protease as being strongly associated with diarrhea among the EAEC strains tested [62]. Further investigation is required to define the contribution of SepA to the pathogenesis of EAEC.

Current Progress in Prevention and Treatment

One of the hallmarks during EAEC infection of the gastrointestinal tract is the release of IL-8, resulting in neutrophil recruitment and gastroenteritis [51, 52]. However, an incomplete understanding of the effect of EAEC adherence to intestinal epithelial cells (IECs) and the subsequent innate responses elicited has resulted in an inadequate development of effective treatments. Recent studies have characterized and identified the EAEC processes that might be contributing to the mechanisms underlying EAEC-induced mucosal inflammatory responses. One study found that EAEC adherence contributes to IECs antimicrobial innate immunity, inducing a strong expression of IL-8 and CCL20, consistent with its greater in vivo propensity to induce inflammatory diarrhea [63]. A subsequent study found that polymorphonuclear neutrophils (PMN) transepithelial migration promoted an enhanced attachment of EAEC to intestinal epithelial cells and raised the possibility that EAEC-induced PMN infiltration may favor colonization and, thus, pathogenesis of EAEC in the host [64].

EAEC infections are often persistent and significantly inflammatory. Moreover, they have resulted in a high worldwide incidence of antibiotic-resistant EAEC isolates [65]. Because of these factors, the antibiotic of choice to treat EAEC-induced diarrhea is ciprofloxacin, an antibiotic with bactericidal activity against a wide spectrum of bacteria. Interestingly, it has been shown that subminimum inhibitory concentrations of ciprofloxacin inhibited EAEC adhesion to glass- and tissue-cultured cells [66]. However, if the EAEC surface charges changed—for example, in a mutant lacking the surface protein dispersin—then the sensitivity to ciprofloxacin was significantly reduced when compared with the same activity in a wild-type strain. Therefore, maintenance of the EAEC surface’s hydrophobicity is required to maintain the effectiveness of ciprofloxacin treatment [66]. Unfortunately, recent reports indicate an increased resistance to nearly all of the antibiotics tested against EAEC, including ciprofloxacin [67], implying that a continuous surveillance of EAEC susceptibility patterns worldwide and development of novel antimicrobial therapies are required to combat EAEC infections.

Conclusion

Significant progress has occurred in recent years relative to understanding the pathogenic processes of these enteroadherent *E. coli* categories affecting the small intestine, and undoubtedly this knowledge will continue evolving. However, a larger emphasis on the effectiveness of novel treatments is required to ultimately reduce the number of cases and outbreaks still affecting endemic regions. Because of the wide nature of clinical symptoms observed with these enteroadherent *E. coli* strains and the decreased diagnostic sensitivity of these pathogens, future assays that target stable traits might improve diagnostic sensitivity. To facilitate diagnosis and patient management, future methods would also ideally allow for an assessment of the organism's potential to cause severe disease. Discovery of pathogen-specific virulence factors in those so-called "atypical" strains or from outbreak-producing isolates is required to increase our repertoire of factors that can be incorporated into effective vaccines. Furthermore, as nucleotide sequences of more *E. coli* strains become available, comparative genomic studies might identify targets that can be used to improve detection, virulence profiling, and vaccine development for these pathogenic *E. coli* categories.

Finally, a new category of *E. coli* linked to inflammatory bowel disease has emerged as a potential enteroadherent pathogen. The so-called *adherent invasive E. coli* (AIEC) category represents a group of isolates found in the ileal mucosa of patients with Crohn's disease (CD) that do not possess the classical virulence factors found in other *E. coli* pathotypes. Unlike nonpathogenic commensal *E. coli*, the AIEC isolates have been shown to efficiently adhere to and invade epithelial cells and macrophages, to survive and replicate within macrophages, without triggering host cell death, and to induce the release of large amounts of TNF- α from infected macrophages [68–71]. The ability of AIEC to further stimulate production of proinflammatory cytokines, such as IFN γ and TNF- α , suggests that these bacteria might contribute to the persistence and the chronic inflammatory response observed in CD patients. However, since further work is required to establish the direct link between development of CD and the pathogenesis of AIEC associated with this human disease, this new *E. coli* category was not further discussed in this review.

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