# Gastrointestinal (GI) Bacteria I and II

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## Lecture Objectives

A large and diverse flora (>$10^{11}$ organisms/gram of stool) resides within the human gastrointestinal tract. Only a few of these species are capable of causing disease. The GI bacteria will be divided into two parts.

- In the first part, the leading bacterial causes of gastroenteritis (i.e. diarrhea) and differences in their mechanisms of pathogenesis will be reviewed.
- In the second part, some of organisms that reside in the gut, but cause infectious diseases other than diarrhea will be discussed.

**GI Tract -Innate Immune Mechanisms:**
Microorganisms entering by the oral route, more than any other, have to compete with the well-adapted, substantial normal flora of the mouth and intestine. Most organisms that are swallowed are destroyed by acid and various secretions of the stomach. Alkaline pH of the lower intestine can discourage other organisms. The peristaltic action of the intestine ultimately flushes out organisms which have not succeeded in colonization. Bile salt, secretory IgA and lysozyme are present, which kill or inhibit many types of bacteria.

**DEFINITION:** Diarrhea: an increase in stool mass, frequency, and /or fluidity

Over 200 million episodes of acute gastroenteritis occur each year in the US. 76 million GI illnesses per year in the U.S. are due to foodborne pathogens, with over 300,000 hospitalizations and several thousand deaths. Foodborne pathogens account for a significant portion of these infections, but the etiology of these illnesses are not identified in the majority of cases.

**General Pathogenic Mechanisms:**
There are three general pathogenic mechanisms for the development of bacterial gastroenteritis.
1) Ingestion of preformed toxin with rapid onset of illness. These toxins are present in the food at the time of ingestion and act quickly to cause illness (nausea/vomiting/diarrhea) within hours after ingestion (*S. aureus* - see *S. aueus* lecture, *Bacillus cereus)***.**

2) Ingestion of organisms that produce toxins *in vivo* that cause disease. There may be sudden or delayed onset of illness, depending upon the organism and, ingestion of organism that produces a quick acting toxin (*B. cereus, Clostridium perfringens)* or have a more delayed effect (*V. cholerae*, ETEC, *C. difficile*).

3) Infection by enteroinvasive organisms with delayed onset of illness. Caused by ingestion of organisms that are locally or systemically invasive, with incubation periods of 24-72 hours following exposure with a sufficient infective dose or organism. (*Campylobacter jejuni*, *Salmonella spp., Shigella spp*, EHEC, EPEC).

**Treatment-Overview:**

Virtually all bacterial diarrheal diseases are treated with supportive care to replace fluid and electrolytes only. Between 20% and 50% of travelers to developing and industrialized nations will develop at least one episode of diarrhea, making it the most common medical ailment afflicting travelers. Although usually a mild illness, traveler's diarrhea can result in significant morbidity and hardship overseas. Precautions can be taken to minimize the risk of developing traveler's diarrhea, either through avoidance of potentially contaminated food or drink or through various prophylactic measures, including both nonpharmacological and antimicrobial strategies. If diarrhea does develop despite the precautions taken, effective treatment-usually a combination of an antibiotic and an antimotility agent-can be brought by the traveler and initiated as soon as symptoms develop. In the future, vaccines-several of which are in the advanced stages of clinical testing-may be added to the list of prophylactic measures.

## PART ONE: MAJOR ETIOLOGIC AGENTS OF ACUTE GASTROENTERITIS:

### *Vibrio cholerae*

Physiology & Structure:

A. The family *Vibrionaceae*

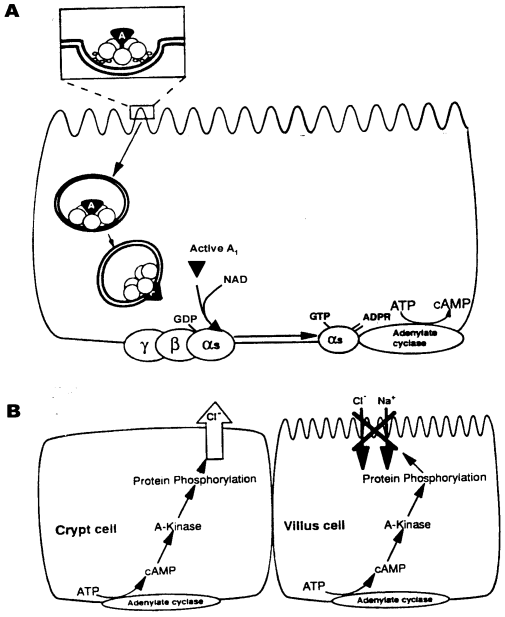
Motile, curved Gram-negative rods. Facultative anaerobes. Growth is stimulated by $Na^+$ but no exacting nutritional requirements. Common inhabitants of aquatic environment (both freshwater and marine)

B. *Vibrio cholerae* O1 & O139

Serological differentiation in this species is based upon the O (LPS) antigen, of which there are >200 varieties. The O1 and O139 serogroups are only ones associated with epidemics and the only ones that invariably produce cholera toxin (CT) and possess a pathogenicity island encoding intestinal colonization factors. (Until the emergence of O139 in 1992, O1 was the only serogroup responsible for epidemic cholera.) *V. cholerae* O1 can be further subdivided into two biotypes, classical and El Tor on the basis of a few biochemical tests. Classical gives more severe disease but is now very rare; El Tor is predominant biotype.

