Culture-Independent Diagnostics: Impact on Public Health

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The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention
Culture-Independent Diagnostics: Impact on Public Health

- Overview of issues
- Strategies for Public Health
- Regulatory role?
Bacterial Culture
Available for many conditions, many microbes
- invasive, respiratory, sexually transmitted, and enteric diseases
- bacteria, RNA/DNA viruses, parasites, fungi, toxins

Multiple technologies (nucleic-acid based, antigen-based, metabolite based)

Multi-analyte panels
## Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>Rapid/culture-independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speed</strong></td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td><strong>Infrastructure needed</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Expertise required</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Labor cost</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Cost of materials</strong></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Culture or standard tests (e.g. microscopy)</td>
<td>Rapid/culture independent tests</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Gold standard</td>
<td>Low to high</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>High</td>
<td>Low to high, almost always different</td>
</tr>
<tr>
<td><strong>Interpretation of positive findings</strong></td>
<td>Usually straightforward</td>
<td>Significant issues</td>
</tr>
<tr>
<td><strong>Range of pathogens detected</strong></td>
<td>All pathogens allowed by growth or test conditions</td>
<td>Limited to specific pathogen tested</td>
</tr>
<tr>
<td><strong>Allows for susceptibility testing &amp; genotyping?</strong></td>
<td>Yes</td>
<td>Generally no</td>
</tr>
</tbody>
</table>
Demise of GC Culture

Rapid NAA test:

- Fast (hours)
- Urine specimen (vs urethral swab)
- Includes *Chlamydia trachomatis*
- High sensitivity
- No susceptibility data
- Specimen incompatible with culture
- Expensive
- Some concerns about false positives
Medical reasons for laboratory testing

- Appropriate treatment
- Prevent unnecessary treatment or procedures
Interim Guidance for the Detection of Novel Influenza A Virus Using Rapid Influenza Diagnostic Tests

For guidance on antiviral treatment see: CDC/ACIP/IDSA

http://www.cdc.gov/h1n1flu/guidance/rapid_testing.htm
Recommendations for Diagnosis of Shiga Toxin--Producing
Escherichia coli Infections by Clinical Laboratories

Importance of Culture Confirmation of Shiga Toxin-producing
Escherichia coli Infection as Illustrated by Outbreaks of
Gastroenteritis --- New York and North Carolina, 2005

*Escherichia coli* O157:H7 and other strains of *E. coli* that produce Shiga toxin are collectively known as Shiga toxin-producing *E. coli* (STEC). The current outbreak of STEC O157 infections associated with eating fresh spinach illustrates the importance of obtaining isolates to identify the source of the infections (1). Laboratory methods that do not require bacterial culture of stool specimens to identify STEC are being used increasingly by clinical diagnostic laboratories, sometimes without subsequent confirmation of a strain by isolating it in culture. This report describes findings from outbreaks of gastroenteritis in 2005 in New York and North Carolina in which clinical diagnostic laboratories initially used only non-culture methods to detect Shiga toxin (Stx). The findings highlight the importance of confirmation of Stx-positive stool specimens by bacterial culture for timely and reliable identification of STEC infections, including *E. coli* O157 and non-O157 STEC, to enable implementation of appropriate public health actions. An important part of that identification is...
Patient Management
Public health reasons for surveillance / outbreak investigation

- Limit transmission
- Control underlying problems
- Monitor trends → informed policy development
Impacts

- Patient Management
- Public Health Programs
  - Requiring accurate case counts
    - Burden
    - Attribution
    - Trends
  - Isolate-requiring
Estimates of Foodborne Illness
Relative rates of laboratory-confirmed infections with *Campylobacter*, *E. coli* O157, *Listeria*, *Salmonella*, and *Vibrio* compared with 1996-1998 rates, by year – Foodborne Diseases Active Surveillance Network (FoodNet), United States, 1996-2010†

