Infectious Diarrheas (Gastroenteritis)

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Table 1 – Types of Enteric Infections by Pathophysiologic Mechanism
Key features and Microorganisms

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<th>Enterotoxin mediated (secretory)</th>
<th>Cytotoxin mediated (inflammatory)</th>
<th>Systemic syndromes</th>
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<td>Disruption of water/electrolyte secretion by GI mucosal cells</td>
<td>Invasion and destruction of mucosal cells</td>
<td>Invasion beyond GI mucosa and dissemination systemically</td>
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<td>No structural damage to GI mucosa, no inflammation</td>
<td>Destruction of GI mucosal cells with inflammation</td>
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<td>Small intestine (organisms generally do not penetrate GI epithelium but remain in lumen)</td>
<td>Large intestine (organisms actually invade but are generally limited to GI mucosa)</td>
<td>Distal small intestine – site of entry (disseminates systemically)</td>
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<tr>
<td>High volume, watery</td>
<td>Dysentery-frequent, small volume stools containing blood and mucus</td>
<td>Systemic illness in which GI symptoms may not be very prominent</td>
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<tr>
<td>NO</td>
<td>YES (PMN)</td>
<td>VARIABLE (MONONUCLEAR LEUKOCYTES)</td>
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<tr>
<td>No fever, leukocytosis; volume depletion predominates</td>
<td>Fever, leukocytosis; volume loss less prominent</td>
<td>Systemic sx/signs predominate-fever, HA, enlarged liver and spleen</td>
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<td>Vibrio cholerae</td>
<td>Shigella species</td>
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<td>Enterotoxigenic Escherichia coli (ETEC)</td>
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<td>Campylobacter jejuni</td>
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<td>Entamoeba histolytica (parasitic)</td>
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I. Significance/Overview

A. GENERAL
1. As a class, diarrheal diseases are greatest cause of morbidity and mortality in the world—particular problem in developing countries in contrast to heart disease, cancer in industrialized nations
2. Diarrheal diseases – overall incidence decreasing
   -worldwide accounts for
     • 2-3 million deaths/year or 5,000 -8,000 deaths/day in children ≤5y/o
     • major cause of death in children (≥50%) ≤5y/o
   -in the U.S., annually accounts for an estimated:
     • 211-375 million episodes or 1.4 episodes/person/year
     • 900,000 hospitalizations, 6000 deaths
3. Following respiratory illnesses, gastroenteritis second most common cause of visits to doctors
4. Historical—during Vietnam war, number of hospitalizations 2° diarrheal diseases equal to or greater than that due to injuries from combat

B. ETIOLOGIC AGENTS
1. *Salmonella, Campylobacter, Cryptosporidium* are leading identified etiologic agents
2. *Norovirus* and other viral agents, pathogenic *E. coli* also common but not routinely identified due to lack of routinely available clinical diagnostic testing

C. VARIETY AND MAGNITUDE OF PROBLEM—RECENT EXAMPLES
   • Outbreaks on cruise ships – affecting up to 40% passengers, also crew and long term care facilities/assisted living facilities identified, most cases on land
   • Potential for large outbreaks in closed or institutional settings; transmission from environmental surfaces or fomites
   • Newly identified prevailing strain (no prevailing strain in most previous years) and availability of RT-PCR for diagnostic testing likely accounts for increased illness attributable to *norovirus*
2. *E. coli O157:H7* -
   • 1993-(500 people) outbreak of bloody diarrhea associated with a fast food hamburger chain in the northwestern U.S.;
   • 1999 - almost 1100 people attending a county fair in upstate NY ill due to a contaminated water supply from an unchlorinated well
   • recent outbreaks associated with unpasteurized fruit juices and swimming in contaminated waters
3. *Cyclospora* - a parasitic infection resulting in ≈100 outbreaks and thousands of cases in 1996-97 linked to raspberries imported from Guatemala; resulted in a ban on such imports
beginning 1998 and no further outbreaks


5. *Cryptosporidium* outbreak in Wisconsin 1993 - (370,000 people) faulty filtration system for city water supply

6. Travelers—-16 million/yr from industrialized to developing countries, 8 million from U.S. alone: 1/3 experience “traveler’s” diarrhea

7. Food-borne outbreaks—-50,000–60,000 cases reported; an estimated 6-80 million cases/year estimated to occur in U.S.

8. Institutional settings - day care centers, hospitals (≥500,000) and others

II. Epidemiologic Factors

A. WHO YOU ARE

1. Age—infants: high risk → dependent on others for food, handling of feces, urine
   — If breast-fed, decreased exposure to contaminated food sources and maternal antibodies in breast milk protective →↑↑ risk at weaning

2. Living conditions—resident of developing country, high risk
   a. Type of housing, crowding
   b. Sanitation facilities
   c. Water sources/food related

