Gastrointestinal gram negative Rods

Different enteric gram-negative rods cause diseases within the GI tract, outside the GI tract, or in both locations:

Diseases caused by members of the genera *Escherichia, Salmonella, Yersinia, and Campylobacter* can be both GI and extraintestinal

Those caused by members of the genera *Shigella, Helicobacter, and Vibrio* are primarily GI

Those caused by members of the genera *Enterobacter, Klebsiella, Serratia, and Proteus* are primarily extraintestinal.

Fecal contamination is commonly important in the transmission of those organisms that cause GI diseases.

ESCHERICHIA COLI

Escherichia coli is part of the **normal flora** of the colon in humans and other animals but can be pathogenic both within and outside of the GI tract.

The differences in the **degree of virulence** of various *E. coli* strains is correlated with the acquisition of plasmids, integrated prophages, and pathogenicity islands.

E. coli has **fimbriae or pili** that are important for adherence to host mucosal surfaces, and different strains of the organism may be **motile or nonmotile**.

Most strains can **ferment lactose** (that is, they are Lac+) in contrast to the major intestinal pathogens, *Salmonella and Shigella* which cannot ferment lactose (that is, they are Lac –).

E. coli **produces both acid and gas** during fermentation ofcarbohydrates.

Facultative anaerobes, **oxidase negative.**

Typing strains is based on differences in three structural antigens: **O, H, and K**.

Clinical significance: intestinal disease

Transmission of intestinal disease is commonly by the **fecal–oral route**, with contaminated food and water serving as vehicles for transmission.

At least five types of intestinal infections that differ in pathogenic mechanisms have been identified.

Enterotoxigenic E. coli: ETEC are a common cause of **traveler's diarrhea**. ETEC colonize the small intestine (pili facilitate the binding of the organism to the intestinal mucosa). And produces enterotoxins which include a heat-stable toxin (ST) and heat-labile toxin (LT). In a process mediated by enterotoxins, ETEC cause prolonged hypersecretion of chloride ions and water by the intestinal mucosal cells, while inhibiting the reabsorption of sodium. The gut becomes full of fluid, resulting in significant watery diarrhea that continues over a period of several days.

Enteropathogenic E. coli: EPEC are an important cause of **diarrhea in infants**, especially in locations with poor sanitation. The newborn becomes infected perinatally. The EPEC **attach to mucosal** cells in the small intestine by use of **bundle-forming pili (BfpA**). Characteristic lesions in the small intestine called **attaching and effacing lesions** (A/E) in addition to destruction of the microvilli, are caused by injection of effector proteins into the host cell by way of a type III secretion system (T3SS).

Enterohemorrhagic E. coli: EHEC bind to cells in the **large intestine** via BfpA and, similar to EPEC, **produce A/E lesions**. However, in addition, EHEC produce one of two **exotoxins** (Shiga-like toxins1 or 2), resulting in a severe form of copious, **bloody diarrhea** (hemorrhagic colitis) in the absence of mucosal invasion or inflammation. Serotype **O157:H7** is the most common strain of E. coli that produce Shiga-like toxins.

Enteroinvasive E. coli: EIEC cause a **dysentery-like syndrome** with fever and bloody stools. **Plasmid-encoded virulence** factors are nearly identical to those of Shigella species. These virulence factors allow the invasion of epithelial cells (Ipa) and intercellular spread by use of actin-based motility. In addition, EIEC strains produce a **hemolysin** (HlyA).

Enteroaggregative E. coli: EAEC also cause **traveler's diarrhea and persistent diarrhea in young children**. Adherence to the small intestine is mediated by aggregative adherence fimbriae. The adherent rods resemble stacked bricks and result in shortening of microvilli. EAEC strains produce a **heat-stable toxin that is plasmid encoded**.

Clinical significance: extraintestinal disease

Urinary tract infection: E. coli is the most common cause of urinary tract infection (UTI), including cystitis and pyelonephritis.