† The position of each line indicates the relative change in the incidence of that pathogen compared with 1996-1998. The actual incidences of these infections cannot be determined from this graph.
Hierarchical scheme for categorizing foods into commodities

- All Food
  - Aquatic
    - Fish
    - Shellfish
    - Dairy
    - Crustaceans
    - Mollusks
  - Land animals
    - Eggs
    - Meat-Poultry
    - Meat
      - Beef
      - Game
      - Pork
      - Poultry
  - Plant
    - Grains-beans
    - Oils-sugars
    - Produce
      - Fruits-nuts
      - Vegetables
        - Fungi
        - Leafy
        - Root
        - Sprout
        - Vine-stalk

Painter et al, J Food Protection 2009
Addressing CIDT: Burden, Attribution, Trends

- Determine extent of issue
- Study test performance
- Redefine case definitions
Impacts

- Patient Management
- Public Health Programs
  - Requiring accurate case counts
    - Burden
    - Attribution
    - Trends
  - Isolate-requiring
    - Subtype-based tracking programs
    - Susceptibility monitoring
    - Subtype-based attribution studies
## Selected Microbial Disease Agents Under Surveillance

<table>
<thead>
<tr>
<th>Agent</th>
<th>Public health surveillance</th>
<th>Isolate significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td>Shigatoxin-producing <em>E. coli</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Genotype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>AST</td>
<td>+++</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Subtype, AST</td>
<td>+++</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Subtype (outbreaks)</td>
<td>++</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Serotype, AST</td>
<td>++</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td>AST</td>
<td>+</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
<td>Subtype (outbreaks)</td>
<td>+</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>AST</td>
<td>?</td>
</tr>
</tbody>
</table>
Making Food Safer to Eat
Reducing contamination from the farm to the table

Each year, roughly 1 in 6 people in the US gets sick from eating contaminated food. The 1,000 or more reported outbreaks that happen each year reveal familiar culprits—Salmonella and other common germs. We know that reducing contamination works. During the past 15 years, a dangerous type of E. coli infection, responsible for the recall of millions of pounds of ground beef, has been cut almost in half. Yet during that same time, Salmonella infection, which causes more hospitalizations and deaths than any other type of germ found in food and $365 million in direct medical costs annually, has not declined. Each year, 1 million people in the US get sick from eating potentially contaminated foods.
Global Meat Trade

Source: Center for Global Food Issues
Nationwide reporting began in 1912.


- **Typhoid Fever**
- **Non-typhoid Salmonellosis**

CDC, National surveillance data.
A National Outbreak of *Salmonella enteritidis* Infections from Ice Cream


May 16, 1996
Nationwide reporting began in 1912.

Reported *Salmonella* infections in the United States, 1920-2006

- **Typhoid Fever**
- **Non-typhoid Salmonellosis**

CDC, National surveillance data
87 labs in the PulseNet USA network

- CDC PulseNet headquarters
- Regional labs
- Local and secondary state labs
- Federal labs

December 2011
The decade's 10 biggest food-borne illness outbreaks (from CNN)

By Jacque Wilson, CNN
updated 8:00 AM EDT, Thu November 3, 2011

1. 2011: Cantaloupes (*Listeria monocytogenes*)
2. 2011: Turkey (*Salmonella*)
3. 2010: Eggs (*Salmonella*)
4. 2010: Celery (*Listeria monocytogenes*)
5. 2008: Jalapeno and Serrano peppers (*Salmonella*)
6. 2008 – 2009: Peanut butter (and peanut-containing products; *Salmonella*)
7. 2006: Spinach (bagged; *E. coli* O157:H7)
8. 2005 – 2006: Tomatoes (4 outbreaks; *Salmonella*)
9. 2004: Tomatoes (*Salmonella*)
10. 2002: Deli meat (*Listeria monocytogenes*)
11. 2001: Cantaloupe (*Salmonella*)
Multistate Foodborne Outbreak Investigations, 2012 (so far)