B. WHERE YOU ARE

1. Living conditions—obviously affected by where you are

2. *E. coli* which make diarrheal-producing toxins and parasites predominantly found in tropical climates—diarrhea due to this type of *E. coli* seen much more commonly in travel from industrialized to developing countries and not vice versa

3. Viral causes of diarrheal illness more prevalent in temperate climates

C. WHEN IT OCCURS

1. Temperate climates—similar to respiratory illnesses, viral exanthems, usually occur in winter months

2. Tropical climates—most occur in summer

III. Host Factors/Defense Mechanisms

A. TRANSMISSION

1. Almost all GI pathogens acquired by fecal–oral route or ingestion of material contaminated by pathogens from other mammalian GI tracts, often human
   - sexual activity also an established route for fecal-oral transmission

2. Infectious inoculum:
   For most organisms, ingestion of a large number (10^5–10^6) organisms required to produce disease; exceptions are *Shigella* (10–100 organisms), shiga-toxin producing *E. Coli* (*E.coli* O157:H7), viruses – norovirus, rotavirus, and parasites (*Giardia/Entamoeba*)
a. Organisms requiring large numbers to produce disease generally require growth in food or water → no direct person-to-person transmission unless immunocompromised
b. Organisms requiring very small numbers to produce disease are readily transmitted directly by person to person contact, e.g., *Shigella* in day-care centers

**B. HOST DEFENSE MECHANISMS—GENERAL**

1. Hygiene— influenced by age, water supply, sanitation facilities

2. Gastric acidity
   a. Normal gastric pH <4 will kill 99.9% bacteria within 30 min
   b. Neutralization of gastric pH decreases size of inoculum causing disease by 10,000-fold
   c. risk factors: antacids, H₂ blockers, achlorhydria, gastric resection

3. GI motility
   a. Physically decreases ability of pathogens to adhere and flushes out offending organisms
   b. Antimotility drugs—Lomotil, etc.—may actually prolong fever, diarrhea, and shedding of organisms

4. Normal flora—particularly anaerobes in GI tract
   a. 99.9% normal GI tract flora are anaerobes (10¹¹ organisms/gm feces)
      — *Bacteroides, Clostridia, Peptostreptococci*
   b. Aerobic bacilli—*E. coli, Proteus, Klebsiella*
   c. Mechanisms
      1. Compete for attachment sites, nutrients
      2. Produce substances toxic for pathogens
      3. Induce low-level immunity, e.g., antibodies with cross-reactivity with pathogens
   d. Alteration of normal flora increases risk for GI infections
      — Antibiotic use eradicates normal flora and allows colonization of pathogens → *C. difficile, Salmonella*
      — Newborns who have not yet acquired normal flora → Enteropathogenic *E. coli,* generalized ↑ risk for GI infections

5. Intestinal immunity—nonspecific
   — GI tract presents high risk for entry of microbial pathogens because of constant ingestion of water, food potentially containing bacteria
   — Mucous membranes composed of a single layer of cells with secretory/absorptive functions vs. skin, which consists of multiple layers of dead cells
   — Not surprisingly, GI tract has evolved a sophisticated defense system
   a. Mucous—produced by Goblet cells
      1). Composed of polysaccharides and proteins which trap bacteria and prevent them from adhering to cells lining GI tract
      2). Has CHO residues similar to those found on cell membranes and competitively binds bacteria which have cellular receptors for GI tract cells
      3). Contains lysozyme (degrades peptidoglycan), lactoferrin, and lactoperoxidase, all of which are harmful to bacteria
   b. Rapid turnover of mucosal cells - originate in crypts of intestinal surface, migrate to tips of villi, and sloughed into lumen
      — Makes it difficult for bacteria to attach to GI tract and to mediate pathogenic effects
   c. Tight junctions between mucosal cells prevent bacterial penetration
d. Paneth cells—located in crypts of small and large bowel
   — Produce peptides which are toxic to bacteria; non-oxidative, similar to defensins, and lysozyme

6. Mucosa-associated antibody production (MALT) (Fig. 1)
   — Gastrointestinal tract–associated lymphoid tissue (GALT)—comprised of M-cells, associated with underlying clusters of macrophages, T-cells, and B-cells called follicles; large aggregates of these immune cellular components make up Peyer’s patches
   — Found in small and large intestine, tonsils, upper respiratory tract
   a. M-cells—part of mucosal surface
      — Phagocytic cells which ingest bacteria and transport them to underlying macrophages
      — Microbes usually killed by phagocytic cells but M-cells are exploited by, and provide portal of entry for, certain bacteria—Shigella, Salmonella
   b. Macrophages process microbial antigens → present them to T-cells → interact with B-cells → their production of IgA
      - Analogous to process that occurs systemically
   c. IgA produced in submucosal space binds to receptors on the basal surface of mucosal cells, acquiring a portion of the receptor in this process, is transported through the cell and secreted into the gut lumen, in the mucus

