Listeria spp. and Listeria monocytogenes in Foods

Kristi McCallum
Rocky Mountain Food Safety Conference
May 24 & 25, 2016
Genus *Listeria*

- Gram-positive, non-spore forming bacilli
  - Demonstrate a tumbling motility (flagellar-driven motility)

- Facultatively anaerobic = capable of anaerobic respiration if oxygen is absent

- Peritrichous flagella (flagella around the entire surface) are produced at < 30°C – characteristic tumbling motility
  - Not at temperatures >/= 37°C

- Not heat tolerant

- Optimal growth is 30-37°C

Photo Source: www.foodsafetynews.com
Genus *Listeria*

- The genus *Listeria* contains 6 species
  - *L. grayi*
  - *L. innocua*
  - *L. ivanovii**
  - *L. monocytogenes***
  - *L. seeligeri*
  - *L. welshimeri*
Pathogenic species

L. monocytogenes
- Humans
- Animals

L. ivanovii
- Animals
- Mainly in ruminants
Serotypes of *L. monocytogenes*

- There are 13 serotypes of *L. monocytogenes*
  - Serotype is a group of related microorganisms that are classified based on their cell surface antigens 😊

- More than 90% of the isolates which are associated with illness are serotypes
  - 1/2a
  - 1/2b
  - 4b

Microbial Ecology of *Listeria*

- Widely found in nature from the soil, water, vegetation and in animal waste

- Typically found in foods such as raw and pasteurized milk, cheeses (especially soft cheese), ice cream, produce, deli meats, raw/cooked fish and poultry and cured meats

- Thrives in wet, cold environments
  - Grow at temperatures as low as 4°C (refrigeration) to 37°C (body temp)

- Not heat resistant
  - Killed by cooking/pasteurization

- Not typically found in dry food processing environments

- Grows well in anaerobic and aerobic conditions

- Grows well in mildly acidic (pH 4.4) and basic conditions (pH 9.9)
Listeria monocytogenes – Human Pathogen

- The CDC estimates that there are ~1600 illnesses and 260 deaths annually due to listeriosis

- Mortality rate amongst reported cases is 21%

- In the United States, listeriosis accounts for only 0.29 cases per 100,000 population but accounts for 19% of deaths due to foodborne illness

## Current Leading Causes of Foodborne Illness in the United States - CDC 2013

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Cases</th>
<th>Hospitalizations</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Incidence*</td>
<td>No.</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>6,621</td>
<td>13.82</td>
<td>1,010</td>
</tr>
<tr>
<td>Listeria</td>
<td>123</td>
<td>0.26</td>
<td>112</td>
</tr>
<tr>
<td>Salmonella</td>
<td>7,277</td>
<td>15.19</td>
<td>2,003</td>
</tr>
<tr>
<td>Shigella</td>
<td>2,309</td>
<td>4.82</td>
<td>450</td>
</tr>
<tr>
<td>E. Coli O157</td>
<td>552</td>
<td>1.15</td>
<td>210</td>
</tr>
<tr>
<td>Non-O157STEC</td>
<td>561</td>
<td>1.17</td>
<td>76</td>
</tr>
<tr>
<td>Vibrio</td>
<td>242</td>
<td>0.51</td>
<td>55</td>
</tr>
<tr>
<td>Yersinia</td>
<td>171</td>
<td>0.36</td>
<td>55</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>19,056</td>
<td>4,200</td>
<td>80</td>
</tr>
</tbody>
</table>

*Per 100,000 population according to CDC

Source: CDC MMWR, Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006–2013April 18, 2014 / 63(15);328-332
Listeriosis

- Listeriosis is an infection caused by consuming food contaminated with *L. monocytogenes*.

- There are 2 types of listeriosis:
  1. **Non-invasive**: In most cases involving healthy individuals, listeriosis causes fever, muscle aches, and gastrointestinal symptoms.
  2. **Invasive** listeriosis is when the bacteria spread beyond the gastrointestinal tract and cause very serious infection.
Febrile Listerial Gastroenteritis

- Non-invasive

- Typically occur 24h after ingestion of a high dose of bacteria

- Symptoms include fever, diarrhea, nausea, headache and muscle/joint pain and last for ~2 days

Source: www.tumblr.com
Invasive Listeriosis

- **Affects individuals who are:**
  - Elderly – adults 65 and older are 4 times more likely to get infection
  - Immunocompromised – cancer patients, liver/kidney disease, diabetes, HIV/AIDS
  - Pregnant women – 10 times more likely to get infection and pregnant Hispanic women are about 24 times more likely to get infection
  - Neonates – baby is born infected
Invasive Listeriosis

