Lysteria monocytogenes, Clostridium difficile, MRSA: The Foodborne Link
Dr. Keith Warriner, University of Guelph
A Webber Training Teleclass

Listeria monocytogenes, Clostridium difficile and MRSA: The Foodborne Link
Keith Warriner
Dept Food Science
University of Guelph
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Foodborne Illness within Ontario

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number Cases</th>
<th>Estimated Cost (CAN$ m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>54000</td>
<td>128</td>
</tr>
<tr>
<td>Salmonella</td>
<td>29000</td>
<td>106</td>
</tr>
<tr>
<td>Yersinia</td>
<td>4300</td>
<td></td>
</tr>
<tr>
<td>VTEC</td>
<td>1800</td>
<td>115</td>
</tr>
<tr>
<td>Shigella</td>
<td>457</td>
<td></td>
</tr>
<tr>
<td>Listeria</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

How to Estimate to Cost of Foodborne Illness
- Lost Productivity: $495/day
- Doctor visit: $75/visit
- Hospital: $770/day
- Chronic illness: $1.68m
- Mortality $9.7m

Economic Impact of Foodborne Illness Cases by Commodity

The food link
- Human pathogens in the environment
- Animal-to-Animal
- Animal-to-Person
- Person-to-Person
- Waterborne
- Foodborne

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The Foodborne Link
- Lysteria monocytogenes
- Clostridium difficile
- MRSA

Listeria monocytogenes
- Gram positive non-spore forming rod
- Facultative anaerobe
- Catalase positive
- Oxidase negative
- hemolytic

- Psychrotrophic
- Growth range 1 - 44°C
- Opt temp 35-37°C
- pH 5.0 – 9.6
  (opt 6 – 8) Survives at pH 4
- Min aw 0.93
- Can survive in 25-30% NaCl solutions

Illness
Healthy individuals: Mild flu
High risk groups (young, pregnant, old, immuno-compromised:
Stillbirth or abortion
Meningitis
Septicemia
Pneumonia

- Infective dose $10^9$
- High risk groups $10^3$
- 30% mortality rate
- Incubation period 1-4 weeks
- Illness can last 1-90 days

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- Approx. 2500 human cases/year in the U.S., resulting in about 500 deaths/year
- Endemic in certain processing environments
- Carriage on raw materials
- Grows at refrigeration temperatures

History of Listeria monocytogenes
- Isolated from diseased rabbit in 1926
- Named after Lord Lister
- Animal Diseases
  - Circling disease
  - Silage sickness
  - Leukocytosis
  - Cheese sickness
  - Tiger river disease.

Human Listeriosis
- Zoonotic
  - Widely distributed in the environment
- Foodborne
  - More common in urban rather than rural populations.
  - Linked to raw milk derived from cows suffering listeriosis.

Confirmation of Foodborne Link
- 1981: Maritime Canada involving 41 cases and 18 deaths
- Coleslaw prepared from cabbage fertilized with sheep manure
- Amongst the most significant foodborne pathogens.

Key Products linked to LM
- Deli meats (1.82%)
- Seafood Salads (4.7%)
- Smoked seafood (4.31%)
- Deli salads (2.36%)
- Luncheon meats (0.89%)
  - (Gombas et al., 2003)

L. monocytogenes: Pilgrims Pride largest recall in history 27.4m lbs deli meats

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Significant Outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
<th>Number of cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981 Canada</td>
<td>Coleslaw</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>1985 USA</td>
<td>Mexican Cheese</td>
<td>142</td>
<td>48</td>
</tr>
<tr>
<td>1992 France</td>
<td>Deli Meat</td>
<td>279</td>
<td>85</td>
</tr>
<tr>
<td>2004-2007 USA</td>
<td>Queso Fresco</td>
<td>135</td>
<td>22</td>
</tr>
<tr>
<td>2008 Canada</td>
<td>Deli Meats</td>
<td>65</td>
<td>20</td>
</tr>
</tbody>
</table>

Listeria Product Recalls

- 2003 – 2007: 19 product recalls
- 2008: 446 product recalls
- 2009: 6 product recalls (deli meat, sandwiches, leeks)
- Increased testing: Product recalls likely to increase

Clostridium difficile

- Gram-positive bacillus
- Spore-forming
- Anaerobic
- Most common nosocomial infection of GI tract

Carriage

- 5% of the population
- Asymptomatic carriers
- Cl. difficile infection (CDI or CDAD)
- 93 cases/100,000
- 2006-2008: >300 deaths within Ontario

Risk Factors for Clostridium difficile Associated Disease (CDAD)

- Exposure to antibiotics causes disruption of protective intestinal microbiota
- Fluoroquinolones (e.g. Levaquin, Cipro) to be strongly linked to CDAD more than any other antimicrobial
- Most cases and outbreaks of CDAD occur in health care settings

Cause?