Fig. 1 Cellular components of MALT or GALT. M-cells and their associated lymphoid cells (T- and B-cells) are sometimes called follicles. Collections of follicles are called Peyer’s patches.
d. Secretory IgA:
   — Can also be passively acquired by infants from breast milk
   — Resistant to degradation by GI proteases
   — Bind bacteria, thus preventing their adherence to GI-tract cells; may also result in aggregation of bacteria, making it easier for normal motility to propel them out of the GI tract
   — Bind bacterial toxins
   — Fc portions bind to phagocytes and mediate clearing of organisms by phagocytosis but do not fix complement

e. GALT-mediated immunity not localized
   1). Although immune response is probably greatest at site of primary stimulation, WBC components of GALT are capable of migrating to other mucosal surfaces and producing IgA at those sites, so localized exposure to microbial pathogen does not limit immune response to that area but results in immunity at other mucosal surfaces and in breast milk
   2). Role/importance of this system exemplified by greater efficacy of oral vs. parenteral vaccines for pathogens which enter via GI route, e.g., Salmonella, polio

7. Localized mucosal cell–mediated immunity— poorly understood
   a. Mast cells
   b. Among mucosal cells, specialized lymphocytes actually interspersed which most closely resemble cytotoxic T-cells in their surface markers

8. Breast milk
   a. Decreases exposure to contaminated environment
   b. Contains antibody (IgA), phagocytes, lactoferrin, lysozyme, low pH,
      — Unfavorable environment for bacteria

IV. Microbial Virulence Factors—General

A. GENERAL CHARACTERISTICS
   Most GI bacterial pathogens are gram-negative aerobic bacilli which are members of the Enterobacteriaceae family → organisms which share homology at the DNA level, organization of genes for virulence factors, certain biochemical properties, e.g., oxidase negative and ferment glucose

B. Genomic level – virulence genes spread on transmissible genetic elements which include plasmids, bacteriophages, pathogenicity islands. Pathogenicity islands are discrete large genetic loci containing sets of virulence genes that are present in the genome of pathogenic bacteria but absent from closely related non-pathogenic strains – often with distinct nucleotide content suggesting their origin from another species.

B. ADHESIN SYSTEMS - ADHERENCE
   1. Generally initial requirement in causing infection/ disease
      — Ability to adhere to GI mucosa correlates with ability to produce disease
   2. Not unique to GI tract infections
   3. Microbial surface molecules/receptors—discussed in other lectures
4. Frequent determinant of predominant site of infection—tropism for particular tissues, e.g.,
strains of *E. coli* which cause diarrhea have surface receptors which are different from
strains of *E. coli* which cause urinary tract infection

5. Pili and non-pilus adhesins
- pili or fimbriae – have pilin as major structural protein (two major types in enteric pathogen-host cell interactions)
  a) chaperone-usher pilus family
  b) type IV pilus family
- non-pilus adhesins – below have immunoglobulin and lectin like domains
  a) invasion – *Yersinia pseudotuberculosis*
  b) intimin – Enteropathogenic *E. coli* (EPEC)

C. **TOXINS** - often produced and delivered to target host cells utilizing Type II and III secretion systems

1. Enterotoxins—toxins which disrupt the absorptive/secretory function of intestinal
  mucosal cells; do not actually kill the cells or cause structural cell damage; frequently do
  not cause any grossly evident or histologic damage to GI tract
  a. A 1° function of GI tract is absorption of fluids and electrolytes
     — 9–10 liters of H2O/day in food and liquids, secretions from saliva, bile, and pancreas
     and all but few hundred ml are absorbed by GI mucosal cells
  b. Disruption of fluid and electrolyte transport of GI tract mucosal cells will result in diarrhea
  c. Organisms—*Vibrio cholerae*, enterotoxigenic *E. coli* (ETEC)

2. Cytotoxins—toxins which actually destroy or kill intestinal mucosal cells and induce an
  inflammatory response due to cellular damage, thus interfering with fluid/electrolyte
  transport functions; frequently result in visible ulceration of GI tract
  — Organisms: *Shigella*, shiga toxin producing *E. coli* (STEC e.g. *E. coli* O157:H7),
     *Clostridium difficile*, *Entamoeba histolytica*

3. Neurotoxins—toxins which cause GI symptoms but do not act directly on GI mucosal
  cells; generally act on autonomic nervous system to induce symptoms or alterations in GI
  tract motility; frequently associated with food poisoning
  — Organisms: *Staph. aureus*, *Bacillus cereus*, *Clostridium botulinum*

D. **INVASIVENESS**
1. Ability to penetrate barrier imposed by GI tract mucosal cells and disseminate systemically
2. Often results in systemic illness, e.g., not limited to GI tract
3. Organisms: *Salmonella*, *Yersinia*

