Medical Microbiology
Enterobacteriaceae and other gram negative bacilli

William H. Benjamin PhD
Clinical Pathology

E. Coli O157:H7 spinach

- 204 cases, 103 hospitalizations, 31 HUS, 3 deaths
- 26 states
- Since 1995, there have been 19 outbreaks of foodborne E. coli O157:H7 for which lettuce or leafy greens were implicated
- Found in cattle 1 mile away
- Found in feral swine in the area
**Salmonella** on restaurant tomatoes

- Sept. 14 – Oct. 2
- 21 states reported 183 cases of illnesses
  - 22 hospitalized
  - AL (1), AR (4), CT (28), GA (1), IN (1), KY (19), MA (50), ME (8), MI (2), MN (14), NC (4), NE (1), NH (14), OH (4), PA (3), RI (6), TN (9), VA (3), VT (8), WA (1), and WI (2)
- **Salmonella enterica** Typhimurium

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**Cadbury Chocolate**

**Salmonella enterica** Montevideo

- 65 cases of *S. enterica* Montevideo in Great Britain
- 6 months before cases occurred Cadbury had a *S. Montevideo* isolate from an ingredient
- Leaking pipe at one of Cadbury’s main factories
- Cadbury’s rational for not acting on the original isolate is that the numbers identified did not reach a recall “threshold”
- Cases matched the PFGE pattern of the original Cadbury isolate and prompted an investigation that lead back to Cadbury
Common Characteristics of Enterobacteriaceae

- Gram negative Rods
- Rounded ends and straight parallel sides
- Colony morphology on sheep blood agar
  - ~2 mm colonies, usually dull gray
  - Other characteristics sometimes present
    - hemolysis
    - mucoid
    - swarming
    - pigmented colonies

Sputum Gram Stain
Gram Negative Rods
E. coli

Gram positive cell wall vs Gram negative cell wall and outer membrane
Gram positive cell wall vs Gram negative cell wall and outer membrane

http://micro.digitalproteus.com/morphology2.php

LPS

- Priming for O₂⁻ Radicals
- Adhesion molecules
- Phagocytosis

- TNF-α
- IL-1
- IL-6
- IL-8
- PAF
- O₂⁻ Radicals

- IL-1
- IL-6
- Adhesion molecules

- Proliferation
- Immunoglobulin

B-cells

- Proliferation
- INFγ
- IL-2

T-cells

- Direct actions
- Stimulation of additional cells
- Recruitment of additional mediators (e.g., complement factors, clotting cascade)

Macrophages/monocytes

Vascular cells

Granulocytes

Stem cells

- Fever
- Hypotension
- Tachycardia
- Tachypnea
- Neutropenia
- etc.

- Multi-organ failure
- Death

Fever
Hypotension
Tachycardia
Tachypnea
Neutropenia
etc.
Endotoxin resistant \((lps^{d})\) Mice

- \(lps^{d}\) mice are not affected by up to 1 mg of LPS, usual lethal dose 50 \(\mu\)g
- Paradoxically the mice are more susceptible to infection by \textit{Salmonella}, dying after <100 organisms vs 10,000 or 100,000 for non- \(lps^{d}\) mice
- Two different spontaneous mutations, one a single bp, one a large deletion.
- The \(lps^{d}\) mutation is in \textit{tlr4} an Endotoxin receptor gene in mice

Antigenic Structure of Enterobacteriaceae

- \textit{S. typhi}
- Flagella (H antigen)
- Capsule (K or Vi antigen)
- Lipopolysaccharide (LPS) or endotoxin
- \(O\) antigen side chain
- Pili (Fimbriae)
- Outer membrane
- Periplasmic space
- Lipopolysaccharide core
- Lipid A
- Peptidoglycan
- Cytoplasm

http://www.life.umd.edu/classroom/baci424/Lectures/LecturePPTs/Enterobacteriaceaeintro.ppt
Antigenic Formulae of Enterobacteriaceae

- **Escherichia coli**
  - O157:H7
  - O75:K100:H5
- **Salmonella enterica** serovar Typhi
  - O9,12(Vi):Hd:-
- **Salmonella enterica** serovar Typhimurium
  - O1,4,5,12:Hi:1,2

Physiology of Enterobacteriaceae

- Facultative anaerobes - ferment glucose
- Oxidase negative
- Nitrate positive - NO$_3$ --> NO$_2$
- Catalase Positive
- Motility is by peritrichous flagella
Oxidation Fermentation Test

Microscan Walkaway
Gram Positive Microscan Panel

Identification of Species and Genera

- Mostly biochemical reactions
  - Fermentation of carbohydrates pH change
  - decarboxylation of amino acids
  - deaminization of amino acids
  - etc.

