Foodborne and Enteric Outbreaks: Microbiological Testing at the Public Health Laboratories

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www.oahpp.ca
Overview

• Laboratory testing for foodborne outbreaks in Ontario
• Public Health Laboratories branch of the OAHPP
  – Tests available and methodologies used
  – Interpretation of results
  – Critical to success
    • Laboratory requisition forms
    • Optimized sampling, specimen collection and transportation
• Other issues
  – Evolving role of the laboratory
    • Real time surveillance, eg PulseNet
    • Changing burden of proof
    • Development of new methods for detection and typing
Escherichia coli O157:H7 Infections Associated with Ground Beef from a U.S. Military Installation --- Okinawa, Japan, February 2004

In February 2004, the Okinawa Prefectural Chubu Health Center (OCHC) and the Okinawa Prefectural Institute of Health and Environment (OIHE), Japan, investigated three cases of Escherichia coli O157:H7 infection in a Japanese family associated with eating ground beef. Public health officials from multiple agencies in Japan and the United States collaborated on this investigation, which resulted in a voluntary recall of approximately 90,000 pounds of frozen ground beef in the United States and at U.S. military bases in the Far East. This was the first reported instance in which Japanese public health officials identified contaminated, commercially distributed ground beef that was produced in the United States. This report summarizes epidemiologic and laboratory investigations conducted by OCHC and OIHE. The results underscore the importance of using standardized molecular subtyping methods throughout the world to facilitate international public health communication and intervention.
Listeriosis Outbreak Canada 2008

57 cases of listeriosis
22 deaths *
*with listeriosis as underlying or contributing cause
### Laboratories Performing Testing for Foodborne Outbreaks in Ontario

#### Clinical Samples
- **Public Health Laboratories/ OAHPP**
  - Confirmation, subtyping, detection of rare organisms
  - Primary specimens in outbreak
- **Community and hospital labs:**
  - Routine stool testing from primary specimens
- **National Microbiology Laboratory/ Health Canada**
  - Specialized testing such as botulism

#### Food Samples
- **Public Health Laboratories/ OAHPP**
  - Testing for support of Public Health Inspectors in investigation of outbreaks and safety investigations
- **Health Canada**
  - Specialized testing eg botulism, Hep A
- **CFIA**
  - Testing of commercial products, manufacturers, plants
- **OMAFRA/ Guelph Laboratories**
  - Testing of agricultural programs

N.B. Lesser role of private labs during outbreaks

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Role of the Public Health Laboratories, OAHPP in Foodborne Outbreak Investigations

• **Primary goals**
  – To support Public Health Inspectors’ foodborne investigations
  – To integrate and communicate results to stakeholders
  – Develop and implement state of the art methodologies to improve laboratory detection of foodborne pathogens
Role of the Public Health Laboratories, OAHPP in Foodborne Outbreak Investigations

- Full capacity to investigate all aspects of foodborne outbreaks
  - Clinical (e.g., stool samples)
  - Environmental (e.g., food and water)
  - Molecular surveillance (aka “finger printing”)

- Serves as reference laboratory to other clinical laboratories
  - E.g., all E. coli O157 across the province is submitted to PHL

- Integrated with PulseNet
  - National and international surveillance system
Overview of Laboratory Sections and Testing Involved in Managing a Bacterial Foodborne Outbreak

• Clinical, Environmental and Molecular Surveillance Laboratories

• Example of the Salmonella Enteritidis PT13 Outbreak (2005)
  – Bean sprouts 101
  – Test procedure for isolating Salmonella from bean sprouts

• Challenges highlighted by this outbreak
Bean Sprout Outbreak 2005

- **May 2005**
  - Increase in Salmonella Enteritidis PT13 in
  - The Reference Enteric Laboratory & NML in Winnipeg

- **Epidemiologists at MOHLTC initiated surveillance activities with the Health Units**
  - Food attribution was not identified

- **October 2005**
  - A dramatic increase in SE PT13 isolates
  - Suspected food attribution was determined and the laboratory began receiving bean sprout samples for testing
## S. Enteritidis PT13 Statistics Year 2004 – March 2006

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<thead>
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<th>Month</th>
<th>2004</th>
<th>2005</th>
<th>PT13</th>
<th>% SE PT13</th>
<th>PT - Other</th>
<th>% PT – Oth</th>
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<td>0</td>
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<td>43</td>
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<td>16</td>
<td>38.1</td>
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<td>430</td>
<td>94.3</td>
<td>26</td>
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<td>December</td>
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<td><strong>Total Oct/Nov/Dec</strong></td>
<td>81</td>
<td>693</td>
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Enterics Laboratory
Enterics Laboratory

- Primary samples are from human clinical specimens
  - stool specimens for **diagnostic testing**
  - isolates received for **reference testing**
- Diagnostic specimens routinely screened for
  - *Salmonella*
  - *Shigella*
  - *E.coli* O157:H7
  - *Campylobacter*
  - pathogenic *Yersinia*
- Reference isolates are submitted from external laboratories and the environmental lab for confirmatory testing and serotyping
Selective Plating / Isolation and Identification

Brilliant Green Sulfa Agar

Novobiocin Brilliant Green Agar
Serological Identification
Question: What organisms are routinely tested for in bacterial cultures of stool?