E. **PATHOPHYSIOLOGY**
Three generalized mechanisms which correlate with virulence factors just described
  1. Enterotoxin-mediated—secretory, noninflammatory diarrheas
  2. Inflammatory or cytotoxin-mediated diarrheas
  3. Systemic syndromes

V. **Enterotoxin-mediated, Secretory Diarrheas**

A. **VIBRIO CHOLERAE** - Cause of cholera; humans only known reservoir and acquired by ingestion
  of water or food infected with organism

  1. Genome sequenced in 2000 consists of two circular chromosomes vs. conventional view that
bacteria contain single circle of chromosomal DNA, both of which contain genes essential to the survival of the organism
   a. larger (about 3 million base pairs) – contains most virulence factors e.g. toxin, adhesion pili,
   b. smaller (about 1 million base pairs) – but also contains “essential genes” and genes involved in transport of essential sugars, metal ions, two component signal transduction, and DNA repair
2. Required components for pathogenicity/virulence
   a. Cholera toxin (CTX) bacteriophage/operon encoding for cholera toxin
   b. Toxin coregulated pilus (TCP) pathogenicity island/operon encoding TCP pili
   - tcp gene complex regulated similarly to those coding for cholera toxin
   - mediate adherence to GI mucosal cells (mutants lacking pili avirulent)
   - Accessory colonization proteins (acf genes)- outer membrane proteins important for colonization of GI tract (mutants lacking acps have decreased ability to colonize mammalian GI tracts and cause disease)
   c. ToxR regulon – network of genes regulating expression of the above
3. Cholera toxin (Fig. 2)
   a. chromosomal and transcriptional regulation/assembly/activation of this toxin discussed elsewhere
      — sensory/response mechanisms
      — ctxAB operon
   b. AB type toxin—composed of 2 types of structural subunits
      A = enzymatic subunit (1) encoded by ctxA
      B = binding subunit (5) encoded by ctxB
      — subunits synthesized separately, excreted into the periplasm of the bacteria, assembled into complexes consisting of 1 “A” subunit and 5 “B” subunits
      — “A” or enzymatic subunit must then be cleaved into two fragments in order to be activated
   c. “B” subunits of excreted, activated, toxin binds to GI mucosal cells by recognizing G\text{M1} gangliosides on host cell surface—sialic acid residues linked to a ceramide lipid
   d. “A1” subunit released from bound toxin and enters host cell by mechanisms not entirely clear
Fig. 2. **A.** Genetic structure of the *ctxAB* operon. *CtxA* encodes the ADP-ribosylating portion of the toxin, and *ctxB* encodes the binding subunit (rbs, ribosome binding site). **B.** Assembly, excretion, and activation by nicking of cholera toxin (CM, cytoplasmic membrane; OM, outer membrane).

e. ADP ribosylation of host cell G proteins to result in net water and chloride secretion by GI mucosal cells (Fig. 3)

1. G proteins are a class of membrane proteins involved in the transduction of a wide range of extracellular signals into intracellular events; made up of multiple subunits which associate and disassociate to activate or deactivate the G protein

2. G\textsubscript{S} is a G protein which regulates the activity of adenylate cyclase and therefore the levels of cAMP in a cell

3. A\textsubscript{1} subunit of cholera toxin ADP ribosylates G\textsubscript{S} → activates G\textsubscript{S} → increases adenylate cyclase activity → increases intracellular cAMP levels → increases Na\textsuperscript{+} and Cl\textsuperscript{-} transport and consequently water out of GI mucosal cells and into the gut lumen, resulting clinically in diarrhea
Fig. 3. Mechanism of cholera toxin–mediated increase in water and chloride secretion by GI mucosal cells

4. Other toxins
   a. Zot toxin (zonula occludens toxin)—disrupts the tight junctions or zonula occludens between GI mucosal cells → disrupts functioning of ion pumps

B. ENTEROTOXIGENIC E. coli
Major cause of traveler's diarrhea, also diarrhea in children in developing countries
1. E. coli is normal inhabitant of GI tract and most strains of E. coli are not pathogenic; strains must possess certain virulence factors in order to be pathogenic and cause disease—not clear-cut classification system
2. Adherence
   — ETEC bind preferentially to differentiated GI mucosal cells with microvilli vs. other bacteria
     a. Type 1 pili—bind mannose residues; role in GI disease not well established but probably contributes to colonization of GI tract with organism
     b. Colonization factor antigens (CFA)—class of pili only found on strains of E. coli which cause diarrheal disease; probably bind to glycoproteins on surface of GI mucosal cells
     c. Bundle forming pili—aa sequence and structure resembles that of V. cholerae
3. Toxin production (2 types)—heat labile toxin (LT) and heat stable toxin (ST)
   — Genes for toxins carried on plasmids and associated with transposons—facilitates genetic transfer, recombination, survival advantage for bacteria
     a. Heat-labile toxin (LT)—very similar to cholera toxin
        1). Same genetic organization (LTA and LTB genes), 75% homology, 1:5 A:B subunit structure, same GM1 host cell receptor, ADP ribosylation of Gs, etc.
        2). Different promoter regions, induced vs. constitutive transcription
        1). Binds to guanylate cyclase on luminal surface of GI mucosal cells → increases intracellular cGMP levels → resulting in net transport of Na+, Cl−, and H2O out of cells into the gut lumen, again resulting in diarrhea

