THE ENTEROBACTERIACEAE

The Enterobacteriaceae

OBJECTIVES
5. List the triad of symptoms that defines hemolytic uremic syndrome (HUS)
6. Describe the reservoir, mode of transmission, clinical presentation, methods of laboratory diagnosis and treatment of disease caused by Shiga-toxin producing E. coli

In your bowels these bacilli may strut.
Most reside there quite peacefully, but
There are times there may be a
Severe diarrhea
When E. coli inhabit your gut.

Poem by Sheila B on OEDILF
Characteristics of Enterobacteriaceae

- Diverse group of Gram negative rods
- Ubiquitous
- Indigenous flora of GI tract
- Colonize respiratory tract of hospitalized patients
- Grow rapidly aerobically/anaerobically
- Simple growth requirements
Biochemical Characteristics

- Facultative Gram-negative rods
- Ferment glucose
- Reduce nitrate to nitrite

The Oxidase Test

- Identify bacteria that produce **cytochrome c oxidase**, an enzyme of the bacterial electron transport chain.
  - Cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) **purple color end product.**
The Oxidase Test

**Note:** All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

- Mnemonic for Oxidase Positive Organisms: **PUNCH**
  - P: Pseudomonas spp
  - U: *Ureaplasma*
  - N: *Neisseria* spp
  - C: *Campylobacter* spp
  - H: *Helicobacter* spp / *Haemophilus* spp / HACEK organisms

MacConkey Agar

- Selective and differential culture media
- Commonly used for the isolation of enteric Gram-negative bacteria.

[Image of MacConkey Agar]

Culture Media in Lab

**General Purpose Media:**
- Basic nutrients for recovery of non-fastidious bacteria

**Enriched Media:**
- Added nutrients to recover bacteria
- Standard agar used in clinical labs to recover bacteria from clinical specimens
  - Examples: Sheep’s blood agar, Chocolate agar (lysed red blood cells)

Culture Media in Lab

**Selective Media:**
- Inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.
- Suppress the growth of some microorganisms while allowing the growth of others
- Include addition of antibiotics, dyes, chemicals, alteration of pH
  - Examples: MacConkey agar, Mannitol Salt Agar

Culture Media in Lab

**Differential Media:**
- Designed in such a way that different bacteria can be recognized on the basis of their colony color.
- Include incorporation of dyes, metabolic substrates, chemicals, pH indicators
  - Examples: MacConkey agar, Mannitol Salt Agar, Blood agar, Chromogenic agars

**Many examples of selective and differential media**
**Mannitol Salt Agar**

**Selective=**
- Inhibit Strep with salt

**Differential=**
- Fermentation of mannitol; pH indicator (yellow vs. red)
- *S. aureus* vs. *S. epi*

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**MacConkey Agar**

**Selective=**
- Inhibit Gram+ with bile salt and crystal violet

**Differential=**
- Fermentation of lactose; pH indicator (pink/purple vs. clear)
- *E. coli* vs. *Proteus*

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**Structure of Enterobacteriaceae**

**H-antigen:**
- more than 50 serotypes; located in peritrichous flagella.

**K-antigen:**
- more than 80 serotypes; located in capsule (important in causing extraintestinal colonization, UTI, and invasive disease.)
Structure of Enterobacteriaceae

LPS (endotoxin) –
3 components

- O-antigen: located on outer most domain. Polysaccharide (repeating monosaccharide trimers in diverse combination and sequences); giving rise to 100-200 distinct serotypes
- Core oligosaccharide that attaches directly to Lipid A
- Lipid A: inner most region of LPS. Responsible for toxicity. When bacterial cells are lysed, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock).

Enterobacteriaceae

Virulence Factors

- Endotoxins
  - Lipopolysaccharides: major factor in pathogenesis - fever, sepsis, shock & multiorgan failure
- Capsule: protects from phagocytosis
- Antigenic Phase Variation
  - Altered expression of K,H Antigens protects from Ab mediated cell death
- Type III secretion systems
  - Facilitates secretion of virulence factors into host cells
- Sequestration of growth factors – eg. Iron scavengers
- Resistance to serum killing = capsule
- Antimicrobial resistance is a significant clinical issue
Enterobacteriaceae

Established Genera

- Escherichia
- Shigella
- Citrobacter
- Proteus
- Morganella
- Pantoea
- Providencia
- Plesiomonas
- Yersinia

5 species

- Important species: E. coli

Habitat:

- Intestines of humans and animals
- Not considered as a free living organism in the environment.

E. coli

- In humans, infants acquire within hours of birth.
- Because of its ubiquitous nature in human and animal feces, the presence of this species in water is considered an indicator of fecal contamination.
- Not considered as a free living organism in the environment.