Choose one or more

- 1) Shigella spp.
- 2) Clostridium perfringens
- 3) All verotoxin producing E coli
- 4) Salmonella spp.
- 5) Yersinia spp.
- 6) Norovirus

Are there any more that are routinely performed?
Answer: Organisms are routinely tested for in bacterial cultures of stool

Red highlighted are correct answers:

• 1) *Shigella* spp.
• 2) *Clostridium perfringens*
• 3) All verotoxin producing *E coli*
• 4) *Salmonella* spp.
• 5) *Yersinia* spp.
• 6) Norovirus
Answer: Organisms are routinely tested for in bacterial cultures of stool?

Typically routine stool cultures are screened for

- *Salmonella*,
- *Shigella*,
- *E coli O157*,
- *Campylobacter*,
- and *Yersinia*

Others that can be requested specifically include

- *Clostridium difficile*
- *Clostridium perfringens*
- Non O157 shiga toxin producing *E coli*
- *Aeromonas*,
- *Pleisiomonas*,
- norovirus PCR,
- parasites…

*NB.* may require different transport media
Environmental
(AKA food and water)
Laboratory
Environmental Microbiology Laboratory

• Specimens received for testing include:
  – Foods submitted by Public Health Inspectors
  – Water from private citizens and beaches,
  – Water for various outbreak investigations
  – Environmental swabs / samples

• Testing performed on foods based on food type and risk

• Tests may include
  – Aerobic plate count, coliforms, E. coli, Total gram negatives, B. cereus, S. aureus, Clostridium perfringens, Listeria, Salmonella, Campylobacter, VTEC, Yersinia and Vibrio

• Testing for Clostridium botulinum is referred to the Health Canada - Botulism Reference Center in Ottawa
Sampling of Food

Heterogeneously distributed
Complex matrices
• Easy to miss rare bacteria in a sample

Concern about cross contamination

Preserving integrity of viable organisms for testing

Figure 1. Single sampling (two-class) attribute plans for sample sizes
n = 3, 5, 10, 15, 20, 30, 60, and c = 0.
Laboratory Testing for Foodborne Outbreaks: Food Specimens

Specimen types
- Food product consumed by ill person
- Open vs closed samples
- Products from producer

Complexities of food testing
- Heterogeneous distribution
- Natural inhibitors, eg pH, salt content

Use “indicators” to determine risk
- Gram negative bacilli
- E coli
- Total aerobic plate count
Methods for Microbiological Diagnosis in Food

Culture is gold standard
- Ensures viability of bacteria
- Enables typing of strains
- Clinical data correlated
- Enumeration required for some bacteria
  - Clostridium perfringens spores

Other methodologies used (eg for toxin testing)
- Enzyme immunoassays
- PCR based technologies
- Nanotechnologies with multiplexing capabilities being explored
Laboratory Testing for Foodborne Outbreaks: Food Specimens

Primary stages of Food Testing
• Processing
• Pre-enrichment
• Selective enrichment
• Plating for culture
• Biochemical confirmation
• Serotyping
• Molecular typing (PFGE)
Sample Processing and Pre-enrichment

Sample size – 25 grams
Pre-enrichment broth added

Sample is mechanically homogenized to maximize organism recovery
Selective Enrichment

Tetrathionate Brilliant Green is an enriched liquid medium which contains bile salts, tetrathionate and Brilliant Green used to inhibit the growth of gram positive and negative normal intestinal flora.
Challenges for Detecting Pathogens from Food

- Often delays, sometimes unable to have ongoing access to suspected foods for sampling
- Most food samples are not fresh
  - This delay in testing could affect the ability to recover pathogenic microorganisms
- Mung bean seeds and sprouts contain adequate nutrients to sustain bacterial growth
  - Routinely contain high numbers of microbial flora including coliforms and fecal coliforms
- Isolation of Salmonella by selective enrichment and plating are challenged by this normal flora
  - Especially if low numbers of pathogen are present
How do bean sprouts become contaminated with Salmonella?