Pathogenesis & Immunity: The cholera vibrio colonizes the proximal small bowel where it secretes cholera toxin (CT) which is responsible for the massive diarrhea; not an invasive disease. Purified cholera toxin is sufficient severe diarrhea in volunteers; bacterium serves as a delivery system.

Cholera enterotoxin (CT). A-B subunit toxin; 5 B to 1 A subunit ratio. The B subunit is responsible for binding holotoxin to $GM_1$ ganglioside receptors on enterocyte; phage-encoded. A subunit enters cell where it separates into $A_1$ and $A_2$ peptides. The $A_1$ then affects a GTP binding protein, ($Gs\alpha$), which regulates adenylate cyclase (AC). $A_1$ transfers ADP-ribosyl moiety from NAD to $Gs\alpha$ causing AC to be permanently turned on. Resulting increase in cAMP alters $Na^+$ and $Cl^-$ transport (blocks $Na^+$ absorption and causes $Cl^-$ efflux through CFTR), which leads to large osmotic driving force for water secretion.




Epidemiology

A. Cholera is the only diarrheal disease capable of pandemic spread. A classic discovery in infectious disease epidemiology was the description by John Snow in 1854 of waterborne nature of disease in London with Broad Street pump. See John Snow web site: http://www.ph.ucla.edu/epi/snow.html

B. Since early 1800s, cholera has spread across the world in pandemics. We're currently in 7th pandemic, which is predominantly El Tor biotype, started in 1961. Reached South America in 1991; in 1990s, cholera reached widest spread of any time in 20th century. Major epidemic in Haiti in 2010.

C. Transmission is fecal-oral route via contaminated food or water. Environmental reservoir thought to be aquatic sources such as brackish water and estuaries, often in association with copepods or other zooplankton, shellfish, and aquatic plants. Short-term excretion following mild or asymptomatic infection (convalescent carrier) is responsible for most cases. Extraintestinal reservoir in estuarine environment, e.g. high nutrient, brackish water and shellfish

D. Host susceptibility. A large inoculum is required for infection, $10^8$-$10^{10}$. Stomach acidity is an important barrier and inocula can be decreased with food or buffers. Hypochlorhydria (due to surgery, antacids, infection with *Helicobacter pylori* which raises stomach pH) predisposes to infection. Immune status is important; high incidence in young children in endemic areas. All ages affected in non-endemic areas

E. Cholera in the U.S. is both endemic and imported. There's an endemic focus in the U.S. Gulf Coast due to a genetically unique strain with occasional small outbreaks associated with seafood consumption. Nearly all U.S. cases are imported.

Clinical Disease (cholera):

A. Wide spectrum of diarrheal illness, from asymptomatic to mild, moderate or severe (cholera gravis). Severe cholera is one of the most rapidly fatal illnesses known. Onset can be so rapid that a previously healthy person can become hypotensive within one hour of onset of symptoms and a victim may die within 2-3 hours without treatment. More commonly, time from first liquid stool to shock is 4-12 hours with death following in 18 hours to several days.

B. Cholera is characterized by voluminous watery diarrhea without abdominal cramps or fever. In severe purges, the stool loses its color and odor and flecks of floating mucus gave rise to term "rice water stool". Fluid loss may exceed one liter/hour. Major symptoms of cholera are due to the depletion of water and salts from the intravascular and extracellular spaces of the body by loss into the gut lumen. Death can result from the dehydration and electrolyte loss leading to shock.

Treatment, Prevention & Control:

A. Treatment is very simple: replace lost water and salts (rehydration), usually by oral route (oral rehydration solution, ORS) but i.v. therapy in severe cases. The untreated case fatality rate is 50 - 60% but with prompt treatment, 1 - 2%.
B. Antibiotics (e.g., tetracycline, doxycycline; trimethoprim-sulfamethoxazole for children) can shorten duration of diarrhea but are not essential.
C. Stop transmission via adequate sanitation (clean water/chlorination), adequately cooked seafood.
D. Vaccination. No cholera vaccines available in the US. Newer oral vaccines are now available in many countries.

***V. parahaemolyticus***

A. The leading cause of seafood-borne bacterial gastroenteritis. Cause of several outbreaks in coastal states. A recent history of seafood consumption should make you think of *V. parahaemolyticus*.

B. Gastroenteritis is mostly watery diarrhea with cramps, nausea, vomiting, sometimes fever and chills, bloody diarrhea (rare). Diarrhea is self-limited, around 3 days. Rarely needs treatment (ORS and tetracycline). Also causes wound infections (about 1/3 of sporadic infections) after exposure to warm seawater (e.g., crab bites)

***V. vulnificus***

A. Epidemiology. The most common and important cause of serious illness associated with *Vibrio* species in U.S., responsible for 95% of seafood-related deaths. This organism is a common inhabitant of coastal waters and shellfish but not all strains are pathogenic.

