Lysteria monocytogenese, 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

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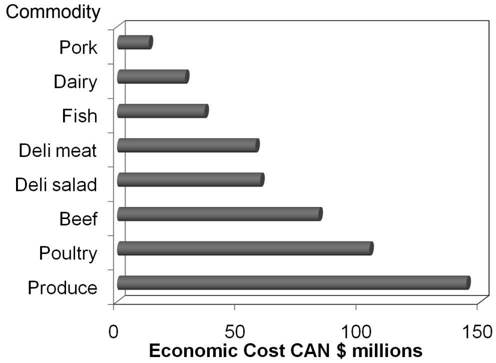
Foodborne Illness within Ontario

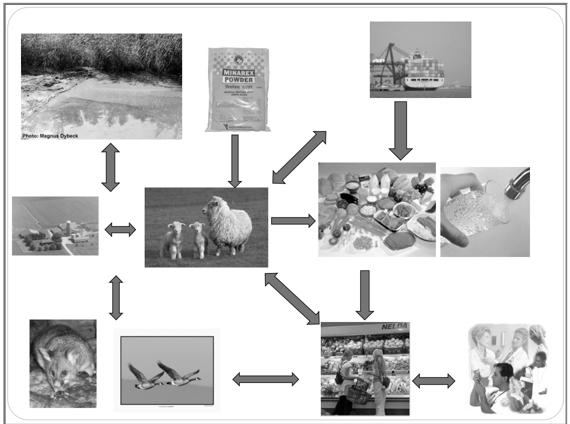
Pathogen	Number Cases	Estimated Cost (CAN$ m)
Campylobacter	54000	128
Salmonella	29000	106
Yersinia	4300	
VTEC	1800	115
Shigella	457	
Listeria	17	

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

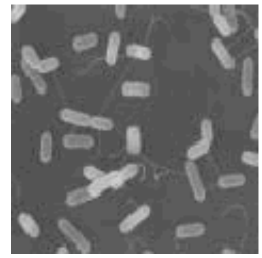
- Foodborne

The Foodborne Link

- *Listeria monocytogenes*
- *Clostridium difficile*
- MRSA

Listeria monocytogenes

- Gram positive non-spore forming rod
- Facultative anaerobe
- Catalase positive
- Oxidase negative
- heamolytic



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

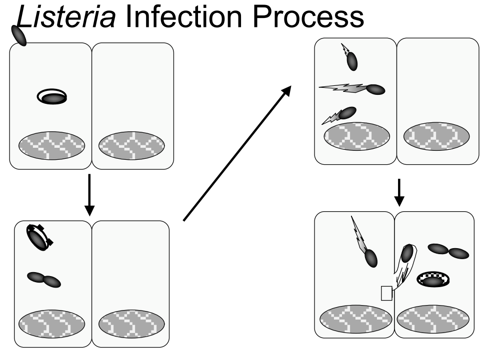
Illness

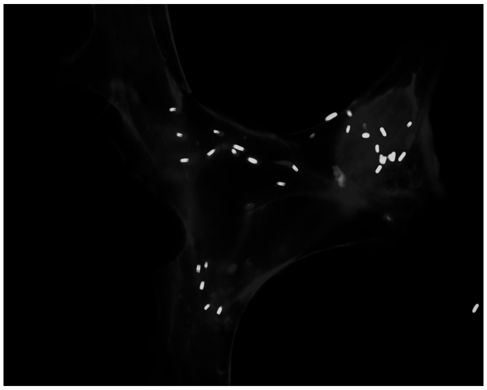
Healthy individuals: Mild flu

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

Listeria Infection Process





- 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
- Commonly linked to wild and domestic animals
- Asymptomatic carriers (10% carriers in the GI tract)

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

Significant Outbreaks

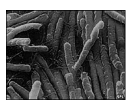
Year	Product	Number of cases	Deaths
1981 Canada	Coleslaw	41	5
1985 USA	Mexican Cheese	142	48
1992 France	Deli Meat	279	85
2004-2007 USA	Queso Fresco	135	22
2008 Canada	Deli Meats	65	20

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 microflora
- flouroquinolones (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