Tracking farm to fork:

- Contamination of the seeds
  - In the field, agricultural water
    - improperly managed animal manure, contact with wild animals and unsanitary production practices or sprout handling by the public
- Contamination during sprouting process
  - Seeds soaked in chlorinated water prior to germination
  - grown in the dark in bins or beds at 21-26C for 4-7 days
  - Sprayed with water every 4 hours to maintain a humid growing environment
- Bean sprouts distributed for human consumption, animal fodder and green manure causing potential for illness

Molecular Surveillance (AKA fingerprinting) Laboratory
Molecular Surveillance Laboratory

• Enteric outbreaks determined by
  – Isolate relatedness/cluster with epidemiological support
  – Human and food attribution

• Standardized methods used
  – Pulse-Field Gel Electrophoresis (PFGE)
  – Phage typing (PT) – performed at the National Microbiology Laboratory (NML) in Winnipeg

• Organisms routinely tested by PFGE include MRSA, VRE, E.coli O157:H7

• Organisms tested upon request for outbreaks include Salmonella, Shigella and pathogenic Yersinia
Pulsed Field Gel Electrophoresis (PFGE): “Fingerprinting” for Foodborne Outbreak Investigation

PFGE strain typing is a tool to support:

– Defining a common cluster of cases
– Potential relatedness among clusters
– Relatedness of a particular case to previously defined cluster
– An association between clinical cases and implicated foods

• Epidemiological information is needed for determination of outbreak
Basic Assumption of PFGE:
Changes in DNA Suggestive of Relatedness to Original Clone
DNA Cut Using Restriction Endonucleases

Diagram showing the cutting of DNA by restriction endonucleases.
DNA fragments Separated by Pulse Field in Agarose Gel

Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14°C
Interpretation of PFGE Patterns

- **Unrelated**
- **Moderately related**
- **Highly related**
- **Identical**

Diagram showing DNA banding patterns with samples 1 to 10.
PFGE Comparison of Isolates in Public Health

• Relatedness determined using specialized software
• Enables comparisons between PFGE from different laboratories
Role of PFGE in Enteric Outbreak Investigation

PFGE strain typing is a tool to support:

– Defining a common cluster of cases
– Potential relatedness among clusters
– Relatedness of a particular case to previously defined cluster
– An association between clinical cases and implicated foods

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Pulsed Field Gel Electrophoresis: 2005 Sprout Outbreak

Lanes 1, 7 and 13 Reference Lanes
Lanes 2 - 6, 8 – 10, Stool samples isolates (SENXAI.0038)
Lanes 11 - 12, Bean sprout isolates
Disadvantages and Potential Pitfalls of the Use PFGE in Outbreaks

• Pragmatic constraints
  – Time consuming (2-3 days to complete after fresh culture)
  – Requires a high-level technical skill

Requires Clinical and Epidemiological Information for Interpretation

• Degree of differentiation may not be appropriate for a particular organism or outbreak
  – Does not work for everything (i.e. clonal patterns)
  – PFGE with one restriction endonuclease may not provide sufficient discriminatory power
  – Some strains are untypable by PFGE
PulseNet

- National and international program of microbiologic typing particularly for enteric organisms
- Standard typing method:
  - Pulsed Field Gel Electrophoresis
  - Secondary methods being introduced
- Most organisms are processed at provincial lab and image uploaded to Winnipeg for Analysis
  - Eg Salmonella
- Use of data for baseline and ongoing surveillance of enteric pathogens
Occurrence of PFGE pattern LMACI.0040 in the PulseNet Canada Database

(secondary enzyme results in parentheses)

NB This is crude data and may not include every isolate

- Oct. 1996: ON=1 (LMAAI.0003)
- Nov. 1999: ON=1 (LMAAI.0004)
- Oct. 2001: CFIA/l Liverwurst sausage

Date received in PFGE lab

- Nov/Dec 2006: 2 (LMAAI.0001)
- March 2007: 1 (LMAAI.0001)
- July 2007: 1 (LMAAI.0001)
- August 2007: 2 (LMAAI.0001)
- Sept 2007: QC=1 LMAAI.0003 =1 (LMAAI.0001)
- Oct 2007 =1 (LMAAI.0001)
- April 2008: =1 (LMAAI.0001), =1 raw sausage (LMAAI.0420)
- July 2008 ON=2 (LMAAI.0539), 1 (LMAAI.0001)
- August 2008 =1 (LMAAI.0001), ON= 6 (4 with LMAAI.0003, 2 with LMAAI.0001)

- PFGE patterns of investigation food samples characterized by Health Canada BMH lab (Dr. Pagotto) as of 2008-08-14: all LMACI.0040, LMAAI.0001
- LMACI.0040 has not been recorded in the PulseNet USA database in the past 120 days, and there are no currently active clusters on their discussion board

From PulseNet Courtesy of Celine Nadon
Alternative Methods for Molecular Epidemiology

