

# *Escherichia Coli* in Diarrheal Disease

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## **General Concepts**

### **Clinical Manifestations**

Depending on the virulence factors they possess, virulent *Escherichia coli* strains cause either noninflammatory diarrhea (watery diarrhea) or inflammatory diarrhea (dysentery with stools usually containing blood, mucus, and leukocytes).

### **Structure, Classification, and Antigenic Types**

These are Gram-negative bacilli of the family Enterobacteriaceae. Virulent strains differ from nonvirulent *E. coli* only in possessing genetic elements for virulence factors. Strains producing enterotoxins are enterotoxigenic *E. coli* (ETEC).

### **Pathogenesis**

Transmission is by the fecal-oral route. Pili (fimbriae) allow the bacteria to colonize the ileal mucosa. Cytotoxic enterotoxins (encoded on plasmid or bacteriophage DNA) induce watery diarrhea. Plasmid-encoded invasion factors permit invasion of the mucosa, and plasmid- or bacteriophage-encoded cytotoxic enterotoxins induce tissue damage; the presence of either of these factors induces a host inflammatory reaction with an influx of lymphocytes and resulting dysentery.

### **Host Defenses**

Gastric acid and intestinal transit time are important defenses. Specific intestinal immunoglobulin A (IgA) develops and appears to be protective.

### **Epidemiology**

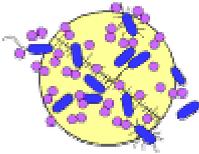
Infection is common where sanitation is poor; both infants and susceptible travelers to developing countries are particularly at risk. The disease is most serious in infants.

## Diagnosis

The diagnosis is suggested by the clinical picture and confirmed by stool culture. Serotyping and tests for virulence factors are occasionally performed for outbreaks.

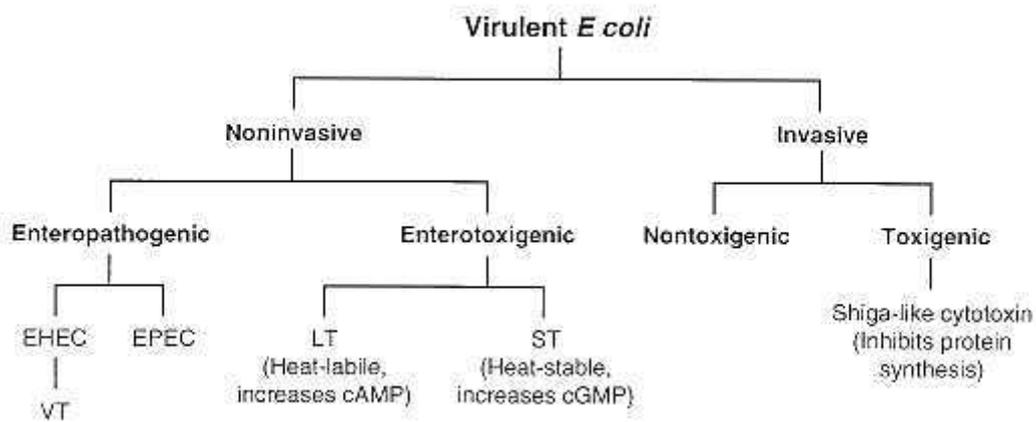
## Control

Prevention depends on sanitary measures to prevent fecal-oral transmission; hand-washing and proper preparation of food; chlorination of water supplies; and sewage treatment and disposal. Parenteral or oral fluid and electrolyte replacement is used to prevent dehydration. Broad-spectrum antibiotics are used in chronic or life-threatening cases.