- Healthy people: good bacteria keeps bad under control.
- Antibiotics kill both the good and bad bacteria → C. difficile growth

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Prerequisites for CDI

- Antimicrobial therapy
- Disturbed colonic microflora
- Acquisition of toxigenic C. difficile
- Toxin A & Toxin B production

Risk Factors for Clostridium difficile Associated Disease (CDAD):
- Other Risk Factors
  - Age greater than 65 years
  - Severe underlying illness
  - Nasogastric intubation
  - Extended hospital stay

Fecal-Oral Route

Endospores or vegetative cells

Virulence Factors

- Major virulence factors:
  - Enterotoxin: toxin A (TcdA)
  - Cytotoxin: toxin B (TcdB)
  - Binary toxin (CDT)
  - Encoded by two genes: cdtA and cdtB
  - Pathogenic role not known

Toxin A and B

Photomicrograph of Colonic Mucosa

Intact Mucosa on left
Damaged Mucosa on right

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Disease Symptoms
- Appear within 4-10 days of taking antibiotics or weeks after discontinuing medication
- Watery diarrhea
- Fever
- Loss of appetite
- Nausea
- Abdominal pain

History
- 1935: First isolation and characterization
  - "difficile" Difficult to culture
- Up to 1980’s: 80% of strains
  - Toxinotype 0
  - A/B toxin negative
  - Binary toxin negative

BI/NAP1/027
- First identified in France in 1998; woman with PMC
- Later found to have been in US in 1980’s
- Not considered to be relevant until 2004!
- In Ontario (in pigs) at least since 2000


States with BI/NAP1/027 Strain of C. difficile (N=38), November, 2007

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Countries in Europe with BI/NAP1/027, November 2007

Community Acquired CDI

Many Patients Developed CDAD without Recent Hospital or Antimicrobial Exposure, Atlanta VA Hospital, 2003-2006

<table>
<thead>
<tr>
<th>Months since hospitalization</th>
<th>No. of patients</th>
<th>No. (%) without antimicrobial exposure within prior 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1 to 4 weeks</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1-3 months</td>
<td>8</td>
<td>1 (25)</td>
</tr>
<tr>
<td>&gt; 3-6 months</td>
<td>6</td>
<td>1 (17)</td>
</tr>
<tr>
<td>&gt; 12 months</td>
<td>44</td>
<td>10 (41)</td>
</tr>
<tr>
<td>Totals</td>
<td>61</td>
<td>20 (33)</td>
</tr>
</tbody>
</table>

What we know about Community Acquired CD
- Reports from Canada, the United States and Europe indicate that the rate of community-acquired Clostridium difficile infection may be increasing.
- A large proportion of cases of community-acquired C. difficile infection are not linked to recent antibiotic therapy, increased age, co-morbidity or prior hospital admission.
- Under reported
- Risk factors remain unknown

The Food Link
- C. difficile is a recognized pathogen in neonatal piglets
- Possible cause of enteritis in calves
- Apparent increase or emergence around 2000
- Little evidence to support link to antimicrobial use
- C. difficile has been isolated from retail meat products

Slide adapted from J. Glenn Songer

Animals
- Horses
- Cattle
- Dogs
- Cats
- Sheep
- Pigs
- Elk
- Cheetahs
- Monkeys

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Cattle
- 7.6% of diarrheic and 14/9% of non-diarrheic calves in Ontario
- 7/8 ribotypes recognized human pathogens
- Ribotype 027 and 017 identified

Pigs
- Cause of diarrhea, esp. in sucklings pigs (Waters 1998, Songer, unpublished data)
- Outbreaks increasingly reported
- Prevalence of colonization unclear
- Pig strains often indistinguishable from human strains, including 027 (Arroyo et al, unpublished data)