B. Two distinct clinical syndromes with different portals of entry
1. Wound infection - Can affect otherwise healthy people. Characterized by cellulitis, sometimes with vesicles or bullae followed by necrosis; sometimes progresses to sepsis and death. Occurs after exposure of wound to seawater during warm months. Classic presentation is that of a patient who has lacerated his hand while cleaning seafood. More severe infections in patients with some type of chronic underlying disease (e.g. diabetes, cirrhosis, leukemia, carcinoma or asthma requiring use of steroids).

2. Primary sepsis – seen in compromised hosts with pre-existing hepatic or other chronic diseases Infection results from eating contaminated seafood. Characterized by chills, fever, prostration, and hypotension; usually secondary skin lesions on the extremities with erythematous or ecchymotic areas - vesicles or bullae - necrotic ulcers.

Prevention - Because of high mortality, patients at risk (cirrhosis and hemochromatosis) should avoid raw shellfish and those at risk with wounds should avoid seawater.

## Diarrheagenic *E. coli*

*E. coli* are members of the Family *Enterobacteraceae*. *E. coli* and other members of this family are normal colonizer of the colon. Many of the opportunistic pathogens from the gut are members of the *Enterobacteraceae* as these may have a competitive advantage when there is inflammation. *Enterobacteraceae* capable of causing invasive infection (outside of GI tract) include Klebsiella, Serratia, Citrobacter, Enterobacter, Proteus and Salmonella species (see below). While most E. coli are not pathogenic, some highly adapted clones have developed with specific virulence properties that are capable of causing disease in healthly individuals. These strains may cause enteric/diarrheal disease, urinary tract infections (local spread from GI to GU tract) and sepsis/meningitis (from bacteremia). There are at least six well defined types that are involved in causing gastrointestinal illnesses:

Physiology and Structure:

A. Gram strain appearance- negative rods. Facultative anaerobe.
B. Key tests for identification include oxidase test (negative), most isolates appear as lactose-fermenters on MacConkey agar (pink colonies). Except for *E. coli* O157: H7, types cannot be differentiated based on phenotype in the laboratory. *E. coli* O157:H7 can be detected on MacConkey-Sorbitol agar.

Pathogenesis and Immunity: There are several distinct syndromes based on differences in the expression of virulence factors.

A. Enterotoxigenic *E. coli* (ETEC): also referred to as toxigenic *E. coli* and are the most common cause of travelers' diarrhea. Produce a cholera-like enterotoxin. ETEC adhere to small bowel enterocytes and induce watery diarrhea by the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins.

B. Enteropathogenic *E. coli* (EPEC): EPEC adhere to small bowel enterocytes, but destroy the normal microvillar architecture, inducing the characteristic attaching and effacing lesion. Cytoskeletal derangements are accompanied by an inflammatory response and diarrhea. 1. Initial adhesion, 2. Protein translocation by type III secretion, 3. Pedestal formation.

C. Enterohemorrhagic *E. coli* (EHEC): EHEC also induce the attaching and effacing lesion, but in the colon. The distinguishing feature of EHEC is the elaboration of protein synthesis-inhibiting Shiga toxin (Stx), systemic absorption of which leads to potentially life-threatening complications following an episode of bloody diarrhea (enterohemorrhagic colitis). Hemolytic-uremic syndrome (HUS), is a disease characterized by hemolytic anemia (anemia caused by destruction of red blood cells),

acute kidney failure (uremia), and a low platelet count (thrombocytopenia). *E. coli* O157 is one the most common serotypes that causes HUS.

D. Enteroaggregative *E. coli* (EAEC) : EAEC adheres to small and large bowel epithelia in a thick biofilm and elaborates secretory enterotoxins and cytotoxins.

E. Enteroinvasive *E. coli* (EIEC) : EIEC invades the colonic epithelial cell, lyses the phagosome and moves through the cell by nucleating actin microfilaments. The bacteria might move laterally through the epithelium by direct cell-to-cell spread or might exit and re-enter the baso-lateral plasma membrane. (Similar to pathogenic mechanisms in Shigella).

Epidemiology:
ETEC- Contaminated water and food. Major cause of childhood diarrhea in developing countries. Leading cause of travelers' diarrhea
EPEC- Person to person transmission. Leading cause of infantile diarrhea in developing countries
EHEC- Food, water and person-to-person transmission. Major cause of bloody diarrhea in developed nations. In 2007, US rate reported as 1.2/100,000 population (range 0.39 to 3.19)
EAEC- Mode of transmission unknown. Important cause of chronic diarrhea in developing countries; emerging cause of acute, chronic and travelers' diarrhea.
EIEC- Contaminated food. Outbreaks in developed countries.