## INTRODUCTION

Escherichia coli is a common member of the normal flora of the large intestine. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals. Strains that acquire bacteriophage or plasmid DNA encoding enterotoxins or invasion factors become virulent and can cause either a plain, watery diarrhea or an inflammatory dysentery. These diseases are most familiar to Westerners as traveler's diarrhea, but they are also major health problems in endemic countries, particularly among infants. Three groups of E coli are associated with diarrheal diseases. Escherichia coli strains that produce enterotoxins are called enterotoxigenic E coli (ETEC). There are numerous types of enterotoxin. Some of these toxins are cytotoxic, damaging the mucosal cells, whereas others are merely cytotoxic, inducing only the secretion of water and electrolytes. A second group of E coli strains have invasion factors and cause tissue destruction and inflammation resembling the effects of Shigella (EIEC). A third group of serotypes, called enteropathogenic E coli (EPEC), are associated with outbreaks of diarrhea in newborn nurseries, but produce no recognizable toxins or invasion factors. Figure 25-1 presents a summary of the diseases caused by virulent E coli.

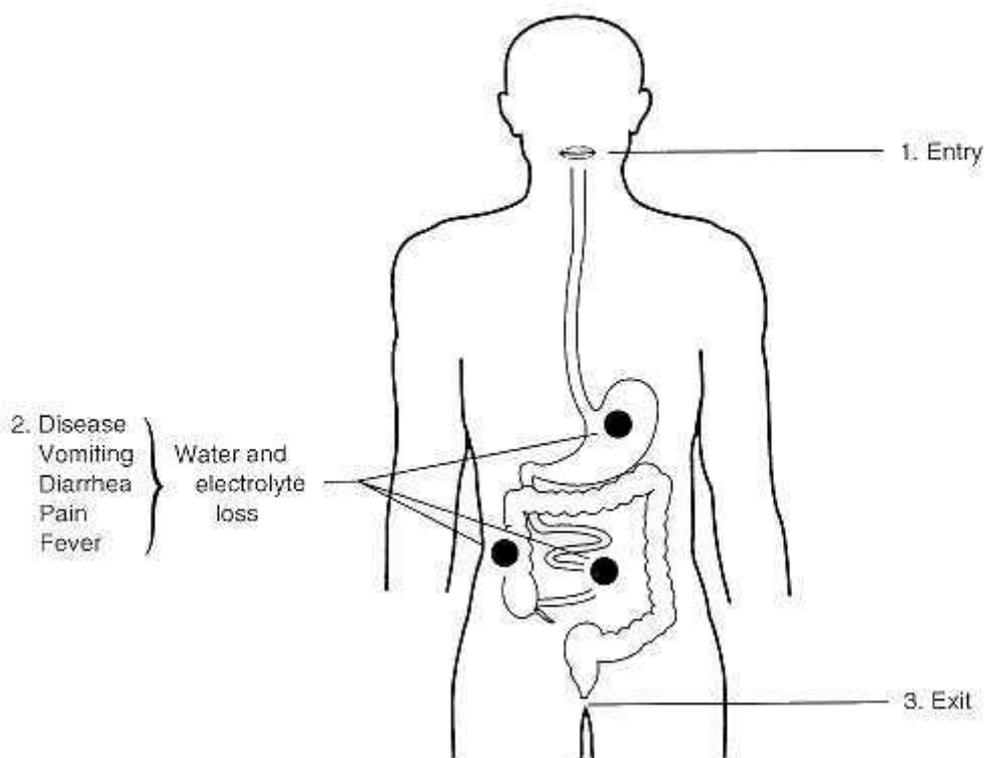


**FIGURE 25-1** Virulence mechanisms of *E coli*.

## **Noninflammatory Diarrheas Caused by Enterotoxigenic *Escherichia Coli***

### **Clinical Manifestations**

The diarrheal disease caused by ETEC is characterized by a rapid onset of watery, nonbloody diarrhea of considerable volume, accompanied by little or no fever (Fig. 25-2). Other common symptoms are abdominal pain, malaise, nausea, and vomiting. Diarrhea and other symptoms cease spontaneously after 24 to 72 hours.