C. CLINICAL SYNDROME
1. History of risk factor(s) for exposure
   — living in, travel to endemic area
2. No fever, systemic findings—no inflammatory response but symptoms and signs of fluid depletion, often massive, predominate—major cause of morbidity and mortality
3. Primarily involves small bowel
4. Profuse watery diarrhea (cholera = “rice water stool”) without fecal leukocytes
5. Gross exam and histopathologically—often no changes seen → no cell destruction

VI. **Inflammatory or Cytotoxin-mediated Diarrheas**

**A. SHIGELLA**

A cause of dysentery, a relatively small-volume diarrhea containing blood and mucus in contrast to profuse watery diarrhea seen with organisms possessing enterotoxins
1. Humans are primary reservoir
2. Seen in developing countries, also in U.S. in institutionalized settings, e.g., day care centers
3. Highly infectious—10–100 organisms enough to cause disease vs. 10⁸ organisms required by most GI pathogens → allows direct person-to-person transmission; not readily killed by acidic pH of stomach
4. Invasion of colonic mucosa—role of M-cells
   — *Shigella* in the gut lumen are taken up and transcytosed by M-cells → adhere and invade neighboring GI mucosal cells from their abluminal or basolateral surface prior to their uptake by macrophages
5. Steps in cellular pathogenesis (Fig. 4)
   a. Attachment to host cell
   b. Uptake of organism into host cell—actin rearrangement
   c. Replication of organisms within host cell cytoplasm → kills cell
   d. Spread to adjacent cells
6. Adherence/invasion of cell – Type III protein secretion system
   — Structural genes located in plasmids; regulatory genes are chromosomal
7. Type III Protein Secretion System
   - direct or contact dependent delivery of microbial proteins into a host cell analogous to an “injection with a syringe”
   - directs translocation of bacterial proteins into host cells → activates host cell signalling pathways resulting in a variety of host cell responses including:
     a. reorganization of actin cytoskeleton
     b. cytokine production
     c. induction of programmed cell death
   a. Invasion plasmid antigens (Ipa)—class of proteins expressed on *Shigella* surface and in some instances secreted extracellularly onto the surfaces of host GI epithelial cell via a Type III protein secretion system —probably bind to integrins
     1. Processing/expression on cell surface/ excretion of Ipa proteins are regulated by another set of genes called mxi genes (membrane expression of invasion)
     2. Required for adhesion/invasion—mutations in these genes result in a loss of *Shigella* invasiveness
     3. Induce rearrangement of host cell cytoskeleton (actin, myosin) at the site of bacterial adherence, which results in the bacteria being engulfed and contained within a phagocytic vacuole in these normally nonphagocytic cells
8. Intracellular replication
   a. *Shigella* must get out of phagocytic vacuole and into cytoplasm to replicate
   b. Ipa B—*Shigella* lacking this gene are unable to get out of vacuoles and replicate
      1. Encodes a pore-forming cytotoxin which is inserted in the vacuole membrane and leads to the disruption of the vacuole—activated at acidic pH
   c. *Shigella* rapidly multiply intracellularly (30–40 min doubling time)

Fig. 4  Steps in adherence to, and invasion of, cultured cell lines by *Shigella* spp. The virulence factors thought to be important at each stage are indicated.

9. Cell to cell spread
   a. IcsA (intercellular spread A) protein—*Shigella* outer membrane protein which is localized to one end of the bacteria → induce the polymerization of host cell actin filaments at this end → propel the bacteria through the host cell cytoplasm to the cell membrane and actually protrude into the adjacent cell cytoplasm
   b. IcsB protein—involves in the lysis of the protrusion which allows entry of *Shigella* into a neighboring cell and further replication and spread
   c. direct cell-to-cell spread may allow *Shigella* to escape the phagocytic/immune cells directly below GI mucosal cells

10. Mucosal cell death
a. Intracellular replication of *Shigella* eventually kills host cells → depletion of cellular nutrients, interference with respiration, energy metabolism, or causes apoptosis of macrophages and monocytes

b. Cellular, mucosal destruction results in erosion of blood vessels and provokes an inflammatory response with recruitment of PMN's, elaboration of cytokines, etc., thus producing diarrhea containing blood and leukocytes; disruption of epithelial impermeability also facilitates bacterial invasion at distant sites

11. Shiga toxin—role in pathogenesis of dysentery is not clear
   — *Shigella* mutants lacking toxin still capable of producing full spectrum of dysentery but somewhat less severe than wild type organisms