Clinical Disease:
ETEC- acute watery diarrhea
EPEC- severe acute diarrhea or dysentery (severe diarrhea with blood and pus) and vomiting, may be persistent
EHEC- watery and bloody diarrhea, may be complicated by hemolytic uremic syndrome
EAEC- mucoid diarrhea, often persistent
EIEC- watery diarrhea or dystentery

Laboratory Diagnosis:
EHEC strains can be routinely detected using culture and immunologic assays in the clinical microbiology laboratory. Stool samples can be cultured on selective and differential media (MacConkey-sorbitol) for detecting O157:H7 serotypes. For other stx producing strains, toxin can be detected directly in stool samples using immunoassays.
Other types of *E. coli* are difficult to diagnose in the clinical microbiology laboratory because there are no commercial diagnostic tests for use. Most diagnostic procedures have been performed in research settings using molecular amplification techniques, or using tissue culture assays.

## ***Shigella spps***.

Physiology and Structure: Facultative anaerobe, Gram-negative, appears rod-shaped on Gram stain. Member of the *Enterobacteraceae*. Key tests for identification include oxidase-negative, non-motile, non-lactose fermentor (on MacConkey agar). There are four recognized species of *Shigella* that cause gastrointestinal infection: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. From a genetics point of view, *Shigella* and *E. coli* are the same organism but continue to be differentiated for epidemiologic purposes, recognition of clinical disease, and potential treatment differences.

Pathogensis and Immunity: Steps of *Shigella* spp. involve translocation by the bacterium of the intestinal epithelium and development of the infectious process leading to bacillary dysentery. *Shigella* spp. cross M (microfold) cells of the follicle-associated epithelium that covers the lymphoid nodules associated with the colonic mucosal tissues. In this subepithelial location, *Shigella* spp. cause extensive apoptosis of macrophages. This process allows escape of bacteria into the tissues and efficient basolateral entry to epithelial cells, followed by cell-to-cell spreading, which generates efficient intracellular colonization. Caspase-1-mediated apoptosis can also initiate inflammation through the release of mature interleukin-1β (IL-1β). The inflammatory mechanism is considerably amplified by the presence of intracellular bacteria that activate the NOD1 (nucleotide-binding oligomerization domain protein 1) pathway through the release of peptidoglycan (PGN), which induces NF-κB activation and chemokine expression (CXCL8, CXC-chemokine ligand 8). Affects primarily the distal colon; acute mucosal inflammation and erosion and purulent exudate. Segment of colon show pale, granular, inflamed mucosa with patches of coagulated exudate from a massive recruitment of PMNs inducing rupture of the epithelial barrier. Although locally invasive, Shigella does not cause bacteremia or disseminate.

Epidemiology: Strictly a human pathogen (no animal reservoir) from fecal-oral transmission. Third most common bacterial enteropathogen reported in the United States. Reported 2007 rate of 6.26/ 100,000 population (range 0.89 to 6.26). Most cases of shigellosis in the United States are caused by *S. sonnei* (>75 percent), with *S. flexneri* the next most frequent isolate. *S. dysenteriae* was the most common isolate both in Europe and the United States at the beginning of this century but is now rare. In the United States, *S. dysenteriae* infection is generally limited to imported cases from Mexico and Central America or from laboratory contamination. Shigellosis in the United States is most common in day care centers and areas with crowded living conditions such as urban centers or residential institutions from person to person spread.

Clinical Disease: Shigellosis has one of the more severe presentations of the common enteric pathogens. A locally invasive organism that causes an "inflammatory" type diarrhea. Shigella is a pathogen that primarily infects the lower intestinal tract. Patients with Shigella gastroenteritis typically present with high fever, abdominal cramps, and bloody, mucoid diarrhea (dysentery). Prominent signs and symptoms include abdominal pain, mucoid or bloody diarrhea, fever and vomiting. The incubation period is usually 1-3 days after exposure. Disease outside of the GI tract is unusual.

Laboratory Diagnosis: Culture is the method of choice for diagnosis. Differential and selective media are used to isolate *Shigella* spp. from stool samples. Special transport media (Cary – Blair) is used to collect the stool sample and send to the clinical microbiology laboratory for analysis.

Treatment, Prevention & Control: No vaccine. Most episodes are self-limited but severe disease (dysentery) or disease in compromised patients will benefit from antibiotic treatment. Ampicillin and trimethoprim-sulfamethoxaole resistance now common. Fluoroquinolone resistance remains rare in the US but increasing in other parts of the world.

## *Salmonella* spps.

Physiology and Structure: facultative anaerobe, Gram-negative, appear rod-shaped on Gram stain. Member of the *Enterobacteraceae*. Key tests for identification include oxidase-negative, motile, non-lactose fermenter (on MacConkey agar). There are two recognized species of *Salmonella*, *S. enterica* and *S. bongori*. Six subspecies within *S. enterica* (I through IV). *S. enterica subsp. enterica* (Subspecies I) contains most of the important human strains. For

epidemiologic purposes, a serotyping system is used to help track *Salmonella* infections and there are >2500 serotypes based on O (LPS) and H (flagellar) antigens.