## FIGURE 25-2 Pathogenesis of E coli diarrheal disease.

### Structure, Classification, and Antigenic Types

ETEC organisms are Gram-negative, short rods not visibly different from E coli found in the normal flora of the human large intestine. Virulence-associated fimbriae are too small to be seen by light microscopy. All ETEC contain plasmids, but this is also not a distinguishing feature unless gene probe techniques are used to detect specific virulence-associated genes on these plasmids.

E coli organisms are serogrouped according to the presence or absence of specific heat-stable somatic antigens (O antigens) composed of polysaccharide chains linked to the core lipopolysaccharide (LPS) complex common to all Gram-negative bacteria. O specificity is determined by sugar or amino-sugar composition and by the sequence of these outer polysaccharide chains. More than 170 different O-specific antigens have been defined since Kauffmann began this method of typing E coli in 1943. In normal smooth strains, which are typable, the core LPS is buried beneath the O antigen. Also occurring are untypable O-minus mutants in which the core LPS is exposed; these are called rough strains. There is considerable cross-reactivity among E coli O antigens; also, many O groups of E coli are cross-reactive or identical with specific O groups of Shigella, Salmonella, or Klebsiella.

Escherichia coli serotypes are specific O-group/H-antigen combinations. The H antigens are the flagellar antigens, of which there are at least 56 types. Escherichia coli isolates may be nonmotile and nonflagellated and hence H negative (H). H typing is important for E coli associated with diarrheal disease for two reasons. First, a strain causing an outbreak or epidemic can be differentiated from the normal stool flora by its unique O:H antigenic makeup. Second, most ETEC belong to specific serotypes (Table 25-1); this relationship facilitates their identification even in isolated cases. The reason for the close association between specific serotypes and the production of plasmid-determined virulence factors remains a mystery.

TABLE 25-1 Major Enterotoxigenic *E Coli* Serogroups and Serotypes Grouped According to Their Colonization Factor Antigens<sup>a</sup>

CFA/I	CFA/II	CFA/IV	Other
O15:H11, O15:H	O6:H16, O6:H	O25:H42	O159:H4
O25:H42	O8:H9, O8:H	O29:H21	O9
O63:H12, O63:H	O8O, O85	O115:H4O	
O78:H11, O78:H12	O115:51	O148:H48	
O78:H, O128ac	O139		
O167:H21			
O153:H12, O153:H45			

<sup>a</sup>No particular ETEC serotype has been associated with CFA/III or longus.

One plasmid-encoded but enterotoxin- and serotype-independent pilus (a long polar structure termed longus) has been reported recently in ETEC; like the CFAs, production of this pilus is restricted to E coli isolated from human sources.

Most E coli isolates also produce heat-labile, surface-associated proteins antigenically unrelated to O and H. These antigens can be seen in electron micrographs as filamentous structures called pili (fimbriae), which are much thinner and usually more rigid than flagella. Commensal E coli strains usually produce so-called common pili, which are defined as a specific set of an antigen. When E coli possessing common pili are mixed with erythrocytes (the standard test uses guinea pig erythrocytes), rapid hemagglutination occurs. This hemagglutination is blocked and also reversed by millimolar concentrations of the carbohydrate mannose.

ETEC possess specialized pili, antigenically unrelated to common pili, which act as ligands to bind the bacterial cells to specific complex carbohydrate receptors on the epithelial cell surfaces of the small intestine. Since this interaction results in colonization of the intestine by ETEC, with subsequent multiplication on the gut surface, these pili are termed colonization-factor antigens (CFAs). Most ETEC isolates produce either CFA/I, CFA/II or CFA/IV, whereas CFA/III and an undetermined number of other CFAs occur on other particular serotypes (Table 25-1). CFA-type pili play a major role in host specificity; for instance, different CFAs (e.g., K88, K99, and 987P) are produced by E coli that cause acute diarrhea in domestic animals.