- Genetic relatedness
  - 16s RNA
  - DNA hybridization
Enterobacteriaceae - host interactions

- Normal flora - beneficial
  - nutritionally
  - competition
  - Immunological stimulation

- Opportunistic infections
- Infections in otherwise healthy individuals
  - Non-inflammatory diarrhea
  - Inflammatory (invasive diarrhea)
  - Invasive systemic infections (typhoid, sepsis)

Escherichia coli

- Normal flora of mammals and birds
- Most common Enterobacteriaceae in gut
- Index of fecal pollution for food or water
  - coliform count
- Lactose positive, indole positive
MacConkey Agar plate
Lactose fermentation

Predisposing factors for UTI

- Female anatomy
- Honeymoon cystitis in the female
- Pregnancy and childbirth
- Male prostatic hypertrophy
- Catheterization or other mechanical manipulations
- Failure to empty the bladder
Other Opportunistic Infections

- Peritonitis from ruptured gut
- Septicemia secondary to UTI or pneumonia
- Wound infections

*Escherichia coli*
Diarrhea

- Estimated 4% of diarrhea in US
- Even higher numbers in the developing world
- Traveler’s diarrhea - 30-40% of visitors to Mexico
**E. coli Enteric Pathogens**

- **EPEC**  
  Enteropathogenic *E. coli*  
  - Infantile diarrhea

- **EAEC**  
  Enteroaggregative *E. coli*  
  - Travelers diarrhea

- **ETEC**  
  Enterotoxigenic *E. coli*  
  - Travelers diarrhea

- **EHEC**  
  Enterohemorrhagic *E. coli*  
  - Hemolytic Uremic Syndrome

- **Enteroinvasive E. coli**  
  - Bacillary dysentery

**Klebsiella pneumoniae**

- Lactose positive
- Nonmotile
- Large capsule (77 K antigens)
- β-lactamase  
  - resistant to ampicillin and carbenicillin  
  - sensitive to cephalosporins
Lactose positive *Klebsiella* sp

*Klebsiella pneumoniae* pneumonia
Predisposing Factors for Enterobacteriaceae Pneumonia

- Hospitalization - nosocomial
- Respirator
- Increased age
- Aspiration of oral secretions
- Alcoholism
- Diabetes mellitus
- Chronic bronchopulmonary disease

*Klebsiella pneumoniae* disease

- Pneumonia
  - 25-50% mortality
  - Thick non-purulent bloody sputum
  - Necrosis and abscess formation
- Septicemia
- Urinary tract infection
- Meningitis
**Enterobacter cloacae**

*E. aerogenes*

- Lactose positive
- Nonmotile
- UTI often nosocomial
- Cephalosporinase - *ampC*
  - Ampicillin resistant
  - Cephalothin resistant

**Disc Diffusion Antimicrobial Susceptibility Testing**
Proteus mirabilis, P. vulgaris

- Urease positive
- Highly motile, swarm on plate
- H₂S positive
- Infections
  - UTI - community acquired
  - Wound infections
  - Pneumonia
  - Septicemia
- Tetracycline resistant
- Ampicillin and Cephalosporin resistant

Urease test
Normal Adult

- 9.0 liters of fluid enters the duodenum
- 1.5 liters crosses the ileocecal valve
- 0.1 liters is passed by the anal sphincter
Osmotic Diarrhea

- Increased intra-luminal osmotic pressure
- Decreased absorption of fluid and solutes
- Causes of osmotic diarrhea
  - Lactose intolerance, *Giardia lamblia*
- Fasting stops diarrhea

Secretory Diarrhea

- Malfunction of Na\(^+\) absorption mechanisms
- Increased Cl\(^-\) secretion
- Mucosal cAMP increases the above two problems
- Bacterial toxins cause increases of cyclic nucleotides in mucosal cells
- Fasting does not stop diarrhea (quickly)
Bacillary Dysentery

- Caused by bacterial invasion of colonic mucosa
- Frequent small volume stools
  - Often containing blood and mucus
- Severe abdominal cramps

Incidence of Food Borne Illness in the US

- Estimated 76 million cases of food-borne illness annually
- 80,000 hospitalizations
- Estimated 5,000 deaths annually
- 7 to 17 billion dollars in medical and social costs
The Risk of Food-borne Disease per meal for selected animal-derived foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Illness episodes/meal</th>
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<tbody>
<tr>
<td>Seafood</td>
<td>1/5,000,000</td>
</tr>
<tr>
<td>Beef</td>
<td>1/200,000</td>
</tr>
<tr>
<td>Poultry</td>
<td>1/20,000</td>
</tr>
<tr>
<td>Raw molluscan shellfish</td>
<td>1/250</td>
</tr>
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Foodborne outbreaks etiology known?