Pathogenesis and Immunity: Strategies that allow *Salmonella* spp. to cross the intestinal barrier, survive in intestinal tissues and spread systemically. *Salmonella* spp. cross M (microfold) cells of the follicle-associated epithelium mainly in the Peyer's patches of the ileal portion of the small intestine but possibly also in the colon. In this subepithelial location, *Salmonella* spp. might cause macrophage apoptosis through effectors injected using a type III secretory system that is encoded by Spi1 (*Salmonella* pathogenicity island 1), thereby also triggering inflammation. *Salmonella* spp. also switch to expression of Spi2, which encodes a type III secretory system that allows injection of effector proteins from the endocytic vacuole into the cell cytoplasm, thereby enabling bacteria to modify the vacuole to a Salmonella-containing vacuole, which supports bacterial survival and multiplication. This provides bacteria with the capacity to both invade epithelial cells basolaterally, owing to expression of Spi1 effectors, and to disseminate systemically.

Epidemiology: The principal reservoirs for nontyphoidal Salmonella organisms include birds, mammals, reptiles, and amphibians (think about pets!). One of the most common agents of bacterial gastroenteritis in the U.S from fecal-oral transmission and contaminated food.. The major food vehicles of transmission are of animals origin, such as poultry, eggs and milk products. Depending on the reporting region, may be the #1 or #2 reported bacterial agent (*Campylobacter* is the other one). Reported rate of 14.92/100,000 in 2007 (range 8.65 to 15.33). In contrast, the typhoidal Salmonella, *S.* Typhi or *S.* Paratyphi, primarily colonize humans, are transmitted via the consumption of fecally contaminated food or water, and cause a systemic illness (typhoid or 'enteric' fever) usually with little or no diarrhea.

Clinical Disease: Salmonella cause a variety of diseases including gastroenteritis, enteric fever, (systemic illness with fever and abdominal symptoms), bacteremia and endovascular infection, focal metastatic infections such as osteomyelitis or abscess, and an asymptomatic chronic carrier state involving the gall bladder.

A. Typhoid fever, which is highly endemic in resource poor countries, usually presents nonspecifically with abdominal pain, fever, chills, and constitutional symptoms; as a result, many other diagnoses may be entertained. During the acute phase of illness, there is fever and bacteremia. This is followed by abdominal pain and rash (rose spots, which are faint salmon colored macules on the trunk and abdomen). Subsequently, there may be hepatosplenomegaly, intestinal bleeding and perforation, related to ileocecal lymphatic hyperplasia of the Peyer's patches, may occur with secondary bacteremia and peritonitis.

B. The much broader group of nontyphoidal Salmonella usually results from improperly handled food that has been contaminated by animal or human fecal material. It can also be acquired via the fecal-oral route, either from other humans or farm or pet animals. Gastroenteritis due to Salmonellae is clinically indistinguishable from gastroenteritis caused by many other pathogens. Furthermore, enteric infection with nontyphoidal Salmonellae may be clinically mild or even asymptomatic, which can complicate clinical decisions about treatment interventions. Salmonella gastroenteritis is characterized by nausea, vomiting, fever, diarrhea (which may be bloody), and cramping, usually occur within 1 to 3 days of ingesting contaminated food or water. A higher ingested dose of bacteria correlates with increases in the severity of diarrhea, the duration of illness, and weight loss. Nontyphoidal Salmonella gastroenteritis is usually self-limited. Fever generally resolves within 48 to 72 hours, and diarrhea within 4 to 10 days, but shedding may lasts for weeks or months especially in children. Immunocompromised individuals and those with certain underlying conditions are more likely to have bacteremic

infection and complications. For example, salmonella causes osteomyelitis in patients with sickle cell disease due to seeding of infarcted bone.

Laboratory Diagnosis: Culture is the method of choice for diagnosis. Differential and selective media are used to isolate *Shigella* spp. from stool samples. Special transport media (Cary – Blair) is used to collect the stool sample and send to the clinical microbiology laboratory for analysis. For practical purposes, laboratories report results as *Salmonella* followed by the serotype name, for example, *Salmonella* serotype Typhi (*Salmonella* Typhi) or *Salmonella* serotype Enteritidis (*Salmonella* Enteritidis). Enteric fever may be difficult to diagnose because the organism is often absent from the stool when the diagnosis is considered.

Treatment, Prevention & Control:
Antimicrobial treatment is not usually indicated for nontyphoidal Salmonella except in severe disease (high fever, severe diarrhea, immunosuppressed individuals) and may prolong shedding. Enteric fever is always treated with antibiotics but multi-drug resistance is increasing in many parts of the world. There are two licensed typhoid vaccines (live oral attenuated and polysaccharide) that offer incomplete protection and should be considered for travelers to endemic regions (see cdc.gov/travel for specific recommendations).

## *Campylobacter jejuni*

Physiology and Structure: Gram-negative, characteristic curved gull or S-shaped rods, Requires special growth conditions including microaerobic atmosphere (5% O2, 10% CO2, 85% N2) and 42$^{o}$C environment. Oxidase positive, catalase positive. Differentiated from other species by ability to hydrolyze sodium hippurate.