   *See below* under shiga toxin producing *E. coli*

**B. SHIGA TOXIN PRODUCING E. COLI (STEC) (E. COLI O157:H7)**

Similar clinical picture to *Shigella*

1. Initially associated with outbreaks of food poisoning associated with fast food chains in developed countries

2. more recently associated with contaminated drinking water and unpasteurized juices, use of recreational waters, and contact with farm animals

3. Found in GI tracts of 1% of cattle and other domesticated animals (sheep, goats) → contamination of meat exacerbated by grinding

4. Although relatively less common than other GI pathogens:
   a. Significance of *E. coli* O157:H7 related to high morbidity and mortality
   b. 25% hospitalized
   c. 6%, (but 15% of children) develop Hemolytic Uremic Syndrome
   d. 1% mortality

5. Adherence
   a. intimin—*E. coli* outer membrane protein which mediates tight binding of organism to host cell and similarly to *Shigella* induces host cell actin rearrangement at site of bacterial binding

6. Shiga-like toxin—almost identical to shiga toxin produced by *Shigella*
   a. Produced by strains of *E. coli* which cause hemolytic uremic syndrome (O157:H7)
   b. AB exotoxin - similar in structure to cholera toxin
   c. Recognizes gal -1,4-gal residue vs. sialic acid
   d. Interferes with host cell protein synthesis by inactivating 60s ribosomal subunit and causes cell damage, eventually death
   e. Role in Hemolytic–Uremic Syndrome and Thrombotic Thrombocytopenic Purpura—complication of dysentery (not limited to O157:H7 but most commonly associated with this organism) characterized by:
      1) hemolytic anemia,
      2) thrombocytopenia,
      3) acute renal failure
   f. Primary target may be blood vessels → vascular damage
      — kill cultured vascular endothelial cells
   g. May contribute to the exuberant inflammatory response
   h. *Shigella* strains which cause hemolytic–uremic syndrome produce unusually high levels of shiga toxin
C. *Clostridium difficile*

1. Cause of antibiotic-associated colitis
2. Gram-positive bacillus, anaerobic vs. other organisms in this section which are gram (-) aerobic bacilli
3. Seen in association with use of *any* antibiotic—eradication of normal flora in the colon → allows colonization and overgrowth of *C. difficile*
4. Hospitalization and advanced age are risk factors
   - ≤5% normal population in the community are colonized with *C. difficile*,
   - increases to 25–70% of hospitalized patients
4. Toxin production—2 types: Toxin A and Toxin B
   - Strains which produce toxin almost invariably produce both toxins
     a. Toxin A
        1). Directly cytotoxic → incubation with any cell type results in retraction and detachment of cells
        2). Mechanism unknown
        3). Causes symptoms in ileal loop assay
        4). Chemotactic for PMN and may mediate much of the inflammatory response seen in this infection
     b. Toxin B
        1). Generally does not cause cytotoxicity or symptoms unless Toxin A also present
        2). Hypothesis: host cells, e.g., GI mucosal cells, may lack receptors for Toxin B (receptor not yet identified) and therefore must be damaged for Toxin B to exert its effects
5. Spore formation—resistant to acidic pH of stomach
   - not metabolically active so resistant to action of antibiotics → clinically results in persistence of infection and recurrence of symptoms following apparent cure
6. Mucosal damage and inflammation can result in a characteristic accumulation of fibrin, mucus, and dead cells on the luminal surface of the colon → grossly appears like yellowish plaques called pseudomembranes, hence term “pseudomembranous colitis”

D. **Clinical Syndrome**

1. History - dysentery vs. watery diarrhea
   - Risk factors for specific organism
     a. *Shigella*—direct contact, travel, institutionalized setting
     b. STEC—ground beef ingestion
     c. *C. difficile*—antibiotic use
2. Exam/labs—
   a. Fever—inflammatory response
   b. Volume loss less prominent than with secretory diarrheas
   c. Leukocytosis
   d. Leukocytes and RBC's in stool → mucosal destruction and inflammation
   e. Tests for specific toxins – Shiga toxin, *C. difficile* toxin
3. Pathology
   a. Colon or large bowel primarily involved
   b. Destruction of GI mucosa and inflammation but limited to GI mucosa
      — further invasion/systemic involvement almost never occurs
c. Can have grossly visible erosions/ulcerations of GI mucosa

VII. GI Infections Which Cause Systemic Illness

Distinguished from infections discussed above by the ability of organisms to invade beyond the GI mucosa and disseminate systemically

A. Salmonella

1. Cause of typhoid fever and common cause of food-borne (esp. poultry, eggs) gastroenteritis

2. Classification—Typhi vs. non-Typhi
   a. typhi—humans are only known reservoir and cause of serious systemic disease, typhoid fever
   b. Non-Typhi—collectively referred to as enteriditis but includes thousands of subspecies, e.g., Typhimurium → ubiquitous; major reservoirs are animals and cause of self-limited disease restricted to GI tract (gastroenteritis) in immunocompetent hosts; can cause systemic disease similar to typhoid fever in immunocompromised host, e.g., HIV-infected patients