Retail Meat
- Ontario (Rodriguez et al., in press)
- C. difficile in 18% of retail ground beef/veal
- Predominant strain, closely related to ribotype 027/NAP1
- CDT+, 18 bp tcdC deletion, toxinotype III
- US (Songer et al, personal communication)
  - ~20% of various processed meats
  - Including ribotype 027

ToxV (BK/NAP7-8/078) Strains; Historically Rare, Recently More Common

Time
- Prior to 2001
- 2001-2005
- 2006

Tox V Isolates
- 10/6000
- 10/600
- 6/125

Human CDAD Caused by Strains Similar to Animal Epidemic Strains, 2001–2006

<table>
<thead>
<tr>
<th>Source</th>
<th>Toxinotype</th>
<th>Binary toxin</th>
<th>Deletion in tcdC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>V</td>
<td>+</td>
<td>39 bp</td>
</tr>
<tr>
<td>Pig</td>
<td>V</td>
<td>+</td>
<td>39 bp</td>
</tr>
<tr>
<td>Human</td>
<td>V</td>
<td>+</td>
<td>39 bp</td>
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<tr>
<td>Pig</td>
<td>V</td>
<td>+</td>
<td>39 bp</td>
</tr>
</tbody>
</table>

Epidemic Animal Strains Share Characteristics with the Human Epidemic Strain

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Human Standard Strains</th>
<th>Human Epidemic Strain</th>
<th>Porcine Epidemic Strains</th>
<th>Bovine Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxinotype</td>
<td>0</td>
<td>III</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>PCR ribotype</td>
<td>001 and others</td>
<td>027</td>
<td>NAP1</td>
<td>NAP1</td>
</tr>
<tr>
<td>PFGE pattern</td>
<td>&lt;80% related to NAP1</td>
<td>NAP7</td>
<td>NAP7 &amp; NAP8</td>
<td>NAP7</td>
</tr>
<tr>
<td>Binary toxin</td>
<td>–</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Deletion in tcdC</td>
<td>–</td>
<td>18 bp</td>
<td>39 bp</td>
<td>39 bp</td>
</tr>
<tr>
<td>TcdC protein</td>
<td>233 aa</td>
<td>65 aa</td>
<td>61 aa</td>
<td>61 aa</td>
</tr>
</tbody>
</table>

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Results from Recent Survey of Ontario Pig Farms
- Samples collected from June – Nov 08
- 52 farms visited, 133 samples screened
- Higher recovery in effluent compared to fecal swabs.
- CD recovered on 15 farms (28% prevalence)
- 20 isolates

Characterization

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Ribotype</th>
<th>Toxin A/B</th>
<th>Binary Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>078</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>027</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Conclusions
- *Clostridium difficile* highly prevalent (28%) on Ontario pig farms.
- Ribotype not linked to epidemic strain found in health care centres
- Possible link to Community Acquired infections.
- Foodborne pathogen?

Methicillin-resistant *Staphylococcus aureus* (MRSA)
Staphylococcus aureus

- Most common cause of nosocomial infection in humans. NCCLS 1999
- Pneumonia, surgical site infections, bacteremia
- Intoxication by heat stable toxin
- Commensal of many species
  - Skin, nasal passages, perineum
- 40% of the population carry S. aureus

MRSA: Humans

- Account for up to 50% of nosocomial infections at some hospitals NCCLS 1999
- 25% of nosocomial infections in US
- Majority of S. aureus are MRSA in many areas.
- Increased mortality, morbidity, costs

Staphylococcus aureus

- Most common cause of nosocomial infection in humans. NCCLS 1999
- Pneumonia, surgical site infections, bacteremia
- Intoxication by heat stable toxin
- Commensal of many species
  - Skin, nasal passages, perineum
- 40% of the population carry S. aureus

Estimated MRSA infections in USA (1999/2000)
- 125,969 hospitalizations with MRSA infection
- 31440 septicemia (10%)
- 29823 pneumonia
- 3.95/1000 hospital discharges
- Overall methicillin resistance rate 43.2%

