Microorganisms Causing Foodborne Disease

Family *Enterobacteriaceae*
Pathogenic *Escherichia coli*

*Escherichia coli*
- Named after German bacteriologist Theodor Escherich
- *E. coli* is a normal inhabitant of the intestine of humans and warm-blooded animals
- It is normally a commensal but some strains have the ability to cause disease
  - Usually gastrointestinal infection

Types of pathogenic *E. coli*
- Enteropathogenic (EPEC)
- Enteroinvasive (EIEC)
- Enterotoxigenic (ETEC)
  - Heat-stable toxin
  - Heat-labile toxin
- Enterohemorrhagic (EHEC)
- Enteroaggregative (EaggEC)
- Diffusely adherent (DAEC)
Characteristics of the genus
- Genetically related to *Shigella*
- Lactose fermentative
- IMVIC (indole, methyl red, voges-proskauer, citrate)
  - Biotype I +++
  - Biotype II +++
  - *Enterobacter aerogenes* is -++
- From antigenic structure
  - O (somatic) serogroups
  - H (flagellar) serovars or serotypes
  - K (capsule) types

Growth and resistance

**Growth**
- Temperature
  - Min 7-8°C
  - Max 44-46°C
  - Optimum 35-40°C
- pH
  - Min 4.4
  - Max 9.0
  - Optimum 6.5 – 7.0
- Aw
  - Min 0.95
  - Optimum 0.995

**Resistance**
- Heat
  - $D_{50} = 0.1$ min
- Acid
  - Depending on type and strain
  - *E. coli* O157:H7 can stand as low as 2.5

EPEC
*Enteropathogenic E. coli*
- Non-invasive and non-toxigenic
- Causes diarrhea by disrupting the absorption-secretion balance in the small intestine
- Symptoms include malaise, vomiting and diarrhea
- Children (< 1 year old) are the most commonly affected
- Not commonly associated with foods
  - Outbreak in Rumania associated with substitute coffee drink
**EIEC**

*Enteroinvasive E. coli*

- Disease similar to dysentery caused by *Shigella*
  - Does not produce shiga toxin
- EIEC invades the epithelial cells of the colon and then grows in these cells
- Intestinal damage consisting of inflammation and ulceration
  - Bloody and mucoid diarrhea
- Foods exposed to fecal contamination from humans are associated
  - Salmon, vegetables, cheese

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**ETEC**

*Enterotoxigenic E. coli*

- Two toxins are produced
  - Heat stable (ST) or heat labile (LT)
  - ST can withstand 100°C for 15 min
  - ST, ST₈, ST₉
  - LT is inactivated at 60°C after 30 min (low pH helps)
- ST I and ST II
  - Specific strains may produce one or the two types of toxin
- All these toxins produce watery diarrhea
  - Mechanism of action changes between toxins
- Common cause of traveler’s diarrhea syndrome
  - Moctezuma’s revenge

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**ETEC**

Outbreaks in the U.S. and vehicles

- 1983
  - Multistate
  - ST
  - Imported cheese
- 1993
  - Rhode Island and New Hampshire
  - ST and LT
  - Garden salad eaten on an airline flight
- 2000
  - Florida
  - Vegetable salad eaten at a theme park
Outbreaks of ETEC on cruise ships

<table>
<thead>
<tr>
<th>Year</th>
<th>Cruise</th>
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<tbody>
<tr>
<td>1995</td>
<td>Crown Odyssey</td>
</tr>
<tr>
<td>1996</td>
<td>Royal Princess</td>
</tr>
<tr>
<td>1997</td>
<td>Royal Princess</td>
</tr>
<tr>
<td>1997</td>
<td>Regal Empress</td>
</tr>
<tr>
<td>1998</td>
<td>Ocean Breeze</td>
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<tr>
<td>2002</td>
<td>M/V Caronia</td>
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</tbody>
</table>

EHEC
Enterohemorrhagic *E. coli*

- Serotype O157:H7 is the most common in the US
- Non-motile O111 and O157 serogroups are common in Australia
- More common than other pathotypes
- Reservoir is the bovine GI tract.