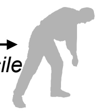
Prerequisites for CDI

Antimicrobial therapy

Disturbed colonic microflora

Acquisition of toxigenic *C. difficile*

Toxin A & Toxin B production



CDI

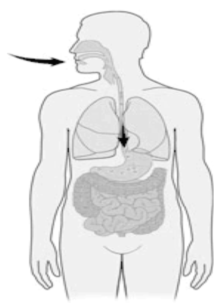
- Advanced age
- Underlying illness

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

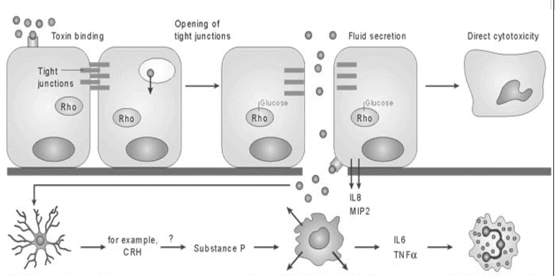
Endospores or vegetative cells



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

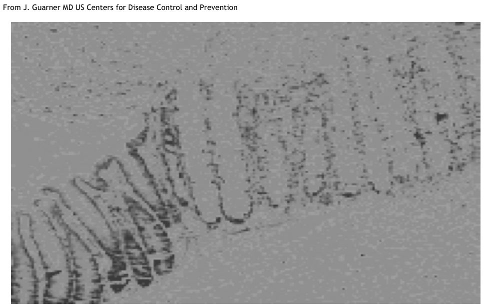
Toxin A and B



Nature Reviews | Microbiology

Photomicrograph of Colonic Mucosa
Intact Mucosa on left Damaged Mucosa on right

From J. Guarner MD US Centers for Disease Control and Prevention



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

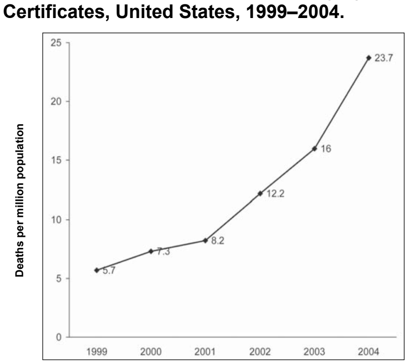
- 1980's BI/NAP1/027, or NAP-1/027
  Toxinotype III

  Increased production of toxin A/B
  Binary toxin
  Fluoroquinolone resistance

BI/NAP1/027

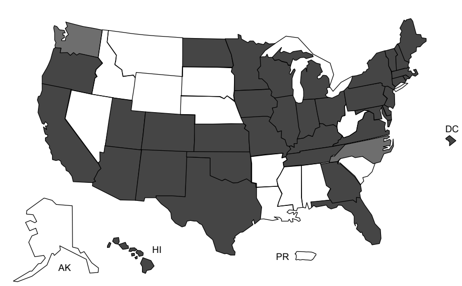
- First *identified* in France in 1998; woman with PMC
- Later found to have been in US in 1980's
- Not considered to be relevant until 2004!
- In Ontario (in pigs) at least since 2000

Yearly *Clostridium difficile*–related Mortality by Listing on Death Certificates, United States, 1999–2004.



From Redelings MD, et al. *Emerg Infect Dis.* 2007;13:1417-1419.

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



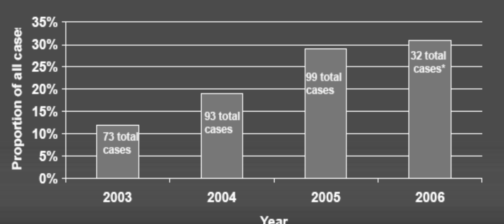
Countries in Europe with BI/NAP1/027, November 2007

Community Acquired CDI

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

Months since hospitalization	No. of patients	No. (%) without antimicrobial exposure within prior 30 days
>1 to 4 weeks	7	0
1-3 months	4	1 (25)
>3-12 months	6	1 (17)
> 12 months	44	18 (41)
Totals	61	20 (33)

Community-associated CDAD is Increasing, Atlanta VA Hospital



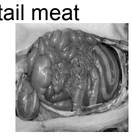
•Through March 31, 2006
•Chi-square for trend: $P<0.05$

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



Rodriguez-Palacios et al. Emerg Infect Dis 2007;13:485-7

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

Rodriguez et al 2006

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

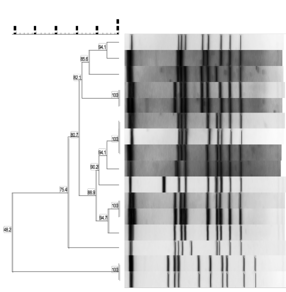
- Ontario (Rodriguez et al , In press)
  - *C. difficile* in 18% of retail ground beef/veal
  - Predominant strain, closely related to ribotype 027/NAP1
    - CDT+, 18 bp *tcdC* deletion, toxinotype III
- US (Songer et al, personal communication)
  - ~20% of various processed meats
  - Including ribotype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

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

Time	Tox V Isolates
Prior to 2001	10/6000
2001-2005	10/600
2006	6/125

Jhung MA, et al. Second International *Clostridium difficile* Symposium, June 6-9, 2007; Maribor, Slovenia.
Jhung MA et al. Emerg Infect Dis 2008;14:1039-45

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



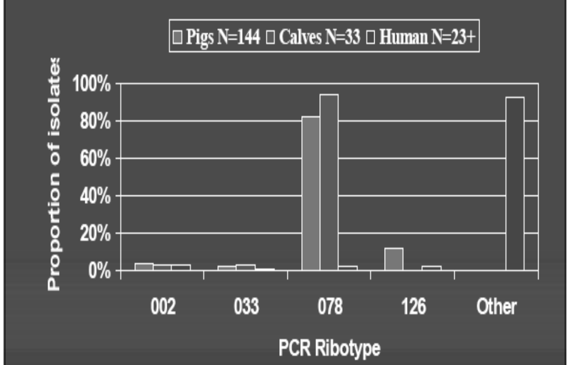
Source	Toxino type	Binary toxin	*tcdC* deletion
Human	V	+	39 bp
Human	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp
Human	V	+	39 bp
Hosp Env	V	+	39 bp
Human	V	+	39 bp
Human	V	+	39 bp
Human	V	+	39 bp
Human	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp
Pig	V	+	39 bp

Jhung MA, et al. Second International *Clostridium difficile* Symposium, June 6-9, 2007; Maribor, Slovenia.