3. Overview of pathogenesis
   — most known about systemic disease 2° lack of good animal model for disease limited to GI tract
   a. Uptake by M-cells, translocation by dendritic cells, or direct invasion of GI mucosal cells
   b. Multiplication in macrophages and lymphocytes submucosally
   c. Hematogenous dissemination
   d. Replication in liver and spleen
   e. Excretion into bile and repeat hematogenous dissemination
   f. Repeat entry into gut lumen

4. Salmonella Pathogenicity Island-1 (SPI-1) Type III secretion system - Invasion
   a. In addition to uptake and transport of Salmonella to submucosa by M-cells, evidence for Salmonella's ability to directly invade nonphagocytic cells, e.g., GI mucosal or hepatocytes, where they replicate intracellularly
   b. invasins—
      1). Group of at least 8 genes which have been identified that confer Salmonella’s ability to invade nonphagocytic cells
      2). May code for a structural protein or participate in the regulation of the secretion of a substance which binds to host cell receptors → ligation of host cell receptors results in increased intracellular calcium concentrations → rearrangement of host cell cytoskeleton → engulfment of organism
      3). Mutations in invasin genes prevent the ability of Salmonella to invade cells in vitro but not yet confirmed by a decrease in virulence in these mutants in vivo

5. Salmonella Pathogenicity Island-2 (SPI-2) – mediates intracellular survival in phagocytes; confers adaptive response by Salmonella and alters biogenesis and dynamics of vacuolar compartment to permit intracellular survival and replication
   a. Must escape GALT to disseminate systemically
   b. Intracellular environment—low pH, oxygen radicals induce an adaptive response in Salmonella;
   c. Resistance to oxygen radicals
1). oxy genes—encode for catalase, superoxide dismutase, among others

d. Resistance to defensins—regulated by phoP/phoQ operon
   — Structural gene not yet identified

e. phoP/phoQ operon is a regulatory locus which controls the expression of a number of virulence genes
   1). Mutation in this locus causes the LD50 to increase by 10,000-fold
   2). Activates some genes and represses other genes
   3) Resistance to low pH—can survive pH of 3
      • in S. typhimurium associated with needle shaped structures on cell surface which may serve as a channel through which bacterial proteins enter target host cells (syringe analogy)

f. mediates apoptosis of macrophages

6. Resistance to complement-mediated cytotoxicity
   — During hematogenous spread
      a. Long O antigen of lipopolysaccharide on Salmonella causes membrane attack complex formed by complement components to form too far away from Salmonella cell membrane to damage or lyse the cell
      b. Outer membrane protein (Rck = resistance to complement killing) which prevents the final steps in the assembly and insertion of the membrane attack complex

7. Virulence of S. typhi associated with the presence of Vi antigen, which is a capsular polysaccharide, but its role in pathogenesis is not clear—unlike most encapsulated organisms, Salmonella wants to be phagocytosed

B. CLINICAL SYNDROME

1. Risk factors—history of exposure, immunocompromised host, hemolytic anemias, young children

2. Systemic illness in which diarrhea is often not a very prominent symptom or is self-limited
   a. Early in infection, often have GI symptoms → nausea, vomiting, diarrhea, which then resolve within a few days
   b. After several days, develop systemic symptoms → fever, headache, adenopathy, enlarged liver and spleen, hypotension
   c. Prolonged course—weeks

3. Laboratory—
   a. Anemia, decreased white blood cell count, elevated liver function tests,
   b. ± WBC's in stool
   c. (+) Blood cultures for Salmonella (80% in first week), (+) stool cultures only 60% after first week of illness

VIII. Viral Causes of Gastroenteritis

May be distinguished from enteroviruses whose route of infection is via GI tract but resulting clinical syndromes are not GI

A. GENERAL

1. 40% cases infectious diarrhea in U.S.

2. Much less known regarding their pathogenesis as these agents more recently identified, often cannot be propagated in vitro, and lack of good animal models
3. Involve small bowel and generally cause histologic alterations and sloughing of GI mucosal cells but no clear-cut inflammatory response, e.g., no fecal leukocytes
4. Not associated with any known toxin production but cause functional changes in electrolyte/fluid secretion, absorption of CHO's

**B. ROTAVIRUS**
1. Most common cause of diarrhea in infants and young children
   — 35% hospitalizations of children < 2y/o
2. Virulence factors
   a. Low infectious inoculum < 100 organisms required to produce disease
   b. Segmented ds RNA genome
      a. VP4 surface protein—viral hemagglutinin
      b. VP7 – outer capsid glycoprotein
3. Diagnosis—detection of viral antigen in stool