Pathogenesis and Immunity: The essential lesion in *Campylobacter* enteritis is an acute inflammatory enteritis, which commonly extends down the intestine to affect the colon and rectum. Histology shows acute inflammation of the mucosa with edema, infiltration by polymorphonuclear leukocytes, and crypt abscess formation. For infection to become established, campylobacters must first survive gastric acidity to colonize the jejunum and ileum. Lowering of gastric acidity, therefore, facilitates infection, which is well-established in relation to *Salmonella* infections. Abdominal pain in *Campylobacter* enteritis may be intense, continuous, and radiate to the right iliac fossa (`pseudo-appendicitis') and the pain is caused by terminal ileitis and mesenteric adenitis. The changes seen in *Campylobacter* enteritis are indistinguishable from those of other acute bacterial infections of the gut, such as those caused by *Salmonella* and *Shigella* infection.

Epidemiology: One of the most common reported causes of bacterial diarrhea in the U.S. Depending on the reporting region, may be the #1 or #2 agent reported (Salmonella is the other one). Reported rate of 12.79/100,000 in 2007 (range 7.39 to 28.21). GI tract of fowl is an important reservoir and improperly cooked poultry is a main vehicle of transmission.

Clinical Disease: Incubation period of usually 1-3 days, followed by fever, abdominal cramping which may be severe, diarrhea (watery or bloody) lasting several days to one week. Like the other common enteric pathogens, illness is self-limited and usually does not require antimicrobial therapy. 10-20% have a relapse or prolonged disease that mimics Inflammatory bowel disease. One of the rare complications following campylobacter linked directly to the infection is Guillain-Barre syndrome (GBS), an acute, self-limited, immune mediated attack on the peripheral nervous system resulting in ascending motor paralysis. GBS following campylobacter infection is thought to occur because *Campylobacter jejuni* produces a ganglioside-like structure in its

outer core of the lipooligosaccharide and the immune response to these ganglioside mimics cross-reacts with relevant ganglioside targets in peripheral nerve tissue. This immune reaction leads to demyelination or axonal degeneration of the peripheral nerves.

Laboratory Diagnosis: Stool culture using selective media designed specifically for isolation of Campylobacter species. Gram-stain of the stool sample in acute disease may be helpful in rapid diagnosis.

### *Clostridium difficile*:

Physiology and Structure: Anaerobe, gram-positive spore forming large rods. Not readily detected by cultured. Common inhabitant of the gut but normally present in low numbers.

Pathogenesis and Immunity: *C. difficile* disease usually follows after patients have been treated with any one of a number of broad spectrum antibiotics, which disrupt the intestinal flora and allows the organism (in colonized patients) to increase and express its toxins and other potential virulence determinants leading to disease. *C. difficile* produces two major toxins TcdA (toxin A) and TcdB (toxin B), which target the Ras superfamily of small GTPases for modification via glycosylation. Both are glucosyltransferases that inactivate Rho, Rac and Cdc42 in target cells. Both enterotoxins enter the cell through receptor-mediated endocytosis and require an acidified endosome for translocation. The receptor for TcdA include the disaccharide Galß1-4GlcNac, found on I, X and Y blood antigens present in a variety of cells. The receptor for TcdB is not known. Both enterotoxins cause cytopathic effects, with TcdB in general having more potent effects on cells. TcdA is capable of increasing the permeability of colonic epithelial layers and may effect the expression of chemokines in human intestinal epithelial cells. Both toxins are able to disrupt tight junctions of epithelial barriers, and enhance migration of neutrophils into the intestines. While TcdA has been considered the primary factor in CDAD, there is evidence that TcdB may also act as a potent enterotoxin in the absence of TcdA. The occurrence of TcdB+ TcdA- strains causing clinical disease supports a role for TcdB in the pathogenesis of disease.

Epidemiology: One of the most common causes of antibiotic associated diarrhea in healthcare settings and is being more recognized as a community acquired infection. It is estimated that 3 to 29% of patients in the hospital who receive antimicrobial agents go on to develop CDAD. Of particular concern is the recent circulation of an epidemic strain B1-NAP1 in U.S. healthcare facilities (toxin type III that is positive for binary toxin). Patients infected with this strain may have more severe disease compared with non-B1/NAP1 strains. This may be because strains producing binary toxin may produce TcdA and TcdB at higher quantities than non B1-NAP1 strains. Patients with nosocomially acquired *C. difficile* disease incur significantly higher hospital costs and length of stay and has been estimated to exceed 1 billion dollars in the U.S. Patient related factors included age (increased risk in elderly), surgery, chemotherapy, laxatives, defects in humoral immunity. Bacterial related risk factors (eg emergence of highly virulent strains such as the Nap1 strain).

Clinical Disease: *C. difficile* causes a spectrum of illness from asymptomatic carriage to fulminant, relapsing and fatal colitis. Endoscopic evidence of pseudomembrane formation in the colon indicates severe disease. Onset of symptoms may be a few days to months following antimicrobial therapy. Diarrhea may be mild or severe. Other findings include fever, abdominal cramping, leukocytosis. Toxic megacolon is a severe, life threatening complication from CDAD. A significant proportion of patients treated with oral vancomycin experience relapsing disease.

The relapse rate of *C. difficile* disease among treated patients has increased in recent years and is associated with the emergence of the Nap1 strain of *C. difficile*.

