E. COLI INTESTINAL PATHOGENIC STRAINS

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DEFINITION

Bacteria belonging to the species *E. coli* are a normal component of the intestinal microbiota.

The majority of *E. coli* are harmless commensals, but specific isolates have acquired pathogenicity genes that enable them to cause diseases.

Enteric infections caused by E. coli may involve the small intestine, colon, or both, depending on the organism's genetic codes for virulence traits.

Virulence Traits

- Variety of toxins
- Adherence factors
- Secreted mediators
- Work together to perturb host intestinal physiology

Six major pathotypes of diarrheogenic *E. coli*

Enterotoxigenic (ETEC)
Enteroinvasive (EIEC)
Enterohemorrhagic (EHEC)
Enteropathogenic (EPEC)
Enteroaggregative (EAEC)
Diffusely adherent (DAEC)
These pathotypes can overlap; for example, some strains can express Shiga-like toxins that are characteristic of EHEC without the usual associated adherence factors; these are collectively known as shigatoxigenic *E. coli* (STEC) – like ST-EAEC

ESCHERICHIA COLI

Small catalase-positive

Oxidase-negative

Gram-negative bacillus in

the family Enterobacteriaceae

Reduces nitrates ferments

glucose and lactose,

is either motile (with peritrichate flagella)

or nonmotile

E. Coli is the predominant member of the Gammaproteobacteria in the intestinal tract of humans and other mammals

ESCHERICHIA COLI

The lipopolysaccharide cell wall of *E. coli* contains immunostimulatory lipid *A* attached to a core oligosaccharide chain

Most *E. coli* have immunogenic carbohydrate chains known as *O* antigens attached to this core glycolipid to produce 173 *O* serogroups

There are also at least 56 distinct flagellar (H) antigens based on variable domains of the flagellin gene

These O, H, and K antigen combinations have allowed serotyping of thousands of different strains, which historically was the simplest way to distinguish them

DIFFERENT TYPES OF ENTERIC *E. COLI* INFECTIONS

TYPE	MECHANISM	PREDOMINANT O SEROGROUPS	GENETIC CODE	DETECTION	CLINICAL SYNDROMES
ENTEROTOXIGENIC *E. COLI* (ETEC)					
Heat-labile toxin (LT)	Activates intestinal adenylate cyclase	6, 8, 11, 15, 20, 25, 27, 63, 80, 85, 139	Plasmid	Gene probe, PCR for LT	Watery diarrhea, traveler's diarrhea
Heat-stable toxin (STa: STh or STp)	Activates intestinal guanylate cyclase	12, 78, 115, 148, 149, 153, 155, 166, 167	Plasmid (transposon)	EIA, suckling mice, 6-hour ileal loop assay, gene probes, PCR	Watery diarrhea, traveler's diarrhea
ENTEROINVASIVE *E. COLI* (EIEC)					
	Cell invasion and spread	11, 28ac, 29, 124, 136, 144, 147, 152, 164, 167	Plasmid (140 MIa, pWR110)	Sereny test, gene probe, PCR for *ipaH*	Inflammatory dysentery
SHIGATOXIGENIC *E. COLI* (STEC)					
Enterohemorrhagic (EHEC)	Shiga-like toxins (SLTs/Stxs) and attaching/effacing ability	26, 39, 113, 121, 128, 139, 145, 157, occ 55, 111	SLT phages and adhesin plasmids; type III secretion system	EIA or PCR for SLT, serotype, cell adhesion with pedestal formation; Vero cell cytotoxicity; sorbitol agar; PCR for *eae*	Afebrile, bloody diarrhea; HUS in some cases
Non-EHEC STEC	SLTs only without attaching/effacing	26, 111, 103, 121, 45, 104, 145	SLT phage; may possess other virulence traits (e.g., O104:H4 EAEC)	SLT EIA or PCR; negative for *eae*	Hemorrhagic colitis, HUS, or benign watery diarrhea
ENTEROADHERENT *E. COLI*					
Typical enteropathogenic (EPEC)	Attach, then efface the mucosa	55, 86, 111, 114, 119, 127, 142	Bundle-forming pili on plasmid and chromosomal LEE	Serotype, focal HEp2/HeLa cell adhesion, pedestal formation, gene probe or PCR *eae*	Infantile diarrhea in developing areas
Atypical enteropathogenic (EPEC)	Attaching and effacing but different microcolony formation	26, 55, 86, 111, 119, 125, 128	Possess the LEE but not bundle-forming pili	Gene probe or PCR for LEE; cell adherence (variable)	Infantile and animal diarrhea in developed areas
Enteroaggregative (EAEC)	Colonize in aggregates; toxins (EAST, Pet), biofilm formation	3, 15, 44, 51, 77, 78, 91	Plasmid (AA); chromosome (Pic/ShET and type VI secretion)	HEp2 cell adherence; AA probe; PCR for *aggR* or other virulence genes; biofilm formation	Endemic persistent diarrhea, acute traveler's diarrhea, sporadic acute diarrhea
Diffusely adherent (DAEC)	Colonize (F 1845 afimbriate adhesin)	86, 75, 15	Chromosomal/plasmid	HEp2 cell adherence; DA gene probe/PCR	Persistent diarrhea in children >18 months old