- Ground Beef - *Salmonella* Enteritidis
- Live Poultry - *Salmonella* Hadar
- Live Poultry - *Salmonella* Montevideo
- Multistate Outbreak – *Escherichia coli* O145 Infections
- Live Poultry - *Salmonella* Infantis, Newport, and Lille
- Dry Dog Food - *Salmonella* Infantis
- Raw Scraped Ground Tuna Product - *Salmonella* Bareilly and *Salmonella* Nchanga
- Small Turtles - *Salmonella* Sandiego, *Salmonella* Pomona, and *Salmonella* Poona
- Raw Clover Sprouts at Jimmy John’s Restaurants - *Escherichia coli* O26
- Restaurant Chain A - *Salmonella* Enteritidis
A large outbreak in one place may be obvious
An outbreak with persons in many places may be difficult to detect, unless cases can be linked.
PulseNet/VetNet Electronic Communication

State and Local Public health laboratories

PFGE patterns (~50,000/yr to PulseNet)

E. coli pathogens

Salmonella sp. and Campylobacter spp.

National databases

State Departments of Agriculture

FDA

USDA

VetNet
Pathogen Specific Surveillance

Case reports

Clinical Microbiology

Public Health

Case interviews

Prevention / control activities

FDA

State/local Vet/Ag labs

PulseNet

OutbreakNet

CDC

USDA

IPHL
Preventing Foodborne Disease

Farm → Transport Processing Distribution → Preparation
Preventing Foodborne Disease

- Farm
- Transport
- Processing
- Distribution
- Preparation

Disease surveillance
Preventing Foodborne Disease

Farm → Transport → Distribution → Preparation

Disease surveillance

Limit ongoing illness
### Largest U.S. Food Recalls in which PulseNet Played a Prominent Role

<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen</th>
<th>Food</th>
<th>Amount recalled</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td><em>Listeria monocytogenes</em></td>
<td>Cantaloupe</td>
<td>Unknown</td>
</tr>
<tr>
<td>2011</td>
<td><em>Salmonella</em> Heidelberg</td>
<td>Ground turkey products</td>
<td>&gt;36,000,000 lbs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella</em> Enteritidis</td>
<td>Shell eggs</td>
<td>&gt;500,000,000 eggs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella</em> Montevideo</td>
<td>Ready-to-eat Italian sausage products/pepper</td>
<td>&gt;1,263,754 lbs</td>
</tr>
<tr>
<td>2009</td>
<td><em>E. coli</em> O157:H7</td>
<td>Cookie dough</td>
<td>300,000 cases of product</td>
</tr>
<tr>
<td>2009</td>
<td><em>Salmonella</em> Typhimurium</td>
<td>Peanut butter/peanut products</td>
<td>&gt;3000 types of products</td>
</tr>
<tr>
<td>2008</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>5,300,000 lbs</td>
</tr>
<tr>
<td>2007</td>
<td><em>Salmonella</em> I 4,5,12:i:-</td>
<td>Frozen pot pies</td>
<td>Millions of pot pies</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen pizza</td>
<td>5,000,000 pizzas</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef (3 outbreaks)</td>
<td>35,400,000 lbs</td>
</tr>
<tr>
<td>2006</td>
<td><em>Salmonella</em> Tennessee</td>
<td>Peanut butter</td>
<td>326,000,000 lbs</td>
</tr>
<tr>
<td>2004</td>
<td><em>Salmonella</em> Enteritidis</td>
<td>Raw almonds</td>
<td>13,000,000 lbs</td>
</tr>
<tr>
<td>2003/’09</td>
<td><em>E. coli</em> O157:H7</td>
<td>Blade Tenderized Frozen Steak</td>
<td>865,046 lbs</td>
</tr>
<tr>
<td>2002</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>27,400,000 lbs</td>
</tr>
<tr>
<td>2002</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>18,600,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>16,900,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>1,100,000 lbs</td>
</tr>
<tr>
<td>1998</td>
<td><em>Listeria monocytogenes</em></td>
<td>Hot dogs, deli meats</td>
<td>35,000,000 lbs</td>
</tr>
<tr>
<td>1998/’08</td>
<td><em>Salmonella</em> Agona</td>
<td>Toasted oats cereal</td>
<td>&gt;3,000,000 lbs</td>
</tr>
<tr>
<td>1997</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen ground beef</td>
<td>25,000,000 lbs</td>
</tr>
</tbody>
</table>
Preventing Foodborne Disease