Uncomplicated cystitis is caused by uropathogenic strains of E. coli, characterized by P fimbriae (an adherence factor) and, commonly, hemolysin, colicin V, and resistance to the bactericidal activity of serum complement.

Neonatal meningitis: E. coli is a major cause of this disease occurring within the **first month of life**. The K1 capsular antigen, which is chemically identical to the polysaccharide capsule of group B Neisseria meningitidis, is particularly associated with such infections.
Nosocomial (hospital-acquired) infections: These include sepsis/ bacteremia, endotoxic shock, and pneumonia.

Laboratory identification
1. Intestinal disease: Because E. coli is normally part of the intestinal flora, detection in stool cultures of disease-causing strains is generally difficult. EIEC strains often do not ferment lactose and may be detected on media such as MacConkey agar.
EHEC, unlike most other strains of E. coli, ferment sorbitol slowly, if at all, and may be detected on MacConkey sorbitol agar.
Molecular techniques, such as pCR) may be employed to identify E. coli strains producing Shiga-like toxins.
2. Extraintestinal disease: Isolation of E. coli from normally sterile body sites (for example, the bladder or cerebrospinal fluid) is diagnostically significant. Specimens may be cultured on MacConkey.

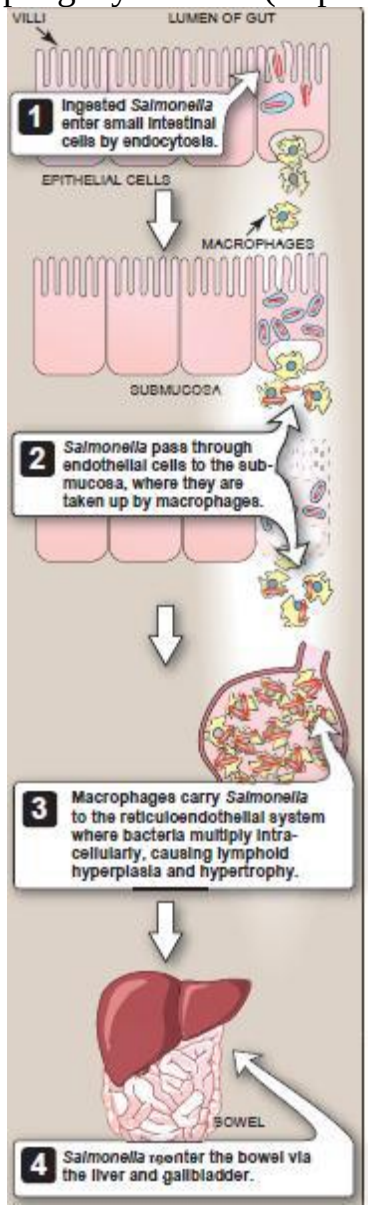
Prevention and treatment
Intestinal disease can be prevented by care in selection, preparation, and consumption of food and water.
Maintenance of fluid and electrolyte balance is of primary importance in treatment.
Antibiotics may shorten duration of symptoms, but resistance is nevertheless widespread.

SALMONELLA
Members of the genus Salmonella can cause a variety of diseases, including gastroenteritis and enteric (typhoid) fever.

All strains affecting humans are grouped in a single species, Salmonella enteritidis, which has approximately 2,500 different serotypes, or serovars, including the clinically significant serotypes *Typhimurium* and *Typhi*.
Most strains of Salmonella are Lac– and produce acid and gas during fermentation of glucose. They also produce H2S from sulfur-containing amino acids.

Pathogenesis
Salmonella invade epithelial cells of the small intestine. Disease may remain localized or become systemic, sometimes with disseminated foci.

The organisms are facultative, intracellular parasites that survive in phagocytic cells. (steps in pathogenesis as illustrated in the figure)



Clinical significance

Salmonella infection can cause both intestinal and extraintestinal diseases.