AA = aggregative adherence; DA = diffuse adherence; EIA = enzyme immunoassay; HUS = hemolytic-uremic syndrome; LEE = locus of enterocyte effacement; PCR = polymerase chain reaction.

Mechanisms of *E. coli* pathogenesis

- Secretion of enterotoxins (ETEC)
- Shigella-like tissue invasion (EIEC)
- Epithelial necrosis as a result of Shiga-like toxins (SLTs SLT-1/2 or Stx1/Stx2) causing food-borne hemorrhagic colitis (EHEC and STEC)
- The classically recognized EPEC serotypes are neither enterotoxigenic nor invasive but rather attach and efface the epithelium
- Enteroadherent *E. coli* exhibit aggregating (EAEC) or diffuse adherence (DAEC) traits, and EAEC in particular is associated with prolonged diarrhea in children in tropical developing areas, in patients infected with HIV, and in acute diarrhea in outbreak settings and travelers from developed areas

EPIDEMIOLOGY

- Specific single-nucleotide polymorphisms in the lactoferrin, osteoprotegerin, CD14, and interleukin-8 genes are associated with traveler's diarrhea caused by ETEC, EAEC, or both
- Enteric *E. coli* infections are acquired by the fecal-oral route
- It is believed that a human reservoir is required for most recognized types of EPEC and ETEC
- The infectious dose of ETEC in volunteers is 10^6 to 10^{10} organisms, meaning that it usually requires multiplication in contaminated food or water vehicles for its transmission, rather than spreading directly from person to person
- Contaminated water and food probably represent the major sources of their acquisition, primarily in warm or wet seasons

EPIDEMIOLOGY

- The highest age-specific attack rates of ETEC are found in young children, especially at the time of weaning, when ETEC accounts for anywhere from 3% to 39%
- A traveler visiting tropical areas has a 30 to 50% chance of acquiring traveler's diarrhea during a 2- to 3-week stay unless untreated water or ice and uncooked foods such as salads are strictly avoided
- ETEC produces the heat-stable toxin STa, the heat-labile toxin LT, or both
- A second cause of traveler's diarrhea is EAEC, now reported in 19 to 33% of affected travelers to India or Mexico

EPIDEMIOLOGY

- Typical EPEC strains have been recognized primarily in poor urban areas, especially among hospitalized infants in their first year of life, with apparent cross-infection in hospital nurseries
- EHEC frequently colonizes commercial livestock but does not infect them
- EHEC (O157:H7 and others) infections were first attributed to eating undercooked hamburgers, but subsequent large outbreaks have been associated with contamination of unpasteurized apple juice, spinach, seed sprouts, and other vegetable items
- More recently, a 2011 outbreak of shigatoxigenic EAEC strain O104:H4 (ST/EAEC) associated with fenugreek sprouts sickened almost 4000 people in Europe