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**E. coli**

**Clinical Syndromes**

- Most infections are endogenous—patient’s own flora
- Gram negative sepsis—most common GNR
- Urinary tract infections – 80% of community acquired UTIs
- Wound infections
- Pneumonia in immunocompromised hospitalized patients
- Meningitis in neonates
- Gastroenteritis

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**E. coli**

**Key Characteristics**

- Gram negative rods
- Lactose fermenter
- Beta hemolytic

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**Strains of E. coli Causing Gastroenteritis**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pathogenic Phenotype</th>
<th>Signs and Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterotoxigenic E. coli (ETEC)</td>
<td>Elaboration of secretory toxins (LT, ST) that do not damage the mucosal epithelium that stimulate hypersecretion of fluids</td>
<td>Secretory diarrhea (Traveler’s Diarrhea) similar to V. cholerae. Profuse watery diarrhea is predominant symptom. Often accompanied by mild abdominal cramps. Dehydration and vomiting occur in some cases.</td>
</tr>
<tr>
<td>Enteropathogenic E. coli (EPEC)</td>
<td>Adhere to epithelial cells in localized microcolonies and cause attaching and effacing lesions</td>
<td>Usually occurs in infants. Characterized by low-grade fever, malaise, vomiting, and diarrhea, with a prominent amount of mucus, but with no gross blood.</td>
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Strains of *E. coli* Causing Gastroenteritis

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<td>Enteropathogenic <em>E. coli</em> (EAEC)</td>
<td>Adhere to epithelial cells in a pattern resembling a pile of stacked bricks</td>
<td>Infants and travelers. Symptoms include <em>watery</em> diarrhea with blood and mucus, vomiting, dehydration and less commonly, abdominal pain.</td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>Invade epithelial cells</td>
<td>Inflammatory diarrhea (Dysentry) similar to <em>Shigella</em>. Hallmarks are fever and colitis. Symptoms are urgency and tenesmus; blood, mucus, and many leukocytes in stool.</td>
</tr>
<tr>
<td>Enterohemorrhagic <em>E. coli</em> (EHEC) Shiga Toxin producing <em>E. coli</em> (STEC)</td>
<td>Elaboration of cyto-toxins (Shiga toxins, Stx1 and Stx2). Primarily caused by <em>E. coli</em> O157:H7</td>
<td>Bloody diarrhea without WBCs. Often no fever. Abdominal pain is common. May progress to hemolytic uremic syndrome (HUS).</td>
</tr>
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Shiga-Toxin Producing *E. coli* (STEC)

- Produce one or more Shiga toxins
- CDC estimates that each year STEC causes >250,000 illness
- *E. coli* O157 most commonly identified serotype; at least 50 other serotypes
- Incidence and major serotypes of non-O157 strains causing diarrhea and HUS often not determined

Look for toxin, not serotype to identify STEC
Serotyping

- Latex agglutination
- Identify presence of different capsule, cell, and flagellar antigens to "identify" the serotype of the organism
- Ab-Ag interaction
  - O antigen
  - H antigen

Shiga Toxin Overview

- Found in *Shigella dysenteriae* 1 and *E. coli* (called Stx1 in *E. coli*)
  - Also called verotoxin
- Stx1 and Stx2
  - same mode of action
  - antigenically distinct
  - Stx2 associated with more severe disease
- AB5 Toxin
Shiga Toxin Overview

A subunit
- N-glycosidase that modifies the RNA component of the ribosome to inactivate it
- Halt to protein synthesis, leading to the death of the cell.
- Killing of cells leads to a breakdown of the lining and to hemorrhage.

B pentamer
- Bind to the cellular receptor, globotriaosylceramide, Gb3, found primarily on endothelial cells.

E. coli O157

Transmission
- STEC is shed in feces of cattle, sheep, deer, and other ruminants = reservoir
- Human infection is acquired via
  - contaminated food or water
  - via direct contact with an infected patient
- Low inoculum required
E. coli O157
Illness and Modes of Transmission

- Foodborne outbreaks most commonly associated with undercooked ground beef
- Sporadic cases:
  - unpasteurized milk, apple cider, lettuce and other produce, petting zoos, state fair show barns, contaminated water
- Largest U.S. outbreak occurred in Western USA in January 1993. Over 500 confirmed cases, 41 with HUS, 4 deaths. Source was undercooked hamburgers from one fast-food chain

STEC non-O157 Disease

- More than 150 serotypes identified. Most frequently isolated serotypes in North America are: serotypes O26, O45, O103, O111, and O121
- 2011, E. coli O104:H4 outbreak in Germany.
- Outbreak resulted in more than 4,000 cases and 50 deaths in 15 other countries; 20% HUS.
- Novel strain, with uncommon serotype, plasmid-encoded ESBL, and genes from enteroaggregative E. coli.
- Origin: fenugreek sprouts
**E. coli O157**

