Shigella, Salmonella, and Campylobacter

The Shigellae

The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.

Morphology and Identification

Growth Characteristics

- All shigellae do not ferment lactose (except of Shigella sonnei)
- Shigellae form acid from carbohydrates but rarely produce gas.

Pathogenesis and Pathology

- *Shigella* infections are almost always limited to the gastrointestinal tract
- Shigellae infection are highly communicable
- The infective dose is on the order of $10^3$ organisms (it usually is $10^5$–$10^8$ for salmonellae and vibrios).
- The essential pathologic process is invasion of the mucosal epithelial cells (eg, M cells) by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells (actin polymerization). Microabscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a “pseudomembrane” on the ulcerated area. As the process subsides, granulation tissue fills the ulcers, and scar tissue forms.
Toxins
A. Endotoxin
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.
- In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine.
- Patients with Shigella flexneri or S sonnei infections develop antitoxin that neutralizes S dysenteriae exotoxin in vitro.
- Acting as an enterotoxin, it produces diarrhea as does the *E coli* Shiga-like toxin, perhaps by the same mechanism.
- Acting as a “neurotoxin,” this material may contribute to the extreme severity and fatal nature of *S dysenteriae* infections and to the CNS reactions (ie, meningismus, coma).
- the invasion of the large intestine, resulting in later dysentery with blood and pus in stools. The toxic activity is distinct from the invasive property of shigellae in dysentery.
After a short incubation period (1–2 days), there is a sudden onset of **abdominal pain, fever, and bloody-watery diarrhea**. The diarrhea has been attributed to an exotoxin acting in the small intestine.

**Mechanism of Action of shiga toxin**

- The toxin acts on the lining of the blood vessels, the vascular endothelium.
- The B subunits of the toxin bind to a component of the cell membrane known as Gb3 and the complex enters the cell.
- When the protein is inside the cell, the A subunit interacts with the ribosomes to inactivate them. The A subunit of Shiga toxin reacts with the rRNA component of the ribosome to inactivate it and so stop protein synthesis leading to the death of the cell.
- The vascular endothelium has to continually renew itself, so this killing of cells leads to a breakdown of the lining and to hemorrhage. The first response is commonly a bloody diarrhea.
- The toxin is effective against small blood vessels, such as found in the digestive tract, the kidney, but not against large vessels.

**Diagnosis:**

**Specimens**
- Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.
- Serum specimens, if desired, must be taken 10 days apart to demonstrate an increase in the titer of agglutinating antibodies.

**Culture**
- The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or Salmonella–Shigella agar), which suppress other Enterobacteriaceae and gram-positive
organisms. Colorless (lactose-negative) colonies are inoculated into TSI agar.

- Nonmotile colonies should be subjected to slide agglutination by specific Shigella antisera.

**Serology**

- Normal persons often have agglutinins against several Shigella species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is not used to diagnose Shigella infections.

**Immunity**

- Infection is followed by a type-specific antibody response. Injection of killed shigellae stimulates production of antibodies in serum.
- IgA antibodies in the gut may be important in limiting reinfection; these may be stimulated by live attenuated strains given orally as experimental vaccines.

**THE SALMONELLA**

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause enteritis, systemic infection, and enteric fever.

**Morphology and Identification**

- Most isolates are motile, never ferment lactose or sucrose, Form acid and sometimes gas, produce H2S.
- Survive freezing in water for long periods.
**Pathogenesis and Clinical Findings**
- Animal reservoir for human infection is the poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others.
- The organisms almost always enter via the oral route, usually with contaminated food or drink.
- The mean infective dose to produce clinical or subclinical infection in humans is $10^5$–$10^8$ salmonellae (but perhaps as few as $10^3$ S. typhi organisms).