Farm → Transport → Processing → Distribution → Preparation

Disease surveillance
Limit ongoing illness
fix underlying problems
Industries Stimulated to Change by PulseNet-Triggered Investigations

Ready-to-eat & "ready-to cook" foods
- Peanut products
- Sprouts
- Leafy greens
- Other vegetables
- Mellon
- Poultry
- Beef
- Eggs
- Tree nuts
- Spices
The foodborne disease surveillance system is to the food industry what radar is to automobile drivers – it is the “threat” of being caught that helps drive compliance with best safety practices.

from Mike Doyle, Ph.D;
CDC Public Health Grand Rounds November 2009
Under-appreciated Sources of Infection

- *Salmonella* in reptiles and amphibians
- Multiple pathogens from live bird markets
- *Salmonella* from microbiology lab exposure
- *Salmonella* in “feeder” mice
- *Vibrio vulnificus* after fish handling
- *Salmonella* in baby chicks and ducks
- *Salmonella* in owl pellets
- STEC in petting zoos
PulseNet Protocols

- *E. coli* O157
- Non-O157 STEC (VTEC)
- *Salmonella*
- *Shigella*
- *Listeria monocytogenes*
- *Vibrio cholerae*
- *Vibrio parahaemolyticus*
- *Campylobacter jejuni/coli*
- *Clostridium perfringens*
- *Clostridium botulinum*
- *Yersinia pestis*

www.pulsenetinternational.org
Bacterial isolates from humans uploaded to PulseNet USA, and identified clusters, 1996-2011†

† Data are preliminary and subject to change
* data type information may not be complete for these years
PulseNet-Based Surveillance

- Each year PulseNet U.S.A. identifies:
  - ~1,500 clusters at local/state level
  - 200 multi-state clusters investigated
Incidence and Outbreaks of *Listeria*, 1978-2010
(from FoodNet and Foodborne Disease Outbreak Surveillance System)

- **Before PulseNet**
  - 1978-1997 (20 years)
  - 5 outbreaks (2 multistate)
  - Average 54 cases/outbreak

- **Era of PulseNet**
  - 1998-2004 (7 years)
  - 13 outbreaks (4 multistate)
  - Average 22 cases/outbreak

- **Era of Listeria Initiative**
  - 2005-2010 (6 years)
  - 19 outbreaks (4 multistate)
  - Average 18 cases/outbreak

Healthy People 2020 Goal: 2 outbreaks per 1 million
Costs and Benefits of a Subtype-Specific Surveillance System for Identifying *Escherichia coli* O157:H7 Outbreaks

Elamin H. Elbasha,* Thomas D. Fitzsimmons,*† and Martin I. Meltzer*

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and †Colorado Department of Public Health and Environment, Denver, Colorado, USA

We assessed the societal costs and benefits of a subtype-specific surveillance system for identifying outbreak-associated *Escherichia coli* O157:H7 infections. Using data from Colorado, we estimated that if it averted five cases annually, the system would recover all its costs.