Laboratory Diagnosis: TcdA is usually detected in stool samples using an immunoassay (eg ELISA assay) and TcdB and be detected using an immunoassay or bioassay (ie toxin neutralization in cell culture system). However, these assays detect only 70-85% of cases and multiple stool samples may be required to increase detection. Another process in common use including at HUP is a two-step procedure. The first step is a sensitive ELISA to detect glutamate dehydrogenase in the stool- a byproduct of Clostridial growth, along with other bacteria. If this is positive, then a PCR is performed on the stool to detect the genes *tcdB, tcdA.*

Treatment, Prevention & Control:
CDAD is usually treated by discontinuing treatment with offending antimicrobial agents and in seriously ill patients, oral therapy with vancomycin or metronidazole is instituted. There are a number of alternative strategies for treating CDAD. Treatment with probiotics (eg. *Lactobacillus* spp., *Saccharomyces boulardii*) shows conflicting results. Others include fecal implants, anion-exchange resins absorption of toxin, and pulse-dosing with vancomycin.

## PART TWO: ETIOLOGIC AGENTS OF THE GUT NOT CAUSING ACUTE GASTROENTERITIS:

### Anaerobes (ex. *Bacteroides fragilis*)

General Overview:
Major Sites for Anaerobes (including non-spore formers):
-Oral Cavity ($10^9$ ml in gingival crevices)
-GI tract (in the colon there are 1000 anaerobes per aerobe)
-Female genital tract
Pathogenesis of anaerobic infection:
Infection generally results from disruption of mucosal surface followed by infiltration of resident flora into a sterile site. Strict anaerobes will thrive only at sites that are poorly perfused and not exposed to air. There are many species of anaerobes. Thus, most anaerobic infection is polymicrobial in nature. Varied gut microbes consisting of facultative organisms and anaerobes may synergize to be able to cause infection.
Anaerobes Characteristics

- Definition-organisms that fail to grow in air including microaerphilic conditions (10% $CO_2$)
- Foul odor, gas production
- Organisms vary in tolerance for exposure to air depending on levels of superoxide dismutase, which removes toxic superoxide, and catalase, which removes hydrogen peroxide.
- Pathogenic anaerobes tend to be more aerotolerant (example *Clostridium perfringens* - a cause of gas-gangrene in wound infections where there is devitalized tissue)

*B. fragilis* is a common inhabitant of the human gut as well as the most common of the many anaerobes isolated from clinical infections.
Physiology & Structure: *Bacteroides fragilis* -Characteristics:
Gram-negative rod, grows in presence of bile-esculin

Pathogenesis & Immunity: Breaching of the gut wall allows the escape of microbes. When these get into the peritoneal cavity-peritonitis, a generalized inflammation, results. Early in this process the enterobactereacae may predominate. Often these infectious processes are contained and form discrete areas of infection with poor perfusion/oxygenation where anaerobes take over. An example is rupture of the appendix. Abscess formation results from an acute inflammatory response with a neutrophil influx, central necrosis and walling off of the infection (a thick fibrin/collegen containing 'capsule'). The ability of *B. fragilis* to induce abscess formation is thought to be due to its capsular polysaccharide, which resists phagocytosis. In addition, there is a CD4+ T cell reaction ($Th_{17}$) to its capsular polysaccharide that may contributes to abscess formation. Its LPS (endotoxin), in contrast, induces minimal inflammation (1000-fold less active than LPS from *E. coli*).

Clinical Diseases: Infection typically occurs as an abscess in normally sterile spaces where there has been exposure to the contents of the gut lumen.

Laboratory Diagnosis: Successful culture often requires maintaining oxygen-free environment with special transport medium and handling procedures in the clinical microbiology lab. For this reason anaerobes are underdiagnosed and underappreciated as causative agents of infection

Treatment, Prevention & Control: Surgical debridement/drainage of necrotic tissue and antibiotics are the mainstays of therapy. Penicillin has good coverage of anaerobes, although *B. fragilis* now typically resistant due to beta-lactamase expression. Metronidazole (Flagyl) has excellent activity against anaerobes and is the treatment of choice for *B. fragilis*. Therapy should consider polymicrobial etiology of many infections.

### *Helicobacter pylori*

First isolated in 1984 by Barry J. Marshall and J. Robin Warren (Nobel Prize in Medicine 2005) Specialized to colonize stomach and duodenum. Resides in thick mucus lining of stomach – unusual niche provides partial protection from acid, competitors and the host immune responses.

Physiology & Structure: Gram negative curved or spiral rod. Highly motile (flagella). Urease positive (see below)

Pathogenesis & Immunity: Specific adaptations allow for survival in the acid-rich environment (pH 1-2) of the stomach lining. High urease activity – produces bases (ammonia) from urea to neutralize acid in the local environment. Strains expressing Cytotoxin associated antigen (CagA) (60-70%) more likely to cause disease. CagA is secreted into host cells by a type IV secretion apparatus. It inhibits clearance by altering host cell signaling through its tyrosine phosphorylation activity. Induction of chemokines like IL-8 attract neutrophils and result in chronic inflammation, elevated gastrin secretion and increased gastric acid levels. The gene vacA encodes the vacuolating cytotoxin, VacA, a secreted protein toxin that is responsible for the gastric epithelial erosion observed in infected hosts. *H. pylori* strains that express vacuolating activity are more common among patients with peptic ulcer disease and distal gastric cancer than among infected patients with superficial gastritis alone. Chronic superficial gastritis may leads to long-term complications of *H. pylori* infection including peptic ulcer disease (ulcerations), atrophic gastritis (loss of epithelial glands) and gastric adenocarcinoma.

