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Infectious Diarrhea

A. Introduction

Acute gastrointestinal illnesses rank second only to acute respiratory illnesses as the most common disease worldwide. In children less than 5 years old, attack rates range from 2-3 illnesses per child per year in developed countries to 10-18 illnesses per child per year in developing countries. In Asia, Africa, and Latin America, acute diarrheal illnesses are a leading cause of morbidity (1 billion cases per year), and mortality (4-6 million deaths per year, or 12,600 deaths per day) in children.

Most cases of acute infectious gastroenteritis are caused by viruses (Rotavirus, Calicivirus, Adenovirus). Only 1-6% of stool cultures in patients with acute diarrhea are positive for bacterial pathogens; however, higher rates of detection have been described in certain settings, such as foodborne outbreaks (17%) and in patients with severe or bloody diarrhea (87%). This syllabus will focus on bacterial organisms, since viral and parasitic (Giardia, Entamoeba, Cryptosporidium, Isospora, Microspora, Cyclospora) causes of gastroenteritis are covered elsewhere. *Clostridium difficile* colitis will be covered in the lecture and syllabus on Anaerobes.

Diarrhea is an alteration in bowel movements characterized by an increase in the water content, volume, or frequency of stools. A decrease in consistency and an increase in frequency in bowel movements to ≥ 3 stools per day have often been used as a definition for epidemiological investigations. "Infectious diarrhea" is diarrhea due to an infectious etiology. "Acute diarrhea" is an episode of diarrhea of ≤ 14 days in duration. "Persistent diarrhea" is an episode of diarrhea > 14 days in duration, and "chronic diarrhea" is diarrhea that last for >30 days duration.

B. Microbiology

Many different bacteria can cause gastroenteritis. From studies of stool cultures performed in U.S. hospitals, the most commonly isolated bacterial pathogens are *Campylobacter* (42% of isolates), *Salmonella* (32%), *Shigella* (19%) and *Escherichia coli* O157:H7 (7%). Some organisms (*Salmonella* and *Shigella* species) are always associated with disease, while others (*E. coli*) are members of the commensal flora and become pathogenic when they acquire virulence factor genes on plasmids, bacteriophages, or pathogenicity islands.

B.1. Vibrio species

Vibrio are Gram negative bacilli that grow naturally in estuarine and marine environments worldwide, and can survive in contaminated waters with increased salinity and temperature (up to 37°C). There are 12 species of *Vibrio* that have been implicated in human infections, with the most prominent being *Vibrio cholerae, parahaemolyticus* and *vulnificus. V. cholerae,* the etiologic agent of cholera, is subclassified based upon somatic O antigens. *V. cholerae* O1 and O139 are responsible for causing classic cholera which

can occur in epidemics or worldwide pandemics. The seventh cholera pandemic which was caused by *Vibrio cholerae* O1 biotype El Tor, began in Asia in 1961 and spread around the globe to the Americas in 1991, resulting in more than 1 million cases and 10,000 deaths in the Americas, alone. Cholera is spread by contaminated water and food and therefore, usually occurs in communities with poor sanitation. Person-to-person spread is unusual because a high inoculum is required to produce disease. The clinical manifestation of cholera ranges from asymptomatic colonization to severe, rapidly fatal diarrhea that begins 2-3 days after ingestion of the bacilli. The fluid loss from cholera can be profound and result in severe dehydration, metabolic acidosis (bicarbonate loss), hypokalemia (low potassium), and hypovolemic shock. The mortality rate is 60% in untreated patients but less than 1% in those who are promptly treated with replacement of lost fluids and electrolytes. Noncholera vibrios such as *Vibrio parahaemolyticus* can be transmitted through contaminated shellfish and cause a mild to severe secretory diarrhea.

B.2. Shigella species

Shigella are Gram negative bacilli that have four recognized species: *S. sonnei, S. flexneri, S. dysenteriae, S. boydii*. *S. sonnei* enteritis occurs predominantly in industrialized countries while *S. flexneri* occurs in developing countries. *S dysenteriae* results in the most severe infections and *S. boydii* is infrequently isolated. Humans are the only known reservoirs for Shigella. Shigellosis is transmitted by the fecal-oral route, primarily by people with contaminated hands and less commonly through contaminated water or food. Because only a small inoculum is necessary to establish disease (<200 bacilli), shigellosis spreads rapidly in institutions (daycare and custodial institutions) or communities where there are poor sanitary standards. Shigella invades and replicates in the cells lining the colonic mucosa resulting in symptoms ranging from a mild gastroenteritis to dysentery (abdominal pain, small and frequent bowel movements, stool with blood or mucous). *S. dysenteriae* produces an enterotoxin, Shiga Toxin, which disrupts protein synthesis and produces endothelial damage.