1. Gastroenteritis: This localized disease (also called salmonellosis) is caused primarily by serovars Enteriditis and Typhimurium.

Salmonellosis is characterized by nausea, vomiting, and diarrhea (usually nonbloody), which develop generally within 48 hours of ingesting contaminated food or water. Fever and abdominal cramping are common.

In uncompromised patients, disease is generally self-limiting (48 to 72 hours).

2. Enteric or typhoid fever: This is a severe, life threatening systemic illness, characterized by fever and, frequently, abdominal symptoms. It is caused primarily by serovar Typhi.

About 30 percent of patients have a faint and evanescent (transient) maculopapular rash on the trunk (rose spots). The incubation period varies from 5 to 21 days. Untreated, mortality is approximately 15 percent.

Laboratory identification

In patients with diarrhea, Salmonella can typically be isolated from stools on MacConkey agar or selective media.

For patients with enteric fever, appropriate specimens include blood, bone marrow, urine, stool, and tissue from typical rose spots (if they are present).

Campylobacter

oxidase positive, curved, spiral, or S-shaped organisms that microscopically resemble vibrios.

A single, **polar flagellum** provides the organism with its characteristic darting motility. polar flagella are attached at their ends giving **"gull wings" appearance**.

Somatic, flagellar, and capsular antigens all contribute to the numerous serotypes.

There is several spp. have been associated with human diseases, of these, *C. jejuni*, and *C. coli* are the most common and similar enough to be considered as one.

C. jejuni grows well only on enriched media under **microaerophilic** conditions. That is, it requires oxygen at reduced tension (5 – 10%), presumably due to vulnerability of some of its enzyme systems to superoxides. Growth usually requires **2 to 4 days**, sometimes as much as a week.

Campylobacter infect the intestine and can cause ulcerative, inflammatory lesions in the jejunum, ileum, or colon.

<u>Epidemiology</u>

Campylobacter are widely distributed in nature as **commensals of many different vertebrate** species (reservoirs of infection).

Campylobacter are transmitted to humans primarily via the **fecal–oral route** through **direct contact**, **exposure to contaminated meat** (especially poultry), or **contaminated water** supplies.

<u>Pathogenesis and clinical significance</u>

Campylobacter may cause both intestinal and extraintestinal disease.

C. jejuni typically causes an acute enteritis in otherwise healthy individuals following a 1- to 7-day incubation. The disease lasts days to several weeks and, generally, is self-limiting. Symptoms may be both systemic (fever,

headache, myalgia) and intestinal (abdominal cramping and diarrhea, which may or may not be bloody).

Important **virulence factors** include a **cytotoxin** that may be involved in inflammatory colitis and an **enterotoxin** (related to cholera toxin) that results in increased adenylyl cyclase activity and, therefore, electrolyte and fluid imbalance.

Laboratory identification

The illness is typically self limiting after 3 to 5 days but may last 1 to 2 weeks. The diagnosis is confirmed by isolation of the organism from the stool. This requires a special medium made selective for *Campylobacter* by inclusion of antimicrobics that inhibit the normal facultative flora of the bowel (**Skirrow´s media, Campy media**). Plates must be incubated in a **microaerophilic** atmosphere that can now be conveniently generated in a sealed jar by hydration of commercial packs similar to those used for anaerobes. grow best at 42 c but can be cultured at 37 c.

Microscopy: typical **(gull-wings)** shaped gram negative rode, typical **darting motility** of the bacteria under dark field microscopy or phase contrast microscopy.

SHIGELLA

Shigella species cause shigellosis (bacillary dysentery), a human intestinal disease that occurs most commonly among **young children**.

Shigellae are **nonmotile, unencapsulated**. The **40 serotypes** of Shigella are organized into four groups (A, B, C, and D) based on the serologic relatedness of their polysaccharide **O antigens**.