Epidemiology: Person to person transmission especially within families. Oral-fecal transmission most likely. Disease usually requires years of infection. Infection rate increases with age. Most

infected individuals asymptomatic (1/3 of world's population infected). Approximately 20% of infected persons develop ulceration and 1% neoplasia. Primary reservoir is in human. Probably most common human chronic infection. Infections correlate to socioeconomic status (higher rates in developing countries, >80% by age 20), and age (% prevalance approximates age in developed countries). Risk decreases with improved hygiene. Infection persists for life unless treated.

Clinical Diseases: Strong association of *H. pylori* infection with complications seen in upper GI tract (gastritis, peptic ulcers, gastric adenocarcinoma)
Gastritis – Strong association with *H. pylori* association
Gastric ulcers-less common
Duodenal ulcers-common, thought to result from hypersecretion of acid in early gastritis
Increased risk of:
gastric adenocarcinoma,
gastric MALT B-cell lymphoma (rare)
It is the host responses to infection (inflammation of infection sites, hypersecretion of acid) that leads to symptoms/complications?

Laboratory Diagnosis: Microscopy of biopsy (from endoscopy) with special stains of tissue. Urease biochemical test (Urease – breath test with $^{14}C$ or $^{13}C$ urea, followed by detection of labeled $CO_2$). Detection of host antibodies (excellent for screening, not good for determining outcome of treatment).

Treatment, Prevention & Control: Effectiveness highly dependent on compliance. Debate on treatment if there is presence of *H. pylori* but no symptoms. Eradication of *H. pylori* heals ulcers and decreases relapse; also leads to regression of MALT lymphoma. Monotherapy ineffective (leads to resistance). Many drugs that might otherwise suffice are ineffective in the acid environment of the gastric lining. Combination therapy required to raise the gastric pH to render antibiotics more effective.
Example of an effective regimen:
Clarithromycin (macrolide) and amoxicillin plus proton pump inhibitor (omeprazole)
No vaccine. (The chronicity of infection suggests that the immune response to natural infection is not adequate for control.

## Enterococcal spps.

Physiology & Structure: Gram positive cocci in singles, pairs and chains (look like streptococci) - formerly classified among Group D streptococci. Facultative anaerobe, major habitat is the GI tract of humans and other animals because of its ability to grow in high concentrations of bile salts. Also found in environmental sources in hospitals and nursing homes.

Pathogenesis & Immunity: Gut colonization precedes disease in susceptible hosts
Host factors (i.e. debilitation) rather than virulence of the organism is the primary determinant of pathogenesis. Ability to exist as a biofilm important to catheter related infection and difficulty in eradication with antibiotics.

Epidemiology: Two major species *E. faecalis* and *E. faecium*. *E. faecalis* encountered more frequently but *E. faecium* accounts for more multi-resistant strains. Second most common cause of nosocomial infection. Spread within hospital settings rather than from 'endogenous' pre-hospitalization flora source of most strains causing nosocomial infections. Hospital personnel an important reservoir for infecting strains. May be isolated from their hands!

Clinical Diseases: The types of diseases that enterococci can produce include:

1. Urinary tract infection
2. Bacteremia/septicemia (esp. catheter associated)
3. Endocarditis
4. Intra-abdominal/pelvic infections (synergistic with *Bacteroides fragilis*?)

31-37% attributed mortality to enterococcal bacteremia. Ability to adhere to heart valves and renal epithelium accounts for ability to cause endocardits and UTI, respectively. Catheters a major contributing factor to above.

Laboratory Diagnosis: Readily grown in routine culture. α-or nonhemolytic colonies on blood agar.

Treatment, Prevention & Control: Multi-resistance makes this the most problematic issue for enterococcal infections. Resistance spreads because enterococci are common and are adept at conjugative transfer of plasmids and transposons. Vancomycin-resistant strains (VRE) tend to be resistant to other commonly used drugs. VRE acquired a mobile genetic element that allows the organism to detect the effect of glycopeptide antibiotics like vancomycin and express an altered stem peptide(D-ala-D-lac instead of D-ala-D-ala) that still allows for cross-linking but reduces the affinity for vancomycin (altered drug target) by 1000-fold. Increasing dependence on vancomycin and cephalsporins in ICUs has selected for a rapid increase in VRE in these settings. Cephalosporins are commonly used and select for enterococci because of intrinsic resistance. Control of these agents may curtail the problem of VRE. Ampicillin is the drug of choice for non-β-lactamase-producing strains. However, treatment dictated by testing of resistance patterns in the clinical microbiology laboratory. There is no vaccine. If there were who would receive it?