B.3. Campylobacter species

Campylobacter are small, comma-shaped Gram negative bacilli with microaerophilic growth requirements. Thirteen species have been associated with human disease. *C. jejuni, C. coli* and *C. upsaliensis* are the most common causes of Campylobacter gastroenteritis. A variety of animals serve as reservoirs, and humans acquire *C. jejuni* and *C. coli* infection through consumption of contaminated poultry, milk and other foods. *C. jejuni* produces histologic damage to the mucosal surfaces of the small and large intestines through invasion into intestinal cells; however, the exact role of the adhesins, cytotoxins, and enterotoxins detected in *C. jejuni* isolates are not well defined. *C. jejuni* and *C. upsaliensis* infection have been rarely associated with Guillain-Barre syndrome, an autoimmune disorder of the peripheral nervous system characterized by development of symmetrical weakness. The pathogenesis of this disease is believed to be related to antigenic cross-reactivity between the oligosaccharides of Campylobacter and glycosphingolipids present on neural tissue.

B.4. Salmonella species

Salmonella are Gram negative bacilli that have more than 2400 unique serogroups, but are broadly classified into typhoidal species (*S. typhi* and *S. paratyphi*) and nontyphoidal species (*S. enteritidis* and *S. typhimurium* are the most common isolates). *S. typhi* and *S. paratyphi* have no reservoirs other than humans and can cause disease with a very low inoculum. *S. typhi* produces a febrile illness called typhoid fever. After passing through the intestinal cell lining, *S. typhi* is engulfed by macrophages, where it is able to replicate, and get transported to the liver, spleen and bone marrow. After 5-21 days, patients experience a fever with headache, malaise, mayalgias, and a salmon pink rash on the abdomen. Severe manifestations such as sepsis and intestinal bleeding may also develop. *S. enteritidis* colonizes the gastrointestinal tract of virtually all animals. A large inoculum is required for development of symptomatic disease; therefore, infection in humans usually occurs when contaminated foods are improperly stored (left at room temperature) allowing bacteria to replicate. The infectious dose of *S. enteritidis*, however, is lower in certain high risk populations such as the elderly, immunosuppressed, or HIV-infected. *S. enteritidis* infection is characterized by fever, nausea, vomiting, bloody or non-bloody diarrhea and abdominal cramps.

B.5. Escherichia coli

E. coli is a Gram negative bacillus and a facultative anaerobe. The strains of E. coli that cause gastroenteritis are divided into the following 6 groups: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC) or Shiga-like toxin-producing *E. coli* (STEC), enteroaggregative (EAEC), and diffusely adherent E. coli (DAEC). The clinical spectrum of diarrheal disease caused by these strains is dependent upon the characteristics of the secreted enterotoxin and plasmid-mediated virulence factors that allow for attachment and invasion of intestinal epithelium. ETEC produces heat-labile and heat-stable enterotoxins which affect the small intestines and cause a secretory diarrhea. EHEC produces cytotoxic Shiga Toxins (Stx-1 and Stx-2) that destroy intestinal villi and cause dysentery; EPEC causes diarrhea by destroying microvilli in the small intestines, while EIEC causes bloody diarrhea by causing destruction of epithelial cells in the large intestines. Diseases caused by ETEC and EPEC are seen most commonly in developing countries, occurring mostly in infants.

More than 50 serogroups of EHEC have been isolated; however, serotype O157:H7 is responsible for most of the *E. coli* associated gastroenteritis in the United States. EHEC disease is most common in the warm months, and most disease has been attributed to the consumption of undercooked ground beef or other meat products, water, unpasteurized milk or fruit juice. Recently, there have been several well-publicized outbreaks of *E. coli* O157:H7 in the U.S.: 500 people in the Northwest developed bloody diarrhea as a result of ingesting contaminated hamburger meat in 1993; 1100 people attending a county fair in upstate New York developed acute diarrhea as a result of a contaminated water supply from a well in 1999. The clinical presentation ranges from mild, uncomplicated diarrhea to hemorrhagic colitis with severe abdominal pain, bloody diarrhea, and little or no fever. Hemolytic Uremic Syndrome (HUS), a disorder characterized by acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia, is a complication of EHEC infection (especially with Stx-2 production) that occurs in 10% of infected children under

the age of 10. Death can occur in 3-5% of children with HUS, and severe neurological and renal sequelae can occur in as many as 30% of patients.