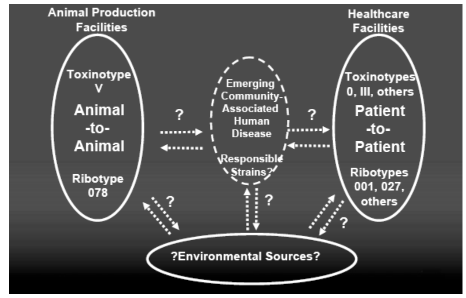
Epidemic Animal Strains Share Characteristics with the Human Epidemic Strain

Characteristic	Human Standard Strains	Human Epidemic Strain	Porcine Epidemic Strains	Bovine Strains
Toxinotype	0	III	V	V
PCR ribotype	001 and others	027	078	078
PFGE pattern	<80% related to NAP1	NAP1	NAP7 & NAP8	NAP7
Binary toxin	–	+	+	+
Deletion in *tcdC*	–	18 bp	39 bp	39 bp
TcdC protein	233 aa	65 aa	61 aa	61 aa

Keel K, et al. *J Clin Microbiol.* 2007;45:1963-1964.



Is there a food link?



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

Number of isolates	Ribotype	Toxin A/B	Binary Toxin
16	078	+	+
1	027	+	+
3	Unknown	+	+

Conclusions

- *Cl 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

- 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 hospitilization
- Intensive care units
- Antibiotic therapy
- Surgery
- Close contact with infected patient

Community Acquired

- Young
- Poor hygiene
- Shared contaminated items
- Crowded living conditions

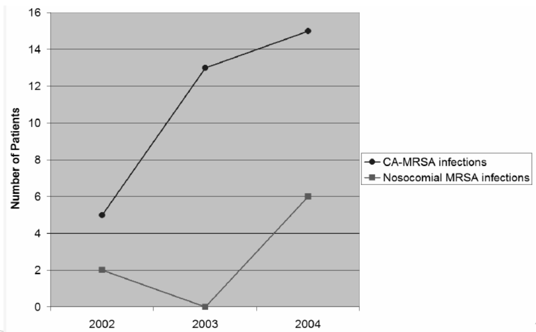
Schools
Correction centres

- 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

Prevalence of CA-MRSA in a Children's Hospital



Community-associated MRSA

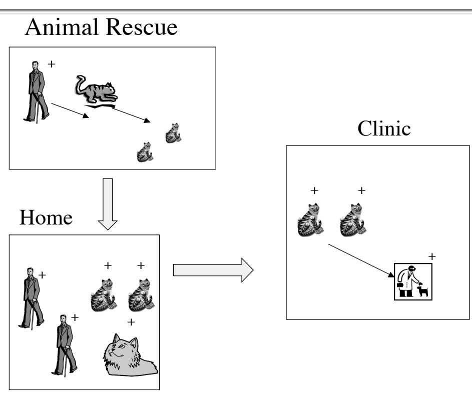
- Skin and soft tissue infections
- Bacteremia (and sequelae)
- Necrotizing pneumonia/fasciitis
- Toxic shock syndrome

CDC Public Health Image Library

MRSA in animals

- Horses
- Household pets
- Pigs
- Cattle
- Other

Household Pets



Cattle

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

- Mastitis in Belgium in 1972 (Devriese et al Res Vet Sci 1975)
- 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)

MRSA in pig farmers/families

- Identification of identical strains of MRSA in pigs and pig farmers/families in the Netherlands (Voss et al 2005)
  - Infected and colonized
  - 23% of pig farmers colonized
- Pig farmers **760** times more likely to carry MRSA than the general Dutch population

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?

MRSA-Food Reports

- Outbreak associated with BBQ pork and coleslaw from deli
- MRSA; enterotoxin C producing
- One food handler colonized with same strain

Jones et al *Emerg Infect Dis 1999*

Retail Meat

- 2/444 (0.5%) retail chicken samples in Japan (Kitai et al J Vet Med Sci 2005)
- 1/69 (1.4%) retail chicken samples in Korea (Lee Appl Env Microbiol 2003)

- Foodborne risks currently unclear
- Risks likely greater from animal and human contacts

- Food production risks likely more relevant for livestock personnel than food handlers and consumers

Conclusions

- Food represents a significant vehical for emerging human pathogens.
- *Cl difficile* likely a foodborne pathogen
- Less evidence for MRSA
- Establishing a foodborne link is first step to control.

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