<table>
<thead>
<tr>
<th>Year</th>
<th>Unknown etiology</th>
<th>Known etiology</th>
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<tbody>
<tr>
<td>1997</td>
<td>32</td>
<td>68%</td>
</tr>
<tr>
<td>1998</td>
<td>28</td>
<td>72%</td>
</tr>
<tr>
<td>1999</td>
<td>27</td>
<td>73%</td>
</tr>
<tr>
<td>2000</td>
<td>31</td>
<td>69%</td>
</tr>
<tr>
<td>2001</td>
<td>37</td>
<td>63%</td>
</tr>
<tr>
<td>2002</td>
<td>37</td>
<td>63%</td>
</tr>
<tr>
<td>2003</td>
<td>38</td>
<td>62%</td>
</tr>
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**Bacterial Foodborne outbreaks US 1997 - 2003**

<table>
<thead>
<tr>
<th></th>
<th>outbreaks</th>
<th>cases</th>
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<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>678</td>
<td>20392</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>157</td>
<td>4550</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>142</td>
<td>8417</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>119</td>
<td>3132</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>81</td>
<td>5105</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>75</td>
<td>1681</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>39</td>
<td>621</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>26</td>
<td>584</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>13</td>
<td>271</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>13</td>
<td>84</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>4</td>
<td>14</td>
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<tr>
<td>multiple</td>
<td>4</td>
<td>103</td>
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<tr>
<td><em>Streptococcus group A</em></td>
<td>1</td>
<td>4</td>
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<tr>
<td></td>
<td><strong>1361</strong></td>
<td><strong>45040</strong></td>
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**Vibrio cholerae**

- 1-5 day incubation period
- Profuse watery diarrhea
- Spread by contaminated water
- Stomach acid offers protection
  - Infectious dose is $10^7$ CFU
- Cholera Toxin
  - B subunit binds intestinal cells
  - A subunit irreversibly activates adenyl cyclase
  - Results in water and electrolyte secretion
- Treatment - oral hydration and electrolyte replacement which is sufficient
Enterotoxigenic *E. coli* (ETEC)

- Watery diarrhea - Secretory diarrhea
- Labile toxin related to Cholera toxin
  - Activates adenylate cyclase (cAMP)
  - Increased permeability to Na\(^+\) and Cl\(^-\)
- Stable toxin
  - Activates guanylate cyclase (cGMP)
  - Decreases absorption of Na\(^+\) and Cl\(^-\)
- Toxins are coded by plasmid DNA
Bacterial food poisoning

- Invasive causes of food poisoning
  - *Salmonella enterica* Serotypes
  - *Salmonella typhi*
  - *Campylobacter jejuni*
  - *Shigella sp*
  - Enteroinvasive *E. Coli*
  - *Yersinia enterocolitica*
  - Enterohemorrhagic *E. coli* (HUS) (O157:H7)
  - *Listeria monocytogenes*

*Salmonella enterica*

- There are 2400 named Serotypes of *Salmonella enterica*
- *Salmonella* causes three syndromes
  - Enteric fever  Typhoid fever - systemic
  - Enterocolitis  Food poisoning - self limiting
  - Bacteremia  Complication of enterocolitis

- Lactose negative
- Most *H₂S* positive
**Salmonella enterocolitis**

- 0.5 to 2 million cases /year in US
- 2000 deaths/year
- Cost to US economy 0.5 to 1 billion $
- Common serotypes
  - *S. enterica* Typhimurium, *S. enterica* Enteritidis, *S. enterica* Newport
- Vomiting and profuse diarrhea 8-48 hr.
- Resolves in 3 to 7 - 10 days
- Source of infection Water, meat, dairy products, eggs and human carriers working as food handlers

**XLD (Xylose Lysine Decarboxylase) plate**

- Black shows $H_2S$
- Clear colonies shows Lactose negative
PulseNet CDC’s Weapon against Foodborne Pathogens

- System where all state public health departments “Fingerprint” selected pathogens for epidemiology
- Whole chromosome is cut with a restriction enzyme that only cuts 20 to 30 times
- Fragments are separated by size giving a pattern for each strain
- The patterns are variable but conserved enough to identify closely related strains