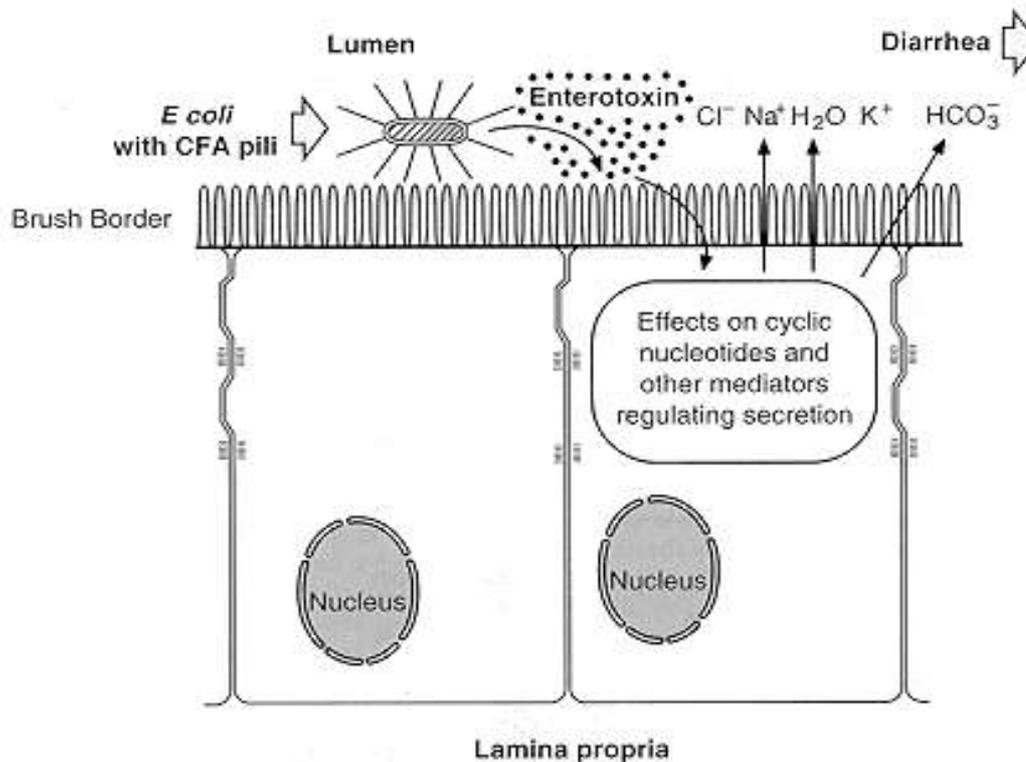
A simple presumptive assay for CFAs on E coli is a test for mannose-resistant (non-common pili) hemagglutination reaction with either human or bovine erythrocytes. However, identification must be confirmed by reaction of the bacteria with antibody directed against a specific CFA or polymerase chain reaction (PCR) assay for specific CFA genes.

Genes coding for the production of CFAs reside on the ETEC virulence plasmids, usually on the same plasmids that carry the genes for one or both of the two types of E coli enterotoxin, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST). Most cases of ETEC diarrhea are caused by E coli possessing a CFA and both LT and ST; fewer are caused by those possessing a CFA and only one toxin (usually LT); and the fewest are caused by E coli that lack a CFA and possess only ST.

## **Pathogenesis**

Escherichia coli diarrheal disease is contracted orally by ingestion of food or water contaminated with a pathogenic strain shed by an infected person. ETEC diarrhea occurs in all age groups, but mortality is most common in infants, particularly in the most undernourished or malnourished infants in developing nations.

