**Virulence/Pathogenesis:** ETEC are noninvasive; the *E. coli* adhere to the small intestine and produce toxins.

**Adhesins:**
1. Long thin pili called colonization factors (CFAI, CFAII, CFAIII, etc.).
   - I, II, and IV are the major CFAs. These are important; are specific for humans.
2. Bundle-forming pilus and Type-1 pili. Role unclear.

**Toxins:** There are two, and these are very ETEC-specific -> after attachment

1. Heat-labile toxin (LT-1): has about 75% sequence identity with cholera toxin (CT). LT is not secreted past the periplasm.
   - Five B (binding) subunits, one A (active) subunit per toxin molecule.
   - LT-1 binds the gangliosides GM1 and GD1b.
   - The A1 portion of the A subunit catalyzes ADP-ribosylation of Gs, raising host cell cAMP levels, leading to fluid and electrolyte secretion.

   - Binds to guanylate cyclase
   - Is processed to this size during secretion by *E. coli.*
   - ST-1 causes increased cGMP in host cell, which has is similar in effect to that of elevated cAMP.

*No vaccine, but can build immunity.*
Genetics: The genes encoding LT and ST are almost always on plasmids.

Transposon.

Immunity: ETEC infection results in immunity.

**EPEC:**

Epidemiology:
- Children and infants in developing countries.
- 20% of diarrhea in bottle-fed <1 year population.
- Humans may be a critical reservoir.

*Not seen in USA*

Virulence/Pathogenesis:

EPEC has a characteristic adherence consequence called the "attaching and effacing" lesion. The microvilli are elongated next to the bacteria, and absent where the bacteria have bound.

Three stages in the development of EPEC attachment:

1. **Nonintimate binding**, mediated by the bundle-forming pilus (BFP).
   - Makes intimin BFP that injects into cell w/ Tir

2. **Contact-dependent secretion** of bacterial proteins into the host cell. This leads to activation of PLC-α1, a rise in intracellular IP3 and calcium, IL-8 secretion.
   - And, loss of microvilli and the formation of a pedestal under the bacterium. (The attaching and effacing lesion.)

3. Intimate attachment between intimin on the bacterial surface, and Tir, a bacterial protein secreted into the host cell.

   - Becomes part of close adherence complex & part of secreted enteropathogenic E. coli (EPEC) strain.
Toxin: in some strains there is a toxin called EspC, an enterotoxin, but its role appears to be minor.

Diarrhea may result from a loss of absorptive activity by the mucosal cells, with contributions from inflammation and increased intestinal permeability.

Genetics:
EPEC carry a pathogenicity island called LEE (locus of enterocyte effacement). LEE encodes intimin, the contact-dependent secretion system and Tir.

![Schematic representation of the EPEC LEE](image)

FIG. 2. Schematic representation of the EPEC LEE. It contains 41 predicted open reading frames arranged in at least five polycistronic operons, namely, LEE1, LEE2, LEE3, LEE4, and LEE5. The diagram shows the genes known to be required for EPEC virulence.

Clinical symptoms:

- Watery diarrhea. Usually self-limiting, but can be chronic in infants.
- Some fever, some inflammatory infiltrate due to limited invasion of bacteria.

Immunity appears to develop to EPEC.

EHEC/STEC:

**Shiga** (?): Toxin E. coli

Epidemiology:

Reservoir: Cattle. Leading to contamination of hamburger. Also manure for fertilization

Other food sources: apple cider, lettuce, alfalfa sprouts, salami, water, milk, etc.

Person-to-person transmission can occur. → *fecal-oral*

A serious disease in developed countries due to hemolytic-uremic syndrome (HUS), a complication of the diarrheal illness.

Both sporadic cases and outbreaks occur.

Incidence in developed countries has increased due to *modern* processing.

Estimate 50 to 200 deaths per year in USA; 62,000 cases.

Mostly O157:H7, → but there are other serogroups.
Disease:

Diarrhea: get watery diarrhea by day 4, and intense abdominal pain; day 6 or so, bloody diarrhea develops. Little fever, no focus-pus. Usually resolves by day 10.

HUS: hemolytic uremic syndrome develops in 8–11% of cases involving young children. See microangiopathic hemolytic anemia, thrombocytopenia, and thrombosis of the glomerular capillaries. It most commonly presents as acute renal failure. Requires transfusion and hemodialysis. Mortality is significant. Do not know why only some patients get HUS.


HEMOLYTIC UREMIC SYNDROME, POSTDIARRHEAL. Reported cases — United States and U.S. territories, 2002

EHEC/STEC virulence/pathogenesis:

Attachment: These *E. coli* also make attaching and effacing lesions (have LEE, intimin, Tir, etc.), but do not make bundle-forming pili.

(A-B toxin)

Shiga toxin (Stx): EHEC encode one or two Shiga toxins (Stx1 and Stx2). (The *E. coli* produced Shiga toxin used to be called Shiga-like toxin, now called Shiga toxin.) However, not all Shigatoxin producing *E. coli* have LEE–thus STEC.

Stx are encoded by a lysogenic bacteriophage.

Shiga toxin is an A:B toxin: 5 B subunits, 1 A subunit.

Receptor: a glycolipid, globotriaosylceramide (Gb3) —

Action: removes an adenine from host cell 28S rRNA, leading to an inhibition of protein synthesis and cell death.