All *shigellae* ferment glucose. With the exception of *Shigella sonnei,* they **do not ferment lactose**. The inability to ferment lactose distinguishes shigellae on differential media. They may also be divided into those that ferment mannitol and those that do not. Most strains **do not produce gas** in a mixed-acid fermentation of glucose.

Epidemiology

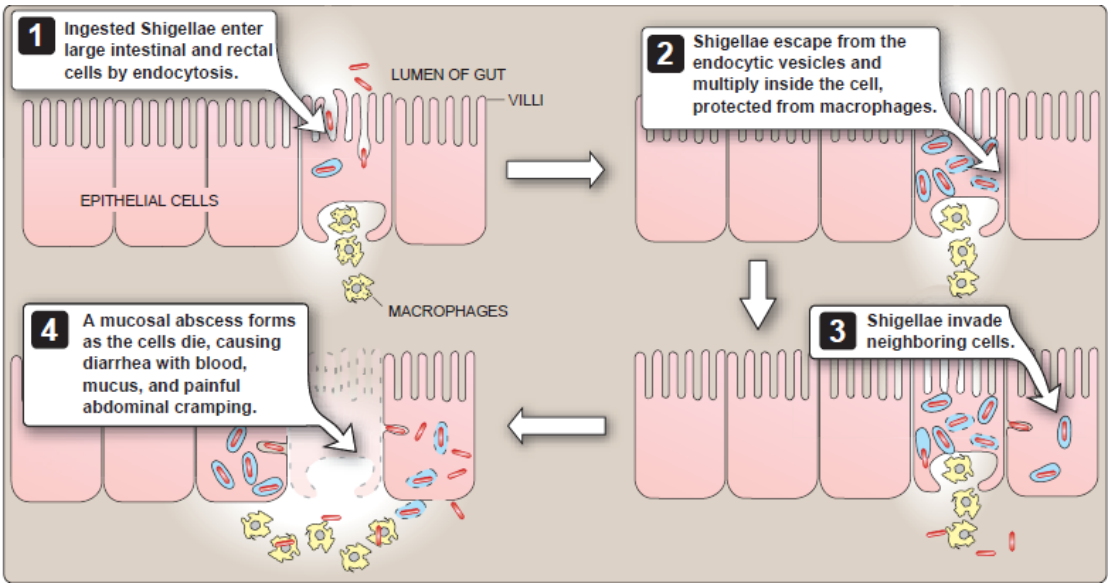
Shigella are typically spread from person to person, with contaminated stools serving as a major source of organisms. Humans are the only natural host for Shigella species.

Shigellosis has a **low infectious dose**: Approximately 10–100 viable organisms are sufficient to cause disease (whereas it usually is 10^5–10^8 for salmonellae and vibrios).

Pathogenesis & Pathology
incubation period (1–2 days)
Shigella infections are almost always **limited to the GIT**

Toxins
A. ENDOTOXIN: contributes to the irritation of the bowel wall.

B. *SHIGELLA DYSENTERIAE* EXOTOXIN
S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut and the central nervous system.



Laboratory identification
During acute illness, organisms can be cultured from stools using differential, selective **Hektoen** agar or other media specific for intestinal pathogens.

VIBRIO
Members of the genus Vibrio are short, curved, rod-shaped organisms.
They are rapidly motile by means of a **single polar flagellum**.
O and H antigens are both present, but only O antigens are useful in distinguishing strains of vibrios that cause epidemics.
Vibrios are facultative anaerobes. The growth of many Vibrio strains either **requires** or is stimulated by **NaCl.**

Vibrio grow in synthetic media with glucose as carbon and energy source. Vibrio tolerate alkaline and sensitive to acid.
Pathogenic vibrios include:
1) *Vibrio cholerae*, serogroup O1 strains that are associated with epidemic cholera. This serotype is further differentiated serologically according to their subsidiary O-Antigen into INABA, OGAWA, HIKOJIMA (the names denoting their histological origen).
2) non-O1 *V. cholerae* and related strains that cause sporadic cases of cholera-like and other illnesses
3) Vibrio parahaemolyticus and other halophilic vibrios, which cause gastroenteritis and extraintestinal infections.