The pathogenesis of ETEC diarrhea involves two steps: intestinal colonization, followed by elaboration of diarrheagenic enterotoxin(s) (Fig 25-3). ST is actually a family of toxic peptides ranging from 18 to 50 amino acid residues in length. Those termed STa can stimulate intestinal guanylate cyclase, the enzyme that converts guanosine 5'-triphosphate (GTP) to cyclic guanosine 5'-monophosphate (cGMP). Increased intracellular cGMP inhibits intestinal fluid uptake, resulting in net fluid secretion. Those termed STb do not seem to cause diarrhea by the same mechanism. One method for testing suspect E coli isolates for ST production involves injection of culture supernatant fluids into the stomach of infant mice and seeing whether diarrhea ensues. Specific DNA gene probes and PCR assays have been developed to test isolated colonies for the presence of genes encoding ST and LT.



**FIGURE 25-3 Cellular pathogenesis of *E. coli* having CFA pili.**

The *E. coli* LTs are antigenic proteins whose mechanism of action is similar to that of *Vibrio cholerae* enterotoxin. LT shares antigenic determinants with cholera toxin, and its primary amino acid sequence is similar.

LT is composed of two types of subunits. One type of subunit (the B subunit) binds the toxin to the target cells via a specific receptor that has been identified as Gm1 ganglioside. The other type of subunit (the A subunit) is then activated by cleavage of a peptide bond and internalized. It then catalyzes the ADP-ribosylation (transfer of ADP-ribose from nicotinamide adenine dinucleotide [NAD]) of a regulatory subunit of membrane-bound adenylate cyclase, the enzyme that converts ATP to cAMP. This activates the adenylate cyclase, which produces excess intracellular cAMP, which leads to hypersecretion of water and electrolytes into the bowel lumen.

LT production is demonstrable by serologic methods, testing for diarrheagenic activity in ligated rabbit intestine, and by testing for specific cAMP-mediated morphological changes in cultured Y-1 adrenal tumor cells or Chinese hamster ovary (CHO) cells.

### Host Defenses

As in any orally transmitted disease, the first line of defense against ETEC diarrhea is gastric acidity. Other nonspecific defenses are small-intestinal motility and a large population of normal flora in the large intestine.

Information about intestinal immunity against diarrheal disease is still somewhat superficial. However, intestinal secretory immunoglobulin (IgA) directed against surface antigens such as the CFAs and against LT appears to be the key to immunity

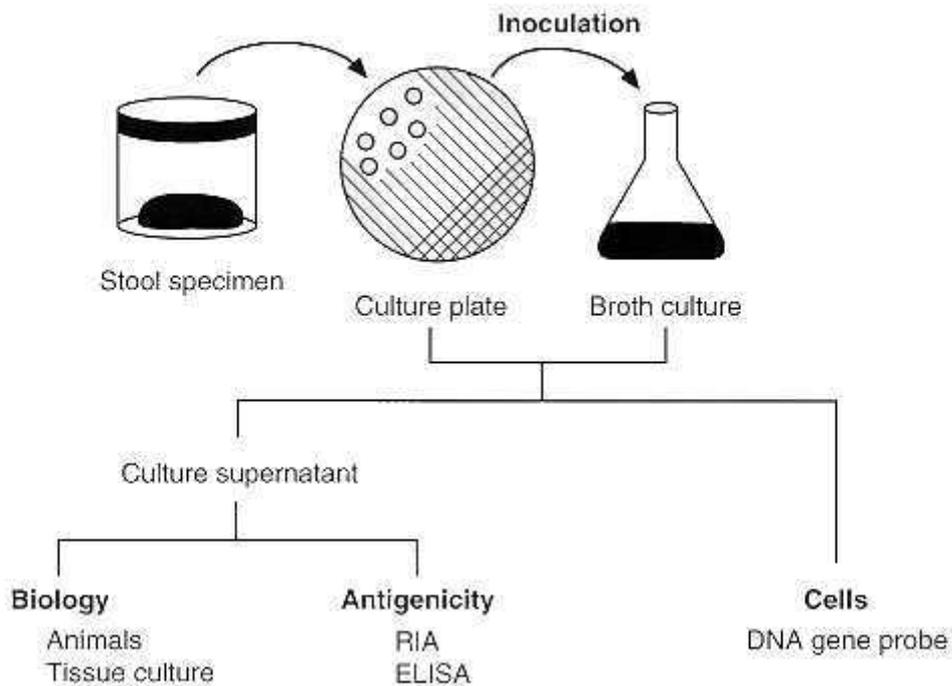
from ETEC diarrhea. Passive immune protection of infants by colostral antibody is important. Human breast milk also contains nonimmunoglobulin factors (receptor-containing molecules) that can neutralize E coli toxins and CFAs.