*Escherichia coli* O157:H7 infections pose a nationally. After the outbreak was traced to the
83 member countries from 7 national and regional PulseNet networks

- PulseNet Canada
- PulseNet USA
- PulseNet Latin America & Caribbean
- PulseNet Europe
- PulseNet Africa
- PulseNet Middle East
- PulseNet Asia Pacific

December 2011
International Outbreaks of Shigellosis in Denmark and Australia in 2007 Associated with Imported Baby Corn from Thailand

By author name and date

Two outbreaks of Shigella sonnei infections simultaneously detected in Denmark and Australia were found to be linked to the same baby corn packing house in Thailand. PulseNet played a key role in confirming this link when

International Outbreak of Salmonella Senftenberg Infection in 2007 Associated with Consumption of Fresh Basil Imported from Israel

Multiple authors, Eurosurveillance, Volume 12, Issue 24, 14 June 2007

PFGE analysis performed according to the PulseNet Salmonella protocol by researchers in Europe and the United States, and shared through the PulseNet International network and the former European Enter-net, was instrumental in delineating this outbreak caused by Salmonella Senftenberg and confirming its source: fresh basil from Israel.

Read original

International Outbreak of E. coli O157 Infections Linked to Ground Beef Patties of a Particular Brand

CDC, October 26, 2007

A joint investigation between the Canadian Food Inspection Agency and the U.S. Department of Agriculture found that a multi-state outbreak of E. coli O157: H7 in the USA was linked to a particular brand of ground beef produced by a Canadian beef-producing firm. The outbreak was significant in terms of its health impact as 217 million pounds of meat were recalled.

International outbreak of Shigella sonnei associated with air travel to Hawaii in 2004

By multiple authors, Japan Journal of Infectious Diseases, July 13, 2006

In September 2004, investigations of two outbreaks of Shigella sonnei took place simultaneously in Japan and the United States. The collaboration through PulseNet International linked both outbreaks to the same source: air travel to Hawaii. In the United States, the Hawaii Department of Health contacted the Minnesota Department of Health (MDH) to inform them of a potential outbreak of S. sonnei associated with domestic and international air travel to Hawaii. PulseNet USA participants in the MN laboratory alerted the rest of PulseNet USA via the PulseNet listserve by posting two different S. sonnei patterns detected with XbaI that were obtained from...
Current PulseNet Methods (PFGE and MLVA) are Isolate-Dependent

(Note: so is whole genome sequencing, and most other methods being considered)
Companies with Multi-Analyte Gastro Panels in Development

- Luminex xTAG panel (in FDA clearance)
  - Bacteria (10)
  - Viruses (3)
  - Parasites (3)
- Verigene (research use only)
- At least 8 other companies have multi-analyte panels in various stages of development
Nationwide reporting began in 1912
Reported *Salmonella* infections in the United States, 1920-2006

- Typhoid Fever
- Non-typhoid Salmonellosis

**Incidence per 100,000 population**

**Years**


**National salmonella serotype surveillance**

CDC, National surveillance data
General Strategies to Address Issue

- **Short-term**: Preserve isolates
- **Longer-term**: Develop culture-independent pathogen characterization methods
- **Very long-term**: exploit paradigm shifting technologies
Reflex Culture

Follow-up culture automatically initiated when positive culture independent-based laboratory test results are observed.

(possible when the specimen collected is compatible with culture)
Short-term: Preserve isolates

- Work with medical industry to make new tests compatible with public health needs
- Consider public health impacts in the device licensure process
- Make reflex culture reimbursable?
- Modify State reportable disease rules
- Develop isolate recovery capacity for PHLs
- Sentinel culture-based surveillance?
Longer-term: Develop culture-independent pathogen characterization methods

- Identify ID/subtype/virulence targets for direct molecular detection and characterization
- Exploit new technologies (metagenomics, single-cell sequencing)
Opportunities

- Faster results (better exposure recall, faster intervention)
- Wider understanding of disease causation
Questions

- How can the public health impact of certain test results be better emphasized as test systems are cleared by FDA?
- Are there ways in which the CLIA program can promote public health recommendations (e.g. supporting CDC guidelines and recommendations)?