**Pathogenicity of Salmonella**
A. The “Enteric Fevers” (Typhoid Fever)
B. Bacteremia with Focal Lesions. Intestinal manifestations are often absent.
C. Enterocolitis
   - This is the most common manifestation of salmonella infection.
   - In the United States, S.Typhimurium and S. Enteritidis are prominent.
   - Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days.
   - Inflammatory lesions of the small and large intestine are present.
   - Bacteremia is rare (2–4%) except in immunodeficient persons.
   - Blood culture results are usually negative, but stool culture results are positive for salmonellae and may remain positive for several weeks after clinical recovery.
   - Once in the intestines, the bacteria invade the cells lining the intestine. The bacteria's virulence factors go to work. An enterotoxin results in the release of fluids from the cell into the lumen. This factor is responsible for the diarrhea and vomiting symptoms. Next, the endotoxin results in the release of endogenous pyrogens from the host cell, causing a fever in the victim. Lastly, the cytotoxin is responsible for the disintegration of the cytoplasm. After the virulence factors have done their duty, the bacteria can move to the liver or spleen, where they are able to replicate.

**Diagnostic Laboratory Tests**
**Bacteriological Methods for Isolation of Salmonellae**
1. Differential medium cultures
2. Selective medium cultures
3. Enrichment cultures
4. Final identification
Epidemiology

- The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are “shedding” organisms.
- Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs.
- The high incidence of salmonellae in commercially prepared chickens has been widely reported.
- The problem probably is complicated by the widespread use of animal feeds containing antimicrobial drugs that favor the proliferation of drug-resistant salmonellae and their potential transmission to humans.

Campylobacter

- Campylobacters cause both diarrheal and systemic diseases.
- Campylobacter infection of domesticated animals also is widespread.
- *Campylobacter jejuni* is a common cause of diarrhea in humans.
Campylobacter jejuni
Morphology and Identification
A. Typical Organisms
*C. jejuni* and the other campylobacters are gram-negative rods with comma, S, or “gull wing” shapes. They are motile, with a single polar flagellum.

B. Culture
- **Skirrow’s medium** contains vancomycin, polymyxin B, and trimethoprim to inhibit growth of other bacteria, O2 (5% O2) with added CO2 (10% CO2).
- Incubation of primary plates for isolation of *C. jejuni* should be at 42°C, thus simplifying the identification of *C. jejuni*.

Growth Characteristics
- *C. jejuni* are positive for both oxidase and catalase.
- Nitrate reduction, hydrogen sulfide production, hippurate tests, can be used for further identification of species.

Antigenic Structure and Toxins
- The campylobacters have lipopolysaccharides with endotoxic activity. Cytopathic extracellular toxins and enterotoxins have been found, but the significance of the toxins in human disease is not well defined.

Pathogenesis and Pathology
- The infection is acquired by the oral route from food, drink, or contact with infected animals or animal products, especially poultry.
- *C. jejuni* is susceptible to gastric acid, and ingestion of about $10^4$ organisms is usually necessary to produce infection.
- The organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools.
- The sites of tissue injury include the jejunum, the ileum, and the colon. Most strains of C jejuni produce a toxin (cytolethal toxin) that hinders the cells from dividing.
- Occasionally, the bloodstream is invaded, and a clinical picture of enteric fever develops. Localized tissue invasion coupled with the toxic activity appears to be responsible for the enteritis.
- The most common routes of transmission are fecal-oral, ingestion of contaminated food or water, and the eating of raw meat. Foods implicated in
campylobacteriosis include raw or under-cooked poultry, raw dairy products.
- Campylobacter is sensitive to the stomach's normal production of hydrochloric acid: as a result, the infectious dose is relatively high, and the bacteria rarely cause illness when a person is exposed to less than $10^4$ organisms.
- Nevertheless, people taking antacid medication (e. g. people with gastritis or stomach ulcers) are at higher risk of contracting disease from a smaller amount of organisms, since this type of medication inhibits normal gastric acid.

**Clinical Findings**
- Clinical manifestations are acute onset of crampy abdominal pain, profuse diarrhea that may be bloody, headache, malaise, and fever.

**Diagnostic Laboratory Tests**

**A. Specimens**
Diarrheal stool is the usual specimen.

**B. Smears**
Gram-stained smears of stool may show the typical “gull wing”–shaped rods. May show the typical darting motility of the organisms.