## Epidemiology

Escherichia coli diarrheal disease of all types is transmitted from person to person with no known important animal vectors. The incidence of E coli diarrhea is clearly related to hygiene, food processing sophistication, general sanitation, and the opportunity for contact. The geographic frequency of ETEC diarrhea is inversely proportional to the sanitation standards. Single-source outbreaks of ETEC diarrhea involving contaminated water supplies or food have been found in adults in the United States and Japan. Adults traveling from temperate climates to more tropical areas typically experience traveler's diarrhea caused by ETEC. This phenomenon is not readily explained, but contributing factors are low levels of immunity and an increased opportunity for infection.

## Diagnosis

ETEC diarrhea is characterized by copious watery diarrhea with little or no fever. The diarrheal stool yields a virtually pure culture of E coli. Since the disease is self-limiting, virulence testing of isolates and serotyping is impractical except in an outbreak situation. Confirmation is achieved by serotyping, serologic identification of a specific CFA on isolates, demonstration of LT or ST production, and identification of genes encoding these virulence factors (Fig. 25-4).



**FIGURE 25-4** Laboratory methods for isolation and identification of ETEC.

## Control

Escherichia coli diarrheal disease is best controlled by preventing transmission and by stressing the importance of breast-feeding of infants, especially where ETEC is endemic. The best treatment is oral fluid and electrolyte replacement (intravenous in severe cases). Antibiotics are not recommended because this practice leads to an increased burden of antibiotic-resistant pathogenic E coli and of more life-threatening enteropathogens.

## Inflammatory Diarrheas Caused by Enteroinvasive, Cytotoxic, and Enteropathogenic Escherichia Coli

### Clinical Manifestation

Diarrhea caused by the enteroinvasive, cytotoxic, and enteropathogenic (EPEC) strains of E coli ranges from very mild to severe. Illness is usually protracted and accompanied by fever. Infection with a few serogroups (O157, O26) is characterized by bloody diarrhea (hemorrhagic colitis). Infection with the Shigella-like serogroups presents as bacillary dysentery (i.e., abdominal pain and scanty stool containing blood and mucus).

### Structure, Classification, and Antigenic Types

As in the case with ETEC, these strains of E coli are not detectably different in structure from E coli of the normal flora. The EPEC serogroups listed in Table 25-2 were the first E coli groups to be recognized as causative agents of diarrhea in infants. Their status as pathogens remained controversial for decades, mainly because the same O groups can be isolated from healthy contacts in outbreaks and from healthy adults. Recent work has proven that these E coli serogroups possess an antigenic adherence factor (termed bundle-forming pilus, or BFP); the gene for BFP is carried by a plasmid termed EAE (enteroaggregative Escherichia coli) plasmid. BFP is responsible for the initial attachment of EPEC to intestinal target cells.

TABLE 25-2 Common Serologic Types of Non-ETEC *E Coli* Grouped According to Their Mechanism of Pathogenesis<sup>a</sup>

Classic EPEC Serogroups	Enteroinvasive Shigella-Like Serogroups	Enterohemorrhagic Serogroups
O18, O26 <sup>b</sup> , O44, O86 <sup>b</sup> , O111 <sup>b</sup> , O112, O114, O119 <sup>b</sup> , O125 <sup>b</sup> , O126 <sup>b</sup> , O127 <sup>b</sup> , O128ab <sup>b</sup> , O142 <sup>b</sup>	O28ac, O29, O124, O136, O143, O144, O152, O164	O26:H11, O111, O55b, O157:H7

<sup>a</sup>Occasional isolates fail to show the expected correlation between serogroup and disease type; this is due to the genetics of virulence factors (i.e., transmissibility of virulence-associated plasmids and bacteriophage between *E coli* strains in vivo as well as spontaneous loss of these factors in vitro).