- **Multiple-locus variable-number tandem-repeats analysis (MLVA)**
  - Looks at repetitive sequences within a genome
  - Isolate 1: TAACCG
  - Isolate 2: TAACCGTAACCGTAACCG
  - Isolate 3: TAACCGTAACCGTAACCGTAACCG
  - Advantages quicker and more automated than PFGE
  - Slightly less discriminatory than PGFE for E Coli O157 performed with 2 enzymes, *XbaI* and *BlnI*.
  - Questionable increase in epidemiologic concordance

- **Multiple-locus sequence typing (MLST)**
  - Sequencing of 5-7 “housekeeping genes”
Genome Sequencing … A tool for the future
Understanding Challenges to Laboratory Detection of Foodborne Outbreaks

• Testing
  – Polymicrobial samples
  – Unequal distribution of organisms in food
  – Time consuming
  – Analytic limitations

• Pre and post analytic
  – Requisition incomplete
  – Sampling and sample integrity
  – Transportation
  – Interpretation of results

• Integration of results from different laboratories
• Legal samples
Components of Laboratory Testing

Pre-analytic
- Requisition
- Sample

Post-analytic
- Interpretation
  • requires understanding of limitations of test method
- Communication of results

Public Health Inspectors Role is Integral to Pre-Analytic and Post –Analytic Components
Laboratories Performing Testing for Foodborne Outbreaks in Ontario

Clinical Samples

- Community and hospital labs: Routine stool testing from primary specimens
- National Microbiology Laboratory/ Health Canada: Specialized testing such as botulism

Food Samples

- Public Health Laboratories/ OAHPP: Testing for support of Public Health Inspectors in investigation of outbreaks and safety investigations
- OMAFRA/ Guelph Laboratories: Testing of agricultural programs
- N.B. Lesser role of private labs during outbreaks

The Challenge:
Linking Clinical and Food Laboratory Results for Outbreak Management
Legal Aspects of Food Borne Outbreak Investigation

• Increasing focus on laboratory evidence with legal cases
• Role of finalized results balanced with public health threat and economic impact
• Legal samples require a legal seal
  – Usually reserved for suspicion of intentional tampering or bioterrorism
  – Chain of custody
• Otherwise, aseptic standardized collection procedures enhances smooth intra-agency transfer
Close link between Epidemiology and Laboratory Testing

- Significance of laboratory results not meaningful without epidemiological link
- Increasing emphasis on laboratory results due to economic and legal ramifications
- As outbreaks handled in real time, often incomplete information
Another Example of Laboratory Support for Foodborne Outbreaks
Salmonella Saintpaul Outbreak USA 2008

- May 22, 2008
  - 4 cases of same PFGE pattern of *Salmonella* Saintpaul reported to CDC by New Mexico Department of Health
  - 15 other isolates not yet characterized
- Total of 1,442 cases to Aug 25th
  - 286 hospitalized, 2 deaths
- 43 states, District of Columbia and Canada
- April 16th-August 11, 2008
FIGURE 1. Number* and incidence rate† of laboratory-confirmed cases of *Salmonella* Saintpaul (outbreak strain), by state — United States, 2008§

* N = 1,442.
† Per 1 million population.
FIGURE 2. Number of laboratory-confirmed cases (n = 1,414) of *Salmonella* Saintpaul (outbreak strain), by date of illness onset — United States, 2008*

*Includes cases with onset information received as of August 25, 2008. Some illness onset dates (n = 366) were estimated by subtracting 3 days from the specimen date. Illness that began during July 29–August 25 might not yet be reported.*
Implicated Foods for *Salmonella* Saintpaul Outbreak

- Jalepeno peppers
- Serrano peppers
- Possibly tomatoes (initial case control OR 6.7)

- Advisories since June 3rd
- FDA warnings
  - June 3- July 16th
    - avoid raw red round, red Roma, and red plum tomatoes
  - July 21st-Aug 25th
    - Recall and advice against consuming jalapeno and serrano peppers grown, harvested or packed in Mexico
What is the Difference Between Jalepeno and Serrano Peppers?
Future Directions

Introduction of Molecular Diagnostics

- More sensitive PCR based methods
- Nanotechnology
- “Point of care” devices
- Could be applied to both clinical and food specimens
Early Integrated Warning Systems

- Computer based triggers to include
  - Laboratory testing submission numbers
  - Laboratory results
  - Linkages with other laboratories
- Potential linkages to other data in real time
  - Syndromic surveillance data
  - Public health reporting data
Summary

- Laboratory services widely available with capacity to detect and type for outbreak support
- All laboratory results require clinical and epidemiological back up
- Accuracy of the testing requires appropriately filled requisition, good sampling techniques, and rapid transportation
- Understand methods available and the strengths and limitations
- Phone us if there are any questions
- Dr. Vanessa Allen 416 235-5806.
Thank you.