C. Pathogenesis
C.1. Bacterial Virulence Factors
C.1.a. Inoculum size

Almost all GI pathogens are acquired by the fecal–oral route or ingestion of material contaminated by pathogens from other mammalian GI tracts. Sexual activity is also an established route for fecal-oral transmission. For most organisms, ingestion of a large inoculum (10^5–10^8 organisms) is required to produce disease; therefore, growth in food or water is a pre-requisite to transmission. The infective dose of *Shigella*, EHEC, *Giardia lamblia* or *Entamoeba histolytica* is much smaller (10–200 organisms); therefore, secondary cases that occur as a result of person-to-person contact are possible.

C.1.b. Adherence

Specific cell-surface proteins that allow bacteria to attach to intestinal walls are important virulence determinants. *V. cholera* adheres to the brush border of small intestinal enterocytes via specific surface adhesins including the toxin-coregulated pilus. Strains of *E. coli* possess highly specialized adhesins, including colonization factor antigens, aggregative adherence fimbriae, bundle-forming pili, intimin, P pili, and Ipa (invasion plasmid antigen) protein that allow them to adhere to intestinal cells.

C.1.c. Toxins

Enterotoxins are toxins which disrupt the absorptive/ secretory function of intestinal mucosal cells without killing the cells or causing structural cell damage. Cholera Toxin is an enterotoxin that consists of two subunits. The B subunit binds to ganglioside (GM1) receptors on intestinal epithelial cells. The A subunit is internalized and activates adenylate cyclase (cAMP), which causes the active secretion of sodium, chloride, potassium, bicarbonate, and water out of the cell into the intestinal lumen. Enterotoxigenic E. coli (ETEC) heat labile toxin (LT-1) is similar to the Cholera Toxin, and consists of one A subunit and five identical B subunits. The B subunit binds to ganglioside (GM1) receptors on the intestinal epithelial cell, and after endocytosis the A subunit translocates outside of the vacuole and interacts with a membrane protein (Gs) that regulates adenylate cyclase. This results in an increase in cAMP levels and enhancement of active secretion of chloride, decreased absorption of sodium and chloride, and production of inflammatory cytokines.

Cytotoxins are toxins which destroy intestinal mucosal cells and induce an inflammatory response secondary to cellular damage resulting in disruption of fluid/electrolyte transport functions. Shiga toxin is produced by *S. dysenteriae*. It has one A subunit and 5 B subunits. The B subunit binds to the host cell glycolipid (Gb3), and facilitates transfer of the A subunit into the intestinal epithelial cell. The A subunit disrupts protein synthesis by preventing the binding of aminoacyl-transfer RNA to the 60S ribosomal subunit. Enterohemorrhagic E. coli (EHEC) strains also express a Shiga toxin (Stx-1, Stx-2 or both) that is encoded by lysogenic bacteriophages. Stx-1 is identical to the Shiga toxin produced by *S. dysenteriae*, and Stx-2 has 60% homology. After binding of the B subunit,

the A subunit is internalized and binds to the 28S ribosomal ribonucleic acid. Disruption of protein synthesis results in destruction of the intestinal cells and villi, decreasing intestinal absorption.

Neurotoxins are toxins which induce GI symptoms or alterations in GI motility indirectly, by affecting the autonomic nervous system. _Bacillus cereus_ produces two enterotoxins: the heat-stable proteolysis-resistant enterotoxin that causes the emetic gastroenteritis and the heat-labile enteroxin that causes the diarrheal gastroenteritis. Other examples of neurotoxins are staph aureus enterotoxin and Clostridium botulinum toxin.

C1.d. Tissue invasion
Inflammatory diarrhea or Dysentery may result not only from the production of cytotoxins, but also from bacterial invasion and destruction of intestinal mucosal cells. Escherichia, Shigella and Salmonella have a common effector system for delivering virulence genes into targeted eukaryotic cells, called the type III secretion system. Salmonella utilizes two separate Type III secretion systems, Salmonella Pathogenicity Island-1 and 2 (SPI-1 & SPI-2), to mediate invasion into the intestinal mucosa. After binding to the M cells, the SPI-1 secretion system introduces salmonella-secreted invasion proteins (Sips or Ssps) into the M cells, resulting in rearrangement of the host cell actin with subsequent membrane ruffling. The ruffled membranes engulf the Salmonellae. Resistant to the acids of the phagosome, Salmonella replicate within the host cell and spread to adjacent epithelial cells and lymphoid tissue. Shigella species utilize a similar system which secretes four proteins (IpaA, IpaB, IpaC, and IpaD) into the macrophage or epithelial cell to induce membrane ruffling. After engulfment of the bacteria, Shigella organisms are able to lyse the phagocytic vacuole and replicate within the host cell cytoplasm.