EPIDEMIOLOGY

- The low infectious dose of EHEC O157:H7 means that person-to-person spread can occur, leading to secondary cases
- EHEC and STEC infections are especially alarming because of the risk of hemolytic-uremic syndrome (HUS)
- HUS can be fatal despite antimicrobial therapy, which in some instances may actually induce SLT production from bacteriophage carried within the organism and hence is generally not recommended. Patients who recover from HUS may also suffer chronic kidney injury as a result

EPIDEMIOLOGY

- The natural reservoir of EAEC is not known, but outbreaks have been traced to contaminated food, and live organisms can be found in drinking water, table salsa, and other consumable items in endemic tropical areas
- High infectious dose is required for acquisition of EAEC, suggesting that direct person-to-person spread may be difficult
- EAEC is an important cause of both acute diarrhea and persistent diarrhea and malnutrition, especially in children in tropical areas and in patients with HIV/AIDS

EPIDEMIOLOGY

- Limited data on EIEC suggest that infectious doses are relatively high, but as with ETEC infections, adequate numbers of organisms have readily been spread in food with high attack rates in outbreak situations
- This distinguishes EIEC epidemiologically from *Shigella*, which is easily spread person to person as well as in contaminated food and water
- DAEC remains the least well understood pathotype and has not consistently been found more often in diarrheal cases than in controls
- Some studies have shown a clear association with acute diarrhea in developing areas, particularly in children 1 to 4 years of age

PATHOPHYSIOLOGY

- Begins with ingestion of the organism in contaminated food or water or rarely direct person-to person spread, in the case of EHEC
- Then faces the normal gastric acid barrier
- ETEC and EIEC appear to be sensitive to gastric acid
- Hypochlorhydria increased the risk of EPEC diarrhea
- EHEC expresses acid tolerance factors that may facilitate its survival in the stomach

PATHOPHYSIOLOGY

- Enteric *E. coli* colonize the involved part of the intestinal tract using specialized adhesins and the coordinated expression of virulence traits
- This can lead to toxin production, intracellular invasion, or other disruptions of host cell physiology
- Incubation period: 14 hours for EAEC and 2 days for ETEC in volunteer studies; epidemiologic studies found an average incubation period of 3 to 4 days for EHEC O157:H7 but 8 days for ST/EAEC O104:H4
- ETEC colonizes the upper portion of the small bowel using fimbriate or fibrillar surface proteins

PATHOPHYSIOLOGY

- The majority of ETEC illness is due to its enterotoxins
- LT, with a molecular weight of about 86,000, has a binding and active subunit and, like the closely related choleratoxin, binds to a monosialoganglioside (GM1) receptor
- The active subunit is an enzyme that ADP-ribosylates the regulatory subunit of adenylate cyclase, leading to constitutive production of cyclic adenosine monophosphate
- The consequently increased chloride secretion and reduced sodium absorption combine to cause net isotonic electrolyte loss that can be as great as 1 liter/ hour
- Besides of dehydration, the only significant pathologic change with ETEC is depletion of mucus from intestinal goblet cells

PATHOPHYSIOLOGY

- EIEC, like the closely related Shigella, can invade and multiply in epithelial cells
- Produce inflammatory colitis and dysenteric or bloody diarrhea
- Numerous polymorphonuclear leukocytes in stool
- The colon shows patchy, acute inflammation in the mucosa and submucosa with focal denuding of the surface epithelium, usually without deeper invasion or systemic spread
- EPEC strains are well-established causes of infantile diarrhea, adherence to epithelial cells (through specialized bundle-forming pili), attachment and effacement of microvilli
- Villous atrophy, mucosal thinning, inflammation in the lamina propria, and variable crypt cell hyperplasia are associated with a reduction in mucosal brush border enzymes and may contribute to the impaired absorptive function and diarrhea

PATHOPHYSIOLOGY

- EHEC, serotype O157:H7 but also serogroups O26, O39, and others, cause type III secretion-dependent intimate adherence and microvillous effacement like EPEC
- They also produce SLTs that are responsible for the characteristic colonic mucosal disruption and hemorrhage as well as the complication of HUS
- These toxins bind to the Gb3 surface ganglioside, leading to internalization and enzymatic inactivation of ribosomes, halting protein synthesis and induce apoptosis
- Gb3 is highly expressed on vascular endothelial cells in the colon, kidney, and brain, which may explain the predilection for HUS to affect these organs