<sup>b</sup>Usually EAF positive; other EPEC serogroups usually EAF negative.

A small but important group of EPEC includes serotypes O157:H7, O26:H11, and some O111 isolates. These cause epidemic hemorrhagic colitis. Serotype O157:H7 is often associated with food-borne outbreaks.

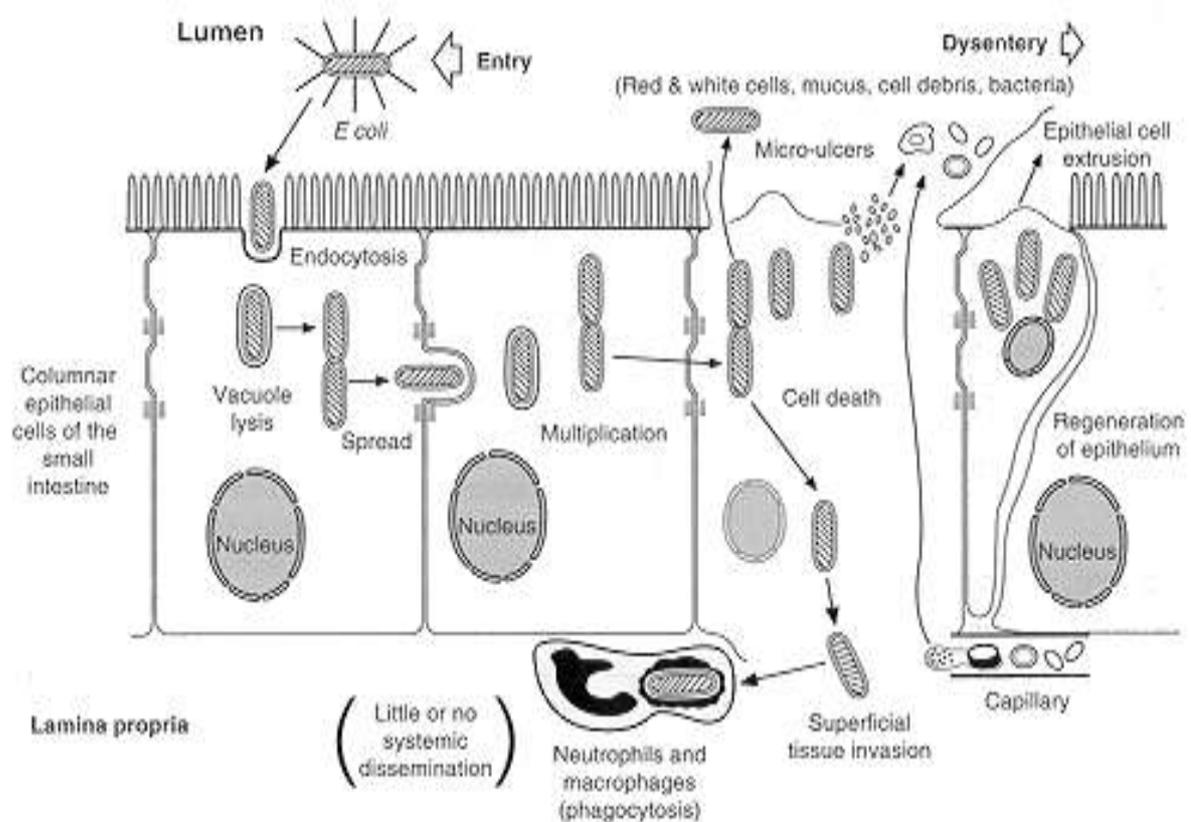
Table 25-2 lists the Shigella-like enteroinvasive E coli serotypes (i.e., those with somatic antigens reactive with specific anti-Shigella typing serum). Also, like Shigella, these E coli strains are non-motile and therefore H negative. These serogroups usually do not harbor ETEC virulence plasmids and therefore are usually CFA negative.