Epidemiology
V. cholerae is transmitted to humans by **contaminated water and food**. In the aquatic environment.
There are two biotypes (subdivisions) of the species *V. cholerae*: classic and El Tor. In contrast to the classic strain, the El Tor strain is distinguished by the production of hemolysins, higher carriage rates, and the ability to survive in water for longer periods. Outbreaks of both strains have been associated with raw or undercooked seafood harvested from contaminated waters.
The infectious dose varies depending on the pH of the stomach, in healthy volunteers **10^8 bacteria** produce infection.
The **small intestine** is the primary site of infection. And *V. cholera* colonizes the epithelium without invasion or apparent damage.
cholera is characterized by **massive loss of fluid** and electrolytes from the body. After an incubation period ranging from hours to a few days, profuse watery diarrhea ("**rice-water**" stools) begins. Untreated, death from severe dehydration causing hypo- volemic shock may occur in hours to days, and the death rate may exceed 50 percent.

Virulance factor
Cholera toxin (CT) composed of AB subunits. Once inside the cell (A)subunit causes changes in the regulation of cell genes and the result flow of ions and water is reversed.

Laboratory identification
V. cholerae grows on standard media such as blood and MacConkey agars. Thiosulfate-citrate-bile salts–sucrose (TCBS) medium can enhance isolation and it required **transport media (Carry Blair media**). The organism is **oxidase positive**.

Helicobacter pylori

H. pylori has morphologic and growth similarities to the campylobacters, with which they were originally classified. The cells are slender, curved rods with polar flagella.
Growth requires a **microaerophilic** atmosphere and is slow (3 to 5 days).

Helicobacter infections are limited to the **mucosa of the stomach**, and most are asymptomatic even after many years.
Burning pain in the upper abdomen, accompanied by nausea and sometimes vomiting, is a symptom of gastritis.
Ulcers may cause additional symptoms, depending on their anatomic location. It is common for gastric and duodenal ulcers to be unrecognized by the patient until they cause frank bleeding or rupture.
A number of unique bacteriologic features: **urease** whose action allows the organism to persist in low pH environments by the generation of ammonia.
Another secreted protein called the **vacuolating cytotoxin** (VacA) causes apoptosis in eukaryotic cells it enters generating multiple large cytoplasmic vacuoles.
Motility provided by the flagella allows the organisms to swim to the less acid pH locale beneath the gastric mucus **(mucinase**), where the urease further creates a more neutral microenvironment by ammonia production.
At the mucosa, **adherence is mediated by surface proteins** on the surface of gastric epithelial cells
The inflammation may be due to toxic effects of the urease or the VacA. The *Cag* protein may contribute by stimulation of cytokines (interleukin-8), and a neutrophil-activating protein (NAP) has been shown to recruit neutrophils to the gastric mucosa
H. pylori is the most common cause of gastritis, gastric ulcer, and duodenal ulcer. In addition, *Helicobacter* gastritis caused by *Cag+ve* strains is acknowledged to be the antecedent cause of gastric adenocarcinoma, one of the most common causes of cancer death in the world.

Lab dx:
Endoscopic examination: biopsy and culture of the gastric mucosa.
The *H. pylori* urease is so potent its activity can be directly demonstrated in biopsies in less than an hour.
Noninvasive methods include serology and a urea breath test. For the breath test, the patient ingests ^{13}C- or ^{14}C-labeled urea, from which the urease in the stomach produces products that appear as labeled CO2 in the breath.
A number of methods for detection of antibody directed against *H. pylori* are now available. Because IgG or IgA remain elevated as long as the infection persists, these tests are valuable both for screening and for evaluation of therapy.

Specimen: gastric biopsy, serum
Smear: Giemsa or silver stain
Culture: Skirrows media, translucent colonies after 7 days of incubation