PATHOPHYSIOLOGY

- The ST/EAEC O104:H4 caused the 2011 European outbreak; this strain was essentially an EAEC that also expressed SLT
- EAEC is defined by a characteristic aggregative adherence pattern to cells and the substrata associated with biofilm formation
- Reside within a biofilm at the epithelial surface, where secreted factors contribute to a damaging host inflammatory response
- DAEC toxicity appears to depend on direct interactions between specialized adhesins (Afa/Dr) and host membrane proteins such as CD55 (decay-accelerating factor) or carcinoembryonic antigen. Many DAEC strains are closely related to uropathogenic *E. coli* with similar virulence traits
- Host risk factors for diarrheogenic *E. coli* infection differ among the various bacterial pathotypes but in general include age, recent antibiotic use, and loss of gastric acid.

CLINICAL MANIFESTATIONS

- ETEC infections generally produce watery diarrhea, particularly in young children and travelers to tropical or developing areas
- Diarrhea may range from mild to severe and cholera-like; it may be life-threatening, especially in small children and elderly individuals, who are particularly prone to dehydration, undernutrition, and electrolyte imbalance (especially hypokalemia and acidosis)
- Malaise, abdominal cramping, anorexia, and occasionally nausea, vomiting, or low-grade fever
- The illness is generally self-limited to 1 to 5 days and rarely extends beyond 10 to 14 days

CLINICAL MANIFESTATIONS

- Infection with EIEC is characterized by inflammatory colitis, often with abdominal pain, high fever, tenesmus, and bloody or dysenteric diarrhea, essentially like that seen with Shigella
- The incubation period is usually 1 to 3 days, with the duration generally self-limited to 7 to 10 days
- Outbreaks of EPEC infection in newborn nurseries have ranged from mild transient diarrhea to severe and rapidly fatal diarrheal illnesses, especially in premature or otherwise compromised infants

CLINICAL MANIFESTATIONS

- Hemorrhagic colitis associated with EHEC classically begins with watery diarrhea that quickly turns grossly bloody, with a conspicuous absence of fever or inflammatory exudate in stool but with significant abdominal pain
- Although this diarrheal illness is self-limited, potentially fatal HUS or thrombotic thrombocytopenic purpura subsequently develops in a significant number of children and older adults
- The incubation period in two outbreaks has been 3 to 4 days (range, 1 to 7 days), and the illness is characteristically self-limited to 5 to 12 days (mean, 7.8 days)
- The clinical manifestations in the ST/EAEC O104:H4 outbreak were similar, although rates of HUS were significantly higher (more than 20%) and women were disproportionately affected

CLINICAL MANIFESTATIONS

- EAEC has been associated with persistent diarrhea and malnutrition in children in developing areas, in HIV/AIDS patients, and in travelers who experience diarrhea (especially those genetically predisposed to greater inflammatory responses)

- DAEC has also been associated with diarrhea with no particular identifying features in children older than 18 months

DIAGNOSIS

- With the exception of EHEC, definitive etiologic diagnosis of *E. coli* diarrhea requires documentation of a specific virulence trait or serotype, which requires specialized immunologic tests, tissue culture, animal bioassay, and molecular testing that are usually available only in research and reference laboratories
- Tests are rarely cost-effective or clinically indicated, except in outbreak or research situations
- Fortunately, a probable diagnosis can often be suspected by the clinical and epidemiologic setting

DIAGNOSIS

- EHEC O157:H7 can be identified with reasonable accuracy by culture on sorbitol-MacConkey agar to identify nonfermenting colonies
- However, it has long been recommended that any stool sample with visible blood should also be tested specifically for SLTs by enzyme-linked immunosorbent assay, polymerase chain reaction, or other molecular methods, which can identify non-O157 serotypes and rare sorbitol-fermenting O157 strains
- In hemorrhagic colitis due to EHEC, sigmoidoscopy, which is rarely indicated, generally reveals only moderately hyperemic mucosa, and barium enema or CT scan may show a thumbprint pattern of segmental or diffuse colonic wall thickening