## **Pathogenesis**

Escherichia coli strains belonging to the classic EPEC serogroups (Table 25-2) bind intimately to the epithelial surface of the intestine, usually the colon, via the adhesive BFP. The lesion caused by EPEC consists mainly of destruction of microvilli. There is no evidence of tissue invasion. Cell damage occurs in two steps, collectively termed attaching and effacing; first is intimate contact, sometimes characterized as pedestal formation; second is loss of microvilli which is the result of rearrangement of the host cell cytoskeleton. Loss of microvilli leads to malabsorption and osmotic diarrhea. Diarrhea is persistent, often chronic, and accompanied by fever. EPEC are negative for ST and LT, but most strains produce relatively small amounts of a potent Shiga-like toxin that has both enterotoxin and cytotoxin activity.

The E coli strains associated with hemorrhagic colitis (enterohemorrhagic E coli, or EHEC) most notably O157:H7, produce relatively large amounts of the bacteriophage-mediated Shiga-like toxin. This toxin is called Vero toxin (VT), or Vero cytotoxin after its cytotoxic effect on cultured Vero cells. Many strains of O157:H7 also produce a second cytotoxin (Shiga-like toxin 2, or Vero toxin 2), which is similar in effect but antigenically different.

The Shigella-like E coli strains are highly virulent; oral exposure to a very small number of these invasive bacteria causes severe illness. The site of the infection is the colon, where adherence is rapidly followed by invasion of the intestinal epithelial cells (Fig. 25-5). An acute inflammatory response and tissue destruction produce diarrhea with little fluid, much blood, and sheets of mucus containing polymorphonuclear cells. Invasive E coli, like Shigella, causes a rapid keratoconjunctivitis when placed on the conjunctiva of the guinea pig eye (Sereny test). Virulent Sereny test-positive isolates carry a large (usually 140-megadalton) plasmid responsible for this property.



**FIGURE 25-5 Cellular pathogenesis of invasive E coli**

## Host Defenses

Host defenses against EPEC are the same as those for ETEC. These defenses are frequently deficient or lacking in the infant and the elderly, which is consistent with the epidemiology of EPEC illness. An important example is the role of the immune system. Passive immune protection of infants by colostral antibody is important; breast-feeding is especially relevant where crowding and poor economic conditions prevail. Infection with these pathogens often excites an inflammatory cell response in the intestine, as is frequently reflected in the diarrheal symptoms.

## Epidemiology

The geographic distribution of all EPEC is generally the same as for the ETEC, with a more severe disease in infants and young children, and so EPEC are much less important in traveler's diarrhea. Common-source community outbreaks are rare in geographic areas with satisfactory sanitation. However, sporadic cases are seen in the United States, Canada, and Europe, and outbreaks occur in these areas, but most commonly in close-contact institutions such as hospital nurseries, day-care centers, and nursing homes.

## Diagnosis

Diagnosis is usually based on the symptomatology described above. Enterohemorrhagic E coli, such as serotype O157:H7, is suspected in the setting of copious bloody diarrhea

without fecal leukocytes or fever, especially when symptoms include hemolytic uremic syndrome, or HUS. *Escherichia coli* serotyping is useful in chronic cases and in outbreaks, because identification of the agent and its antibiotic sensitivity pattern are valuable in these situations. Testing for specific EPEC virulence factors is usually impractical because it can be done only in reference and specialized research laboratories.

## **Control**

Prevention and control are generally the same as for ETEC. Intervention of the fecal-oral transmission cycle is most effective in institutional situations. Broad-spectrum antibiotics are recommended in chronic and/or life-threatening cases.

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