Kuehnert et al Emerg Infect Dis 2005

Emergence of MRSA

Antibiotics Discovered 1928

Industrial Production of Antibiotics 1940's

Emergence of Antibiotic (erythamycin) Resistant SA and introduction of methicillin antibiotic 1950's

MRSA Isolated 1961

Endemic MRSA in Health Care Settings 1970-80's

Community Acquired MRSA 1990's

MRSA

Hospital Acquired
- Prolonged hospitalization
- Intensive care units
- Antibiotic therapy
- Surgery
- Close contact with infected patient

Community Acquired
- Young
- Poor hygiene
- Shared contaminated items
- Crowded living conditions
- Schools
- Correction centres
- Cuts and abrasions

CA-MRSA

- CA-MRSA Genetically Distinct from HA-MRSA
- HA-MRSA: high virulence
- Accounts for 30-40% of MRSA cases
- 40% of Children with MRSA carry CA-strain

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Prevalence of CA-MRSA in a Children’s Hospital

Community-associated MRSA
- Skin and soft tissue infections
- Bacteremia (and sequelae)
- Necrotizing pneumonia/fasciitis
- Toxic shock syndrome

MRSA in animals
- Horses
- Household pets
- Pigs
- Cattle
- Other

Household Pets

Animal Rescue

Cattle
- Sporadic reports of MRSA from cattle internationally
- Europe/Asia
- Under-detection, under-reporting?

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</tr>
<tr>
<td>- Infected and colonized</td>
</tr>
<tr>
<td>- 23% of pig farmers colonized</td>
</tr>
<tr>
<td>- Pig farmers 760 times more likely to carry MRSA than the general Dutch population</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>North America</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Study in Ontario</td>
</tr>
<tr>
<td>- MRSA is present in Ontario pigs</td>
</tr>
<tr>
<td>- Up to 90% prevalence on some farms</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>The Food Link</th>
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</thead>
<tbody>
<tr>
<td>- Direct contamination of foods</td>
</tr>
<tr>
<td>- Enterotoxin-associated disease</td>
</tr>
<tr>
<td>- Colonization of people in contact</td>
</tr>
<tr>
<td>- Colonization of food handlers/preparers</td>
</tr>
<tr>
<td>- Subsequent contamination of food</td>
</tr>
<tr>
<td>- MRSA foodborne illness likely no different from typical Staph aureus, but can it lead to further community dissemination?</td>
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</tbody>
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<table>
<thead>
<tr>
<th>MRSA-Food Reports</th>
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<tr>
<td>- Outbreak associated with BBQ pork and coleslaw from deli</td>
</tr>
<tr>
<td>- MRSA: enterotoxin C producing</td>
</tr>
<tr>
<td>- One food handler colonized with same strain</td>
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<td>- 2/444 (0.5%) retail chicken samples in Japan (Kitai et al J Vet Med Sci 2005)</td>
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<td>- 1/69 (1.4%) retail chicken samples in Korea (Lee Appl Env Microbiol 2003)</td>
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*0.18% of milk samples in Korea (Kwon J Antimicrob Chemother 2005)*

*2 diary herds in Hungary (Kaszanyitzky et al Acta Vet Hung 2004)*

*12/894 (1.3%) milk samples from Korea (Lee Appl Env Microbiol 2003)*

*0.18% of milk samples in Korea (Kwon J Antimicrob Chemother 2005)*

*Identification of identical strains of MRSA in pigs and pig farmers/families in the Netherlands (Voss et al 2005)*

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*MRSA in pig farmers/families*

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*North America*

*Study in Ontario*

*MRSA is present in Ontario pigs*

*Up to 90% prevalence on some farms*

*The Food Link*

*Direct contamination of foods*

*Enterotoxin-associated disease*

*Colonization of people in contact*

*Colonization of food handlers/preparers*

*Subsequent contamination of food*

*MRSA foodborne illness likely no different from typical Staph aureus, but can it lead to further community dissemination?*

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*Outbreak associated with BBQ pork and coleslaw from deli*

*MRSA: enterotoxin C producing*

*One food handler colonized with same strain*

*Retail Meat*

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Conclusions
- Food represents a significant vehical for emerging human pathogens.
- \textit{C. difficile} likely a foodborne pathogen
- Less evidence for MRSA
- Establishing a foodborne link is first step to control.

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- OMAFRA Sustainability Program
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