The 2006–07 peanut butter outbreak

- 11/2006 increased incidence of *Salmonella enterica*
  - Tennessee PulseNet was used to determine that one strain was over represented (normal av 52 cases/yr)
- Nov – Dec - 26 patients in multiple states were given a food-consumption survey 200 items
  - 48% turkey
  - 85% peanut butter
- Multistate case control study 2/5 – 2/13 – 65 patients
  - Patients - peanut butter 81% controls 65% OR 1.9
  - Peter Pan - Patients 67% controls 13% OR 10.9
- 2/14 plant ceased production and recalled all produced after 5/2006
- S. Tennessee found in 21 unopened and opened jars and environmental samples in Georgia plant 7/06 – 12/06
- Heated to 70°C but in high-fat low-water conditions *Salmonella* can survive
Enteric Fever (Typhoid Fever)

- *Salmonella enterica* Typhi, S. Paratyphi
- Humans are the only hosts
- 200 - 500 cases/year in US
- Millions of cases/year worldwide
- Mortality
  - <5% in US
  - 10% in the third world. Antibiotic resistance is common in some countries
Pathogenesis of Typhoid Fever

- Oral ingestion of *S. enterica* serotype Typhi
- Passage through the gastric acid barrier
- Mucosal attachment and internalization via M-cells overlying Peyer’s patches
- Translocation to lymphoid follicles and mesenteric lymph nodes
- Primary (silent) bacteremia
- Seeding of liver, spleen, lymph nodes, gall bladder
- End of incubation period
- Secondary bacteremia and symptom onset

Typhoid Vaccine

- Killed whole *S. typhi*
  - Side effects are localized and systemic symptoms mostly because of LPS
  - 50 to 94% effective depending on exposure and personal history
- Live vaccine Ty21a
  - chemically mutagenized strain
  - mixed results 60-70% protection
  - some studies no protection
**Campylobacter jejuni and C. coli**

- Gram negative curved rods
- 1 to 7 day incubation
- Fever, bloody diarrhea, abdominal pain
- More common/less severe than *Salmonella*
- Carried by animals - food animals and pets
- Most human infections are from contaminated food or water
- Raw milk and eggs have been implicated
- Treatment is rehydration
- Cooking and cleanliness are the best defense

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**Enteroinvasive *E. coli* (EIEC)**

- Causes bacillary dysentery
- Large virulence plasmid related to *Shigella* virulence plasmid
- O-antigens and adhesins are coded on the virulence plasmid
**Enterohemorrhagic *E. coli* (EHEC)**

- Diarrhea usually mild
  - 5-10% have hemorrhagic colitis
  - hemolytic uremic syndrome (HUS)
- The most common outbreak strain
  - O157:H7 (sorbitol negative)
- At least 100 other strains have the toxin but seem to be less virulent

**Hemolytic Uremic Syndrome (HUS)**

- Shiga like toxin AB₅ type of toxin
  - A subunit enzymatic activity that removes an adenosine from 28s rRNA
  - B subunit targets Gb3, or Pk antigen CD77
    - Activates apoptosis in target cells, endothelial cells, B cells
- renal glomeruli contain a high density of Gb3 in infants
- Acute renal failure
- hemolytic anemia
- Thrombocytopenia
- 3% mortality
Shigella species

- Closely related to *E. coli*
- Four named “species”
- Lactose negative
- Nonmotile
- No gas formed from glucose

Bacillary Dysentery

- Oral inoculation 1-3 days high numbers in small bowel
- High volume watery diarrhea
- Invade colon epithelium
- Dysentery - small volume bloody-mucoid stool
- Resolves in 2 - 7 days in healthy adults
- Treatment - supportive
  - Ampicillin, Bactrim
**Shigella Epidemiology**

- Humans are the only normal host
- 200 organisms are an infectious dose
- $10^8$ CFU/gram of infected stool
- 4 F of *Shigella* transmission
  - feces, fingers, food, flies, water
- Modern sanitary facilities and hand-washing
- Epidemics where sanitation breaks down
  - wars, day care centers, institutions, cruise ships
- 15% of pediatric diarrhea in US

**Incidence of Shigella species**

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella dysentery</em></td>
<td>grpA</td>
<td>&lt;2%</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>grpB</td>
<td>18%</td>
</tr>
<tr>
<td><em>Shigella boydii</em></td>
<td>grpC</td>
<td>&lt;2%</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>grpD</td>
<td>80%</td>
</tr>
</tbody>
</table>
**Yersinia enterocolitica**

- Enteric infection
  - mucosal ulcerations in terminal ilium
  - inflamed mesenteric nodes
  - virulence depends on invasion of intestinal cells - red and white cells in stool
- Symptoms abdominal pain/diarrhea lasting 3 week
- Confused with appendicitis
- Transmission to humans by contaminated food

**Yersinia pestis**

- Causes bubonic and pneumonic plague
- Bubonic is contracted from the bite of an infected flea
- Pneumonic is passed from person to person or cat to person
- Plague killed 25 million in 14 century Europe