EHEC Syndromes

- Hemorrhagic colitis
  - Self-limiting, acute, bloody diarrhea
  - Duration varies form 4 – 10 days
  - Symptoms start with stomach cramps and watery diarrhea
  - Incubation period is 1 – 2 days, sometimes 3 – 8 days
  - Usually there is no fever and no leukocytes are found in the stools
EHEC Syndromes

• Hemolytic Uremic Syndrome (HUS)
  – Sometimes preceded by bloody diarrhea
  – Three characteristic features
    • Acute renal failure
    • Hemolytic anemia
    • Thrombocytopenia
  – Leading cause of kidney failure in the US and western Europe
  – Approx. 10% of <10 year old children undergo HUS from hemorrhagic colitis
  – Fatality rate is 3-6%
  – 13% show long-term kidney damage

• Thrombotic thrombocytopenic purpura (TTP)
  – Largely confined to adults
  – Platelet microthrombi start forming
  – Kidney and brain endothelia are particularly vulnerable
  – Neurological symptoms result from blood clots in the brain
  – Life-threatening condition with high fatality rate
    • 95% for untreated cases, 10-20% for patients with early diagnosis and treated with plasma infusion and plasma exchange

EHEC resistance to acid and heat

• *E. coli* O157:H7 is less resistant to heat than *Salmonella*
• *E. coli* O157:H7 is more resistant to acid than most Gram-negative pathogenic bacteria
Growth of *E. coli* in the presence of acetic acid (Diez-Gonzalez and Russell, Microbiol., 143:1175, 1997)

Reduction of *E. coli* O157:H7 by 0.5% lactic acid as affected by temperature

Outbreaks of *E. coli* O157:H7 illness by vehicle U.S.A., 1982-1997

Detection limit

<table>
<thead>
<tr>
<th>Source</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produce</td>
<td>11%</td>
</tr>
<tr>
<td>Various foods/water</td>
<td>7%</td>
</tr>
<tr>
<td>Produce</td>
<td>11%</td>
</tr>
<tr>
<td>Meat products</td>
<td>32%</td>
</tr>
<tr>
<td>Ground beef</td>
<td>85%</td>
</tr>
<tr>
<td>Roast beef</td>
<td>6%</td>
</tr>
<tr>
<td>Salami</td>
<td>3%</td>
</tr>
<tr>
<td>Venison</td>
<td>6%</td>
</tr>
<tr>
<td>Unknown</td>
<td>25%</td>
</tr>
<tr>
<td>Non-food sources</td>
<td>25%</td>
</tr>
</tbody>
</table>
Isolation methods

FDA method
- Enrichment of 25 g of sample in 225 ml of EHEC Enrichment broth (EEB)
  - EEB consists of tryptic soy broth added with bile salts No. 3, K2HPO4, cefixim, cefsulodin and vancomycin
- After enrichment streak and surface spread plates of TC SMAC for isolation
  - TC SMAC consists of sorbitol McConkey agar added with tellurite and cefsulodin
  - Black colonies will be typical
- E. coli O157:H7 confirmation
  - O157 antigen latex test followed by H7 antiserum test
  - Biochemical ID for E. coli species
  - Warning: E. coli O157:H7 is negative for β-glucuronidase

USDA method
- 5 portions of 65 g (for ground beef, cooked meat patties or sausages), or 13 25-g portions for outbreak-related samples
- Mix each 65-g portion with 585 ml of mEC broth + novobiocin in a stomacher bag and pummel for 2 min in a stomacher
- Incubate bags with contents at 35 ± 2°C for 20 – 24 h
- Conduct screening test
  - Latex agglutination test, ELISA, etc.
  - Samples testing negative to the rapid screening test can be discarded as negative. Samples positive must follow the isolation procedure
- Conduct immunomagnetic separation
- Inoculate 0.1 ml and surface spread over a plate of Rainbow agar
- Subject typical colonies (black to gray) to latex agglutination test, biochemical identification and serological identification for confirmation of E. coli O157:H7