C.2. Host Defenses
C.2.a. GI tract
Gastric acidity is probably the most important host defense against infectious gastroenteritis, since 99.9% of bacteria will be killed within 30 min at normal gastric pH <4. Neutralization of gastric acidity with antacids and H_2-blockers or medical conditions such as achlorhydria or gastric resection decreases the bacterial inoculum necessary to cause disease by 10,000-fold. Maintenance of normal GI motility also contributes by preventing adherence of pathogenic organisms. Preservation of normal anaerobic bowel flora may also decrease risk of infection by competing against pathogens for attachment sites and nutrients, and through the production of toxic metabolites.

The mucous membrane of the GI tract also provides a sophisticated defense system: Goblet cells produce mucous, composed of poysaccharides and proteins, which prevents bacterial adherence; rapid turnover of mucosal cells which migrate from the intestinal crypts to the tips of the villi also prevents bacterial attachment; and tight junctions between mucosal cells prevent bacterial penetration. Finally, paneth cells, located in the crypts of small and large intestines, produce peptides which are toxic to bacteria.

C.2.b. Immune mechanisms

Gastrointestinal tract–associated lymphoid tissue (GALT), comprised of M-cells, associated with underlying clusters of macrophages, T-cells, and B-cells, are found in the small and large intestine, tonsils, and the upper respiratory tract. M-cells, which are part of the mucosal lining, phagocytize bacteria and transport them to underlying macrophages. Macrophages process microbial antigens and present them to T-cells, which interact with B-cells and stimulate the production of IgA. IgA is produced in the submucosal space and secreted into the gut lumen where it binds bacteria, resulting in prevention of adherence to mucosal cells and mediation of phagocytosis. IgA is resistant to GI proteases and can also bind bacterial toxins.

D. Clinical Manifestations

It is helpful to organize clinical presentations of bacterial gastroenteritis into three groups: watery (noninflammatory) diarrhea, Dysentery (inflammatory diarrhea), and enteric fever (Table 1). Watery diarrhea is usually localized to the small intestines and is enterotoxin-mediated. Dysentery is usually localized to the large intestines, and is either caused by secreted cytotoxins or bacterial invasion. Enteric fever is the result of bacterial invasion and systemic dissemination.

Table 1

Illness	Watery Diarrhea	Dysentery or Inflammatory Diarrhea	Enteric Fever
Mechanism	Noninflammatory, Enterotoxin mediated disruption of water/electrolyte secretion by GI mucosal cells	Inflammatory with cytotoxin mediated destruction or invasion of mucosal cells	Invasion beyond GI mucosa and dissemination systemically
Histopathology	No structural damage to GI mucosa, no inflammation	Destruction of GI mucosal cells with inflammation	
Site of Infection	Small intestine (organisms generally do not penetrate GI epithelium but remain in lumen)	Large intestine (organisms actually invade but are generally limited to GI mucosa)	Distal small intestine – site of entry (disseminates systemically)
Characteristics of stools	High volume, watery	Dysentery-frequent, small volume stools containing blood and mucus	Systemic illness in which GI symptoms may not be very prominent
Presence of fecal WBCs:	No	Yes (PMN)	Variable
Other clinical findings:	No fever, leukocytosis; volume depletion predominates	Fever, leukocytosis; volume loss less prominent	Systemic signs and symptoms predominate-fever, headache, enlarged liver and spleen
Representative organisms:	►*Vibrio cholerae* ►Enterotoxigenic *Escherichia coli* (ETEC) ►*Bacillus cereus* ►*Clostridium perfringens*	►Shigella species ►Enterohemorrhagic *Escherichia coli* (EHEC) ►*Campylobacter jejuni* ► *Salmonella* ►*Clostridium difficile* ►*Entamoeba histolytica*	►*Salmonella* ►*Yersinia*

Three major clues to the diagnosis of infectious diarrheas are: (1) incubation period (2) presence or absence of fever (3) examination of stool for blood and white blood cells. Symptoms that begin within 6 hours suggest an ingestion of preformed toxins, such as *Staphylococcus aureus* or *Bacillus cereus* enterotoxin. Symptoms that begin between 8-14 hours after ingestion are typical of ingestion of food contaminated with *Clostridium perfringens*, which elaborates a heat-labile enterotoxin that causes watery diarrhea and abdominal cramps without fever. Symptoms that begin more than 14 hours after ingestion can result from viral or bacterial infection. Presence of fecal leukocytes alone or in combination with occult blood suggests an inflammatory process with a sensitivity and specificity ranging from 20-90%, depending on the study.