- Has a longer incubation time of 2-3 weeks but can, in some cases be as long as 3 months!
  - A single Listeria outbreak can continue for several months as new cases are reported over a long time period

- Very serious and often deadly illnesses occur
  - Central nervous system infection – septicemia, meningitis, encephalitis
  - Infection during pregnancy – spontaneous abortion, stillbirth
  - Neonatal infection – septicemia, meningitis, stillbirth
Listeriosis – Central Nervous System

- **Symptoms**
  - Headache
  - Stiff neck
  - Confusion
  - Loss of balance
  - Seizures/convulsions

- **Pathogenesis**
  - *L. mono* pass through the wall of the intestines and enter the blood stream. From there, they migrate to the brain and spinal cord.

  - *L. mono* can live inside the body’s specific white blood cells known as macrophages. Once inside, the bacteria hide from the body’s immune system.

  - *L. mono* can multiply within these macrophages and spread to other macrophages.

Source: “Preventing Foodborne Illness: Listeriosis: CDC"
Listeriosis – Pregnant Woman

- **Symptoms**
  - Pregnant women infected with *L. mono* may have mild flu-like symptoms such as fever, muscle aches, diarrhea and upset stomach

- **Pathogenesis**
  - *L. mono* can proliferate in the uterus and vaginal walls, often asymptotically, leading to miscarriage, still birth and preterm birth.
Listeriosis - Neonatal

- **Two forms of infection**

1. Acquired in utero, early-onset sepsis can result in premature birth

2. Acquired through vaginal transmission during birth can result in meningitis in the newborn
Pathway of Infection

Listeria monocytogenes
contaminated food

intestine

lymph node

bloodstream

liver

brain

placenta

spleen

fetus
Listeria penetrate cells of the intestinal epithelial lining.

The bacterial first appear in macrophages along the intestinal wall and then spread to hepatocytes in the liver.

The bacteria stimulate a Cell-mediated Immunity response. Failure to mount a CMI response leads to septicemia and meningitis. **ESCAPE IMMUNE SYSTEM and destroy host cell**

Fraser Broth

Uninoculated Fraser broth

No darkening

Darkening
Esculin-Based Plates

- Oxford, PALCAM, MOX, and LPM\(^+\) are selective and differential for *Listeria* spp.
  - Differentiation based on esculin hydrolysis
    - Ferric ammonium citrate allows for the detection of esculin hydrolysis
    - Esculin hydrolysis causes a blackening of the agar
      - All *Listeria* hydrolize esculin
  - Additionally PALCAM incorporates mannitol to differentiate mannitol positive organisms that may grow
    - Fermentation causes color change in the colony and/or surrounding agar from red to yellow or gray
- These plates all have different means of achieving selectivity
PALCAM Agar

- Typical *Listeria* morphology
  - Greenish-gray to gray colonies surrounded by blackening of surrounding agar

Slide courtesy of Minnesota Dept. of Agriculture
MOX
(Modified Oxford Medium)

- Typical *Listeria* morphology
  - Bluish-gray to gray colonies with darkening of the surrounding agar

Slide courtesy of Minnesota Dept. of Agriculture
Phosphatidylinositol Phospholipase C (PI-PLC)

- Allows for the differentiation of *Listeria monocytogenes* from other *Listeria* spp.

- *L. ivanovii* is indistinguishable from *L. monocytogenes* on most chromogenic agars

  - Both *L. monocytogenes* and *L. ivanovii* express PI-PLC activity
  - *L. ivanovii* is xylose + on Rapid’L mono agar and agar will turn yellow
R & F® *Listeria monocytogenes* CHROMagar

**White** = Negative PI-PLC Activity

**Blue** = Positive PI-PLC Activity

*L. innocua, L. grayi, L. seeligeri and L. welshimeri*

*L. monocytogenes and L. ivanovii*

Slide courtesy of Minnesota Dept. of Agriculture
Bio-Rad RAPID’L. mono Medium

White = PI-PLC (-)/Xylose (-)

White + Yellow zone = PI-PLC (-)/Xylose (+)

Blue = PI-PLC (+)/Xylose (-)

Blue/Green +Yellow Zone= PI-PLC (+)/Xylose (+)

L. innocua & L. grayi

L. seeligeri & L. welshimeri

L. monocytogenes

L. ivanovii

Slide courtesy of Minnesota Dept. of Agriculture
Hemolysis

- β-hemolysis is the complete lysis of red blood cells on the blood agar observed by a lightened or clear zone around the inoculum as seen on this CAMP test.
Questions