Differential Diagnosis

- Self-limited, noninflammatory diarrhea in tropical, developing areas is most likely due to ETEC, EAEC, rotaviruses (young children), or noroviruses (older children and adults)
- Noninflammatory diarrhea in older children or adults in temperate areas is more likely to be due to noroviruses
- Vibrio infections are common in areas endemic for cholera or in any coastal area where inadequately cooked seafood may be eaten
- If noninflammatory diarrhea persists beyond a week, especially with weight loss, other possibilities include *Giardia lamblia*, *Cryptosporidium*, *Cyclospora*, and *microsporidial* infection
- In outbreaks of food poisoning, *Staphylococcus aureus*, *Clostridium perfringens*, and *Bacillus cereus* should be considered

Differential Diagnosis

- Inflammatory colitis with high fever and tenesmus as well as leukocytes, mucus, and blood in the stool may well be due to EIEC but should prompt a stool culture for more common invasive pathogens, such as *Campylobacter jejuni* , *Shigella*, *Salmonella*, *Yersinia enterocolitica*, or *noncholera Vibrio*

- Any patient with diarrhea and a history of recent antibiotic use, gastrointestinal surgery, or parturition should be screened for toxigenic *Clostridium difficile*

- ***EHEC should be strongly considered in any case of bloody diarrhea, particularly in the absence of fever; it is recommended that laboratories now routinely screen for this pathogen in all stool cultures, and they should automatically screen any grossly bloody samples***

- Ischemic colitis and cytomegalovirus colitis can mimic EHEC but should occur only in people at risk (vascular disease and immune compromise or inflammatory bowel disease, respectively)

TREATMENT

- The primary treatment for most *E. coli* diarrhea is replacement and maintenance of water and electrolytes, usually with a simple oral rehydration solution that uses the intact, sodium-coupled glucose or amino acid absorption (or both) to replace the fluid losses

- Antimotility agents reduce the frequency of diarrheal stools but should not be used when fever or bloody diarrhea is present as they can increase the risk of mortality due to toxic megacolon or HUS

- Because most *E. coli* diarrhea is self-limited, the role of antimicrobial agents is debated and remains of secondary importance to rehydration

TREATMENT

- Treatment is generally favored is traveler's diarrhea because strong clinical studies have shown a benefit of antibiotics in reducing the duration of symptoms
- Unfortunately, rising antimicrobial resistance has narrowed the options for empirical therapy
- Azithromycin (500mgx1/3 days), a fluoroquinolone (ciprofloxacin 500mgx2/3-5 days), or rifaximin (200mgx3/3 days) is recommended, with trimethoprim-sulfamethoxazole a somewhat less reliable alternative
- Antimicrobials are not recommended in EHEC infection because of the possibility of increasing the risk of HUS and a lack of evidence of efficacy
- In the treatment of HUS, plasmapheresis has no benefit and the value of inhibition of C5 (via eculizumab) is unresolved

References

- Gregorio GV, Dans LF, Silvestre MA. Early versus delayed refeeding for children with acute diarrhoea. Cochrane Database Syst Rev. 2011;7:CD007296.
- Lazzerini M, Ronfani L. Oral zinc for treating diarrhoea in children. Cochrane Database Syst Rev. 2013;1:CD005436.
- Francavilla R, Lionetti E, Castellaneta S, et al. Randomised clinical trial: Lactobacillus reuteri DSM 17938 vs. placebo in children with acute diarrhoea—a double-blind study. Aliment Pharmacol Ther. 2012;36:363-369.
- Taylor DN, Bourgeois AL, Ericsson CD, et al. A randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrhea. Am J Trop Med Hyg. 2006;74:1060-1066.
- Tribble DR, Sanders JW, Pang LW, et al. Traveler's diarrhea in Thailand: randomized, double-blind trial comparing single-dose and 3-day azithromycin-based regimens with a 3-day levofloxacin regimen. Clin Infect Dis. 2007;44:338-346.

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