**C. CALICIVIRUSES/NOROVIRUSES/NORWALK-LIKE VIRUSES—ss (+) RNA VIRUSES**
1. Recent genomic sequencing indicates that these viruses all belong to the same class
2. Probably most common cause of self-limited gastroenteritis in the general population (otherwise healthy adults and children school age and older)
3. Transmission
   a. Low inoculum <100 organisms
   b. Prolonged shedding - ? up to 2 weeks S/P infection
   c. Environmentally stable – resistant to standard levels of chlorine (10ppm) and heating to 60°C
   d. Foodborne; person-to-person; consumption of contaminated raw oysters from the Gulf of Mexico
4. Diagnosis—Electron microscopy, immunoassays for viral antigen, RT-PCR → no specific treatment required
5. No long lasting immunity – re-infection common

**IX. Parasitic**

See Parasitology course materials

**X. Diagnosis –**

- Specific diagnosis often not necessary because most illnesses are self-limited or viral and last for less than 24 hours;
- Fever, blood or pus in stools, systemic illness, volume depletion or persistent illness are general indications for diagnostic evaluation,

**A. HISTORY**

1. Risk factors for infection with specific agents
   - infant - Rotavirus,
   - travel—ETEC,
   - antibiotic use— C. difficile
2. Presence or absence of systemic symptoms
3. Character of stool
**B. PHYSICAL EXAM**
1. Fever
2. Presence and degree of volume depletion

*C. DIRECT STOOL EXAM*
1. Gross description
2. Presence of fecal leukocytes
3. Exam for parasites
4. Immunoassays
   a. Toxins—*C. difficile*
   b. Viruses—*Rotavirus*

**D. STOOL CULTURE**
Will identify organisms that are generally pathogenic when present
1. *Vibrio cholerae*
2. *Shigella*
3. *Salmonella*
4. *Campylobacter*
5. Pathogenic *E. coli* will generally *not* be identified by routine culture—normal inhabitant of GI tract
   a. STEC or O157:H7—can be diagnosed microbiologically by growth on sorbitol MacConkey agar and serotyping but must generally be requested
   b. ETEC only identified by in vivo assays, e.g., rabbit ileal loop—not practical

**E. BLOOD CULTURE**
—Systemic infections, e.g., *Salmonella*

**XI. Treatment**

**A. FLUID/ELECTROLYTE REPLACEMENT**
1. Major cause of morbidity and mortality from infectious gastroenteritis is fluid and electrolyte imbalance, particularly true of enterotoxin-mediated, secretory and viral gastroenteritis
2. Cornerstone of treatment is replacement of fluids, electrolytes, and glucose
   — Oral replacement often as effective as intravenous replacement despite derangement in GI mucosal electrolyte, water transport → implications for feasibility in developing countries as well as costs and complications of IV therapy in developed countries

**B. NONSPECIFIC SYMPTOMATIC THERAPY**
1. Loperamide, bismuth subsalicylate – only agents with evidence of efficacy and safety
2. Avoid use with inflammatory diarrheas and in children

**C. ANTIBIOTIC THERAPY**
1. In immunocompetent hosts, most GI infections will eventually resolve even in the absence of specific antimicrobial therapy
2. Antibiotics generally shorten duration of symptoms and shedding of organisms and therefore are useful in reducing morbidity and mortality as well as transmission of GI infections
3. Special considerations
   a. STEC *E. coli* O157:H7—antibiotic use may induce toxin production and increase risk for development of hemolytic–uremic syndrome
b. *C. difficile*—antibiotics must be given orally to achieve sufficient levels within GI tract lumen to eradicate organism

c. *Salmonella* gastroenteritis (non-typhi, not systemic)—antibiotics can actually prolong excretion of organisms in stool

— In systemic infections or those in which bacteremia is present such as typhoid fever caused by *Salmonella typhi*, associated with a high mortality rate, benefits of antibiotic treatment outweigh the risks of prolonged fecal shedding of organisms

**D. EPIDEMIOLOGIC CONSIDERATIONS**

1. *Shigella*—patients with highly infectious organism with person-person transmission must not return to institutionalized settings, e.g., day care, preschool, until 3 negative cultures 2 days apart are obtained

**XII. Prevention**

**A. ADEQUATE WATER, SANITATION FACILITIES**

**B. HYGIENE—PERSONAL, HEALTH CARE GIVER, TRAVEL**

**C. ADEQUATE FACILITIES FOR HANDLING, PACKAGING, AND COOKING OF FOODS**

**D. VACCINES**

1. Oral vaccine available for *Salmonella*,

2. Challenges
   a. Host immune mechanisms and microbial determinants of immunity not well understood
   b. Mucosa-associated immunity most effective since this is portal of entry for GI pathogens so orally administered most effective → presents additional challenge of developing vaccine which will survive acid pH of stomach
   c. New pathogens may be constantly emerging as others are eradicated, e.g., *E. coli O157:H7*