PulseNet USA: Overview of the Molecular Subtyping Network for Foodborne Disease Surveillance

Kelley B. Hise
May 2009
Overview

- PulseNet Network
  - What is PulseNet
  - Objectives
  - Basic Elements

- PulseNet Communication and QA/QC
  - QA/QC: PFGE Lab and Data Analysis/CDC Team
  - Certification and Proficiency Testing

- Active Surveillance
  - PulseNet Network and Activity
  - Cluster Searches
  - Epidemiologic Investigations

- Database Uses
  - Pattern Frequencies
  - Trends
What is PulseNet USA?

- Established in 1996, The Molecular Subtyping Network for Foodborne Disease Surveillance
- A national network of >75 state and local public health/food regulatory agency laboratories (USDA, FDA) coordinated by CDC and APHL
- Perform standardized molecular typing of foodborne disease-causing bacteria by Pulsed-field gel electrophoresis (PFGE)
- Dynamic databases of DNA “fingerprints” at CDC—available on-demand to participants
PulseNet Objectives

- To detect foodborne disease case clusters that may be widespread outbreaks
- Provide real-time molecular surveillance of the most important bacterial foodborne diseases
- Assist epidemiologists in investigating outbreaks
  - Separate outbreak-associated cases from other sporadic cases (case definition)
  - Assist in rapidly identifying the source of outbreaks
- Act as a rapid and effective means of communication between public health laboratories
The National Molecular Subtyping Network for Foodborne Disease Surveillance

The map illustrates the network's coverage across the United States, with different areas highlighted to indicate regional centers and laboratories. The map includes symbols for PulseNet Headquarters, State/County/City/Veterinary/Agricultural Laboratories, USDA Laboratories, and FDA Laboratories. Each region is color-coded to denote the specific type of laboratory or network location.
The Three Basic Elements of PulseNet

1. Data acquisition

2. Data analysis

3. Data exchange
Communication and QA/QC

- On-line databases
- CDC Team postings
  - Cluster detection
  - Outbreak investigations
  - Active Cluster Reports/Bundles
  - Technical support
- “PulseNet News” Newsletter
- PulseNet Website
  (www.cdc.gov/pulsenet)
- Annual update meetings

- Standardized protocols and molecular size standards
- QA/QC Manual
- Standardized software and nomenclature
- Training workshops (lab & software)
- Certification and proficiency testing
QA/QC: PFGE Laboratory

- Follow standardized protocols as closely as possible
- Be consistent
- Document
  - Any variations observed in an individual run
  - Temperature of water baths
  - Expiration date and lot numbers of reagents
Before Image Analysis:
- Critically review the appearance of the gel
- Consider all points of the gel
- Print gel image for reference
- Make note of any obvious problems

PFGE Patterns of *E. coli* O157:H7

- Fragment Sizes (in kilobases)
  - 1135 Kb
  - 452.7 Kb
  - 216.9 Kb
  - 76.8 Kb
  - 33.3 Kb

DNA “fingerprints”
*Global Reference Standard*
QA/QC: Data Analysis/CDC Team

- Follow standardized protocols for analyzing TIFFs and marking bands
  - Name TIFFs according to PulseNet protocols
  - Check all band markings before uploading
- Pay close attention to data format
  - Dates: YYYY-MM-DD
  - Age: use Entry Properties screen to enter (YY-MM-DD)
  - Pull downs: Patient Sex, Serotype, Source Type, etc.
- CDC Team
  - Name bundle files according to PulseNet protocols
  - Follow standardized protocols for posting initial and reply postings
Certification

**Purpose:** to allow accurate comparisons within the PulseNet National databases by:
- Ensuring TIFFs of all gel images are comparable and of satisfactory quality
- Ensuring correct normalization and consistent band assignment

**Process**
- Certification sets include: 4 strains and instructions sent to requesting laboratory
- Individual completes the subtyping and submits the TIFF and/or bundle file
- TIFF and/or bundle file are evaluated to determine if they meet acceptable quality
- Report is sent and individuals certified in analysis receive access to that national database (SecurID fob, username, and password)
Proficiency Testing (PT)

- **Purpose:** To ensure laboratories meet/exceed the PulseNet standards of PFGE gel quality and analysis.