Stx is produced in the colon, crosses into the blood stream, travels to the kidney, where it does damage to renal endothelial cells.
Other: hemolysin, proteases. Maybe other adhesins.

Prevention: cook ground beef. Change animal husbandry-feed cattle hay (instead of corn) for a few days prior to slaughter.

* Keep children away from raw beef.

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**EAEC:**

Disease/epidemiology: a serious persistent watery diarrheal disease in children and adults; mostly in **developing countries**.

Virulence/pathogenesis:

* Like ETEC, the EAEC colonize intestinal cells, but are not **invasive**.

  Adherence: they bind in clumps, via AAF pili, (aggregative adherence fimbriae) and another adherence protein called **dispersin**.

Toxins: make an ST-like toxin called **EAST**

Pet: a serine protease that affects the cytoskeleton of colonized cells

Hemolysin: Punches pores in host cell membranes (but not in RBCs).

* All contribute to watery diarrhea

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**EIEC:**

Invasion of the epithelium. Similar to Shigellosis, but less severe.

Uncommon in USA, but a problem in children under 5 in developing countries.

Humans appear to be sole reservoir.

* Virulence genes encoding invasive capabilities are carried on a **large plasmid**.
DAEC:

Disease: causes a diarrhea in children <1
Virulence: make Dr-type fimbriae, which cause DAED to induce host cell to make long extensions which wrap around the bacteria. *mediates attachment to cells (apical surface)*
DAEC also, by an unknown mechanism, cause brush border membrane metabolism problems which may contribute to diarrhea.

*We don't know how it causes sp/*

Treatment of *E. coli* intestinal disease:

Rehydration therapy.

Antibiotics: When necessary: trimethoprim/sulfamethoxazole or quinolones for ETEC, EIEC, or EPEC.

Antibiotics may not help to avert HUS; in fact, there is some belief that antibiotics may increase the risk of HUS.

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<td>Prominent</td>
<td>Absent</td>
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<td>Absent</td>
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</table>

*Abbreviations: ETEC = enterotoxigenic *E. coli*; EIEC = enteroinvasive *E. coli*; EHEC = enterohemorrhagic *E. coli*; EPEC = enteropathogenic *E. coli*; EAEC = enteroaggregative *E. coli*; LT = heat-labile toxin; ST = heat-stable toxins; WBCs = white blood cells.

*In experimental animals EPEC strains produce lesions in the large intestine as well.
*These are much more common in developing countries. ETEC strains are rare in the United States.
Shigella

**Disease:** Bacillary dysentery, found worldwide.

**Microbiology:**
- Closely related to E. coli, gram-negative rod.
- Nonmotile
- Lactose nonfermenters (usually)

Four species: *dysenteriae, flexneri, boydii, sonnei* — do the same thing for the most part.

**Epidemiology:**
- Contaminated food or water; fecal-oral route, person-to-person.
- In USA, children under ten. Often in day-care centers (usually sonnei).
- High infectivity, need only a few 100 bacteria, due to acid resistance.
- Found only in humans (some primates).
- *S. flexneri* in sexually gay men.

**SHIGELLOSIS. Reported cases per 100,000 population, by year — United States, 1972–2002**

**Virulence/pathogenesis:**
- Pathogenicity island
- Large plasmid

Route of bacteria: enter M cells, exit at the basal surface, enter a neighboring mucosal cell via endocytosis. *Shigella* attach to the α5β1 integrin receptor and to the hyaluronic acid receptor. Then: contact-dependent secretion of several effector proteins into host cell which leads to actin lysis and M cell death.
polymerization and formation of membrane ruffles, followed by uptake of the bacterium into a vacuole, from which they almost immediately escape and enter the host cell cytoplasm.

Rapidly multiply inside the host cell.

A protein (IcsA) is secreted from one pole of the bacterium, where it acts to cause actin condensation at that pole. This powers the bacterium through the cell, into the cell membrane forming protrusions that enter the neighboring cell.

The host cells die.

The spread continues into further neighboring cells by the same cycle of events.

IpaB: Secreted by cytoplasmic Shigella, activates Interleukin-1 Converting Enzyme (ICE) within subepithelial macrophages. This leads to activation and secretion of IL-1α and apoptosis of the macrophages.

Toxin role? Shiga toxin is only released by bacterial cell lysis. It does not kill the invaded mucosal cells.

Activity: cleaves at a specific adenine of 28S rRNA. (Same activity as ricin.) Mutants that don't make toxin invade and spread in mucosa like normal bacteria. Still get bloody diarrhea in monkeys.

Back to HUS: Shiga toxin appears to primarily affect vascular tissue, a target that clearly is involved in kidney damage.

Disease: Begins with fever, malaise, anorexia; then watery diarrhea with many WBCs. Then get bloody stools if is flexneri or dysenteriae.

Invades mucosal cells in the colon; multiply and spread laterally to adjacent mucosal cells.

Generally not systemic.

Can get intense inflammatory response due to the invasion of the intestinal mucosa, stools are bloody and full of mucus. IL-8 is released by the intestinal epithelium and mediates recruitment of PMNs.

Treatment: antibiotic resistance a problem; use quinolones and 3rd generation cephalosporins if therapy seems appropriate.

Vaccines: Immunity is short-lived and serotype-specific. Nonetheless, work continues to develop an effective, safe vaccine-trying a LPS-protein conjugate.