**Illness and Complications**

- Causes both bloody and non-bloody diarrhea
- Infections associated with:
  - Hemolytic Uremic Syndrome (HUS)
  - Hemorrhagic Colitis
- Young children and elderly at increased risk for severe complications

**E. coli O157**

**Hemolytic Uremic Syndrome**

- Triad of symptoms:
  - acute renal failure
  - thrombocytopenia
  - hemolytic anemia
- Preceded by bloody diarrhea
- Leading cause of acute renal failure in children
- Occurs in 20% of pediatric O157:H7 cases—often w/GI complaints
- Usually diagnosed a week after onset of diarrhea
Shiga Toxin & HUS

- High concentration of receptors for toxin in intestinal villi and renal endothelial cells
- Most associated with Stx2—destroy glomerular endothelial cells
- Damage to endothelial cells leads to platelet activation and thrombin deposition
- Decreased glomerular filtration and acute renal failure
- Shiga toxin stimulates cytokines that increase expression of the receptor

E. coli O157
Complications of HUS

- 50% require dialysis
- 3-5% die acutely
- 14% develop severe in-hospital complications including stroke, blindness, bowel resection, seizure
- 39% develop chronic kidney abnormalities years later

E. coli O157
HUS Can Occur in Two Settings

- Diarrheal related - classical HUS, vast majority of cases
- Non-diarrheal HUS - much less common, can occur in association with
  - pneumococcal infection
  - chemotherapy
  - transplant immunosuppression
**E. coli O157**

Hemorrhagic Colitis

- Abdominal cramps, watery diarrhea, bloody discharge (vomiting in 50%)
- No significant fever
- Absence of WBC in stool
- 30-65% of patients progress to bloody diarrhea within 2 days of onset of clinical symptoms of abdominal cramps
- Symptoms resolve in 4-10 days

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**Lab Diagnosis**

- Biochemical
- Structural Properties (surface antigens)
- Toxin detection (antigen detection)

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**Sorbitol MacConkey (SMAC) Agar for Isolation of E. coli O157**

- Substitute sorbitol for lactose in MAC agar
- *E. coli* O157 do not ferment sorbitol
- Not effective for all STEC types
Sorbitol MacConkey Agar for Isolation of *E. coli* O157

- Escherichia coli O157 colonies growing on MacConkey Agar with Sorbitol incubated aerobically for 24 hours at 37 deg. C.
- Escherichia coli colonies growing on MacConkey Agar with Sorbitol incubated aerobically for 24 hours at 37 deg. C.

Antigen Detection

- Antigens: Shiga toxin or the O157 antigen
- Direct fecal specimen detection
- Enriched broth culture detection (more sensitive)

BioFire FilmArray – Multiplex PCR

- Integrated Sample Preparation, Amplification, and Detection for 22 Enteric Pathogens including:
  - Shiga-toxin-producing *E. coli* (STEC) stx1/stx2
  - *E. coli* O157

More next lecture.....
Treatment of STEC Diarrhea

- Oral rehydration (Gatorade, Pedialyte), supportive care, and careful monitoring of kidney function.
- HUS and renal failure: can be managed by dialysis.
- Antibiotics NOT given in STEC
- Antimotility agents - NO! NO!
- Chemoprophylaxis with SXT or ciprofloxacin for travelers’ diarrhea NOT recommended

E. coli O157
Treatment with Antimicrobial Agents

- Most strains are very susceptible, however, treatment with antimicrobials has not been shown to shorten the duration or severity of disease and treatment possibly increases the risk of developing HUS


A college student returning from a Spring vacation in Puerto Vallarta, Mexico developed a profuse watery diarrhea accompanied by mild abdominal cramps. His diarrhea is so profuse that he is experiencing symptoms of dehydration. While on vacation he was careful not to purchase food from street vendors but did frequent the local bars and had drinks served with ice. Stool exam revealed no blood or WBC’s. What type of diarrheagenic E. coli is most likely to cause the symptoms experienced by this student?

A. E. coli O157
B. Enterotoxigenic E. coli (ETEC)
C. Enteroinvasive E. coli (EIEC)
D. Enteropathogenic E. coli (EPEC)
E. Enterohemorrhagic E. coli (EHEC)
A healthy 3-year-old child develops diarrhea that lasts about a week, and that is bloody for a few days. His diarrhea resolves, but he remains lethargic, and passes only a little urine. A blood smear shows fragmented red blood cells and no platelets. What etiologic agent of infection would most likely produce the signs and symptoms displayed in this case?

A. E. coli 0157
B. Campylobacter jejuni
C. Salmonella typhi
D. Shigella flexneri
E. Yersinia enterocolitica