Other helpful information include: travel history; foods eaten and whether others who shared the food developed similar symptoms; sick contacts; recent antibiotic exposure; and presence or absence of symptoms such as vomiting or abdominal pain. Organisms that cause traveler's diarrhea vary considerably with location, but ETEC is the most common bacterial cause of watery diarrhea. Causes of bacterial food poisoning are listed in Table 2. Day-care centers have particularly high attack rates for bacterial gastroenteritis. Recent antibiotic exposure is a major risk factor for *Clostridium difficile* colitis. Vomiting is associated with an acute infection with a toxin. Abdominal pain may be most severe in an inflammatory process, although painful abdominal cramps may also develop with electrolyte loss in severe cases of cholera. An appendicitis-like syndrome should prompt a culture for *Yersinia enterocolitica*. Tenesmus (cramps in the rectum felt after a bowel movement) may be a feature of rectal inflammation caused by *Shigella*.

Table 2. Common Food Vehicles for Specific Pathogens or Toxins	
Vehicle	Pathogen or Toxin
Undercooked chicken	*Salmonella* and *Campylobacter* species
Eggs	*Salmonella* (especially *enteritidis*)
Unpasteurized milk	*Samonella*, *Campylobacter*, *Yersinia* species
Water	*Giardia*, *Norwalk* virus, *Campylobacter* and *Crytosporidium*, *Cyclospora*
Fried Rice	*Bacillus cereus*

Fish
  Shellfish
 	
  Sushi
	
	Vibrio species, *Norwalk* virus
	
	Campylobacter species

Beef, gravy	*Salmonella*, *Campylobacter*, EHEC

E. Diagnosis

Most watery (non-inflammatory) diarrheas are self-limited and can be treated without determination of a specific etiology. All patients with fever and evidence of dysentery acquired outside of the hospital should have stool cultured for *Salmonella*, *Shigella*, and *Campylobacter*. *Yersinia* will also grow in routine culture but is frequently overlooked unless specified. Isolation of *Campylobacter jejuni* requires incubation at 42ºC in

microaerophilic conditions. *E. coli* O157:H7 can be identified in specialized laboratories by serotyping or through detection of the Shiga-like toxin. Unlike ova and parasites which are often shed intermittently, these organisms are generally excreted continuously so that repeat specimens are not usually required. Selective testing can improve the yield of stool testing. Fecal specimens from patients with diarrhea that develops after 3 days of hospitalization have a very low yield when cultured for standard bacterial pathogens (*Campylobacter, Salmonella, Shigella*, etc) or examined for ova and parasites. Conversely, specimens from patients who have been in the hospital for ≥ 3 days may yield *C. difficile* toxin in 15-20% of cases. On the basis of these findings, several groups have suggested that fecal specimens from patients hospitalized for > 3 days should not be submitted for routine stool culture or ova and parasites (known as the "3-day rule").

F. Prevention and Treatment
The most critical therapy in diarrheal illness is hydration. Ideally, an oral rehydration solution containing water, salt, and sugar is utilized. Intestinal glucose absorption usually remains intact with diarrheal illnesses; therefore, the intestines can absorb water if glucose and salt are also present to assist in the transport of water from the intestinal lumen.

Since most cases are self-limited, antibiotic therapy is usually not required. One situation in which antibiotics are commonly recommended is in cases of traveler's diarrhea, in which ETEC or other bacterial pathogens are likely causes, and prompt treatment with fluoroquinolones can reduce the duration of illness from 3-5 days to <1-2 days. Empiric antibiotic therapy for febrile diarrheas in other circumstances, however, does not appear to be warranted. In a large, randomized clinical trial of a 5 day course of norfloxacin (a fluoroquinolone), there was only a modest reduction in time to cure with treatment (1.7 versus 2.8 days) that was more pronounced in patients classified as severely ill. There was no difference in mean time to cure in the subset of patients with *Salmonella* infection. Additionally, there is an unproven concern about an increase in risk of HUS with antibiotic treatment of EHEC enteritis. *In vitro* data indicate that certain antimicrobial agents can increase the production of Shiga toxin, and animal studies have demonstrated harmful effects of antibiotic treatment of EHEC infections. Furthermore, while antibiotics may reduce the shedding of susceptible *Shigella* and *Campylobacter* species, they may prolong the shedding of non-typhi species of *Salmonella*. Therefore, given the limited benefits, utilization of empiric antibiotics should be considered only in patients with moderate to severe disease, and in the absence of suspected *C. difficile* colitis or EHEC infection. Empiric therapy usually consists of a 3-7 day course of a fluoroquinolone.