- **Process:**
  - Annually receive one PT isolate for each pathogen in which certified (every certified participant should run PT).
  - Perform subtyping; analyze in-house TIFF (currently only one TIFF is submitted per lab) and a TIFF sent by CDC.
  - Results from analysis of both gels are submitted for evaluation.
PulseNet Laboratory Network

- Participating Labs
- PFGE Patterns & Demographic Data
- PulseNet National Databases (CDC)
- Local Databases
- TAT from receipt to upload: ~4 working days
- Cluster Follow-Up/Communication w/Epis
- Database Management & Reports
- Cluster Detection

*Image of a map with various regions marked and a diagram illustrating the network flow.*
**PulseNet Activity***

*as of January 1, 2009

Over 375,000 PFGE patterns or DNA “fingerprints” submitted to PulseNet databases since 1996

<table>
<thead>
<tr>
<th>Database</th>
<th>Entries Submitted</th>
<th>Patterns submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Enzyme</td>
<td>2nd Enzyme</td>
</tr>
<tr>
<td><strong>Campylobacter</strong></td>
<td>6,008</td>
<td>5,959</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>35,414</td>
<td>34,070</td>
</tr>
<tr>
<td><strong>Listeria</strong></td>
<td>9,918</td>
<td>9,007</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td>221,806</td>
<td>219,026</td>
</tr>
<tr>
<td><strong>Shigella</strong></td>
<td>37,638</td>
<td>37,423</td>
</tr>
<tr>
<td><strong>V. cholerae</strong></td>
<td>312</td>
<td>291</td>
</tr>
<tr>
<td><strong>V. parahaemolyticus</strong></td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td><strong>Y. pestis</strong></td>
<td>2,202</td>
<td>2,202</td>
</tr>
</tbody>
</table>

*as of January 1, 2009
PulseNet Cluster Detection System

- PulseNet is a cluster detection tool, not an outbreak detection system
  - A PulseNet CLUSTER is a group of patterns that are found indistinguishable by PFGE
  - CLUSTERS of cases identified by PulseNet are investigated by epidemiologists
  - If epidemiologic links are found between cases, the cluster is classified as an OUTBREAK
What is a Cluster Search?

- Patterns submitted electronically
- Cluster searches performed
- Visually compare indistinguishable patterns with 1st enzyme, then 2nd (if necessary)
- Patterns/clusters named by CDC

Cluster of indistinguishable patterns by primary enzyme
Subject: 0802LACJPX-1c (JPXX01.0088)_LAC_Typhimurium

LAC has a cluster of 3 Typhimurium isolates that are indistinguishable by XbaI and BlnI. The collection dates are from 12/10/2007 to 1/10/2008. Epi under investigation. The isolate numbers are:

- LAC_Z19766, 2 year old boy, no travel
- LAC_Z19999, 43 year old female, no travel
- LAC_Z20072, 60 year old female, no travel

We have seen this pattern before in LAC. Our epi contact is (name and phone)

LAC08008PN.BDL
Posted: 05 March 2008 09:52 AM
MLVA Analysis

- Sequence-based subtyping
- Can further discriminate common PFGE patterns through highly variable target sequences
- Data may be epidemiologically more relevant than PFGE data
- Results more straightforward
- Currently MLVA results are housed in databases separate from PFGE; however, the ultimate goal is to have them in combined databases
Epidemiologic Investigations:

A large outbreak in one place

- Outbreak may be obvious
- Detected and investigated locally
Epidemiologic Investigations:
A dispersed outbreak in many places

- Detect outbreaks centrally (or locally) through surveillance (widely dispersed, organism too common to notice small increase, identify related cases)
  - Investigation coordinated centrally
- Distinguish from concurrent sporadic cases
  - Provide microbiological evidence of sources of outbreaks
Recent Foodborne Outbreaks With PulseNet Involvement

- *Salmonella Typhimurium* – Peanut Butter products
- *Salmonella Saintpaul* – Raw Produce
- *Salmonella Tennessee* – Peanut butter
- *Salmonella I 4,[5],12:i:-* – Pot pies
- *E. coli O157* – spinach; ground beef
- *Listeria monocytogenes* – Milk

*Just to name a few.......*
1993 Western States *E. coli* O157 Outbreak

- 726 ill
- 4 deaths
- 39 d

2002 Colorado *E. coli* O157 Outbreak

- Outbreak detected 2002
- 44 ill, no deaths
- 18 d
Database Uses: Pattern Trends

Long-term surveillance to see if a pattern is still seen as commonly in the database
Database Uses: Pattern Frequencies

Long-term surveillance to aide in active surveillance—is this a true increase?
What is the relative contribution of each food source to the burden of foodborne illness in humans?
Summary

- **PulseNet Network**
  - What is PulseNet
  - Objectives
  - Basic Elements

- **PulseNet Communication and QA/QC**
  - QA/QC: PFGE Lab and Data Analysis/CDC Team
  - Certification and Proficiency Testing

- **Active Surveillance**
  - PulseNet Network and Activity
  - Cluster Searches
  - Epidemiologic Investigations

- **Database Uses**
  - Pattern Frequencies
  - Trends
Questions?

Thank you for your attention
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