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

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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 Three Basic Elements of PulseNet

1. Data acquisition

2. Data analysis

3. Data exchange
Patient Specimen Collection → Specimen → Cell Suspension + Agarose = Cells Trapped in Plug
-Lyse cells

Finished Product!

Restriction
...TCTAGA...
...AGATCT...

DNA fingerprinting process
Intra-lab Comparison of PFGE Patterns

DNA “fingerprints”

*Global Reference Standard

- Online databases
- CDC Team postings
  - Cluster detection
  - Outbreak investigations
  - Active Cluster Reports/Bundles
  - Technical support
  - Reports
- “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

Fragment Sizes (in kilobases)
PulseNet Laboratory Network

- Participating Labs
- PFGE Patterns & Demographic Data
- PulseNet National Databases (CDC)

TAT from receipt to upload: ~4 working days

Cluster Detection

Local Databases

Cluster Follow-Up/Communication w/ Epis

Database Management & Reports
### 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>Vibrio cholerae</strong></td>
<td>312</td>
<td>291</td>
</tr>
<tr>
<td><strong>V. parahaemolyticus</strong></td>
<td>37</td>
<td>37</td>
</tr>
</tbody>
</table>
PulseNet Activity, 1996-2008

PFGE patterns submitted to PulseNet Databases
Molecular Surveillance

- Subtyping is the differentiation of isolates of the same species.
- PulseNet is a network of public health laboratories that use subtyping to detect, investigate, and control outbreaks of foodborne infections.
- Monitoring the trends of specific subtypes of a specific pathogen by a high discriminatory molecular subtyping method
  - Plasmid profiling
  - Pulsed-Field Gel Electrophoresis (PFGE)
  - Multi-Locus VNTR Analysis (MLVA)
  - SNP-analysis
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
Local Cluster Detection and Follow-Up

Initial Detection

- Perform cluster search within local database
  - Name local patterns and/or download CDC pattern names
  - Look for clusters of 3 or more within past 60 days (120 days for *Listeria*)
- Create a frequency graph for suspected pattern(s)
  - Look at pattern frequency over time
  - >2x increase over a month
  - May take into consideration seasonality of pattern
- Report local clusters internally using in-house procedures
Local Cluster Detection and Follow-Up

Initial Detection Continued…

- Compare pattern(s) to national database (only if necessary*)
  - Use match against server and/or other query tools to see matches in other labs
  - It is not necessary to post matches from other states to CDC Team

- Post message to CDC Team IF at least one of the following:
  - Epidemiologic links
  - Above normal pattern baseline frequency in local database
  - DO NOT include CDC pattern names or line lists

- Monitor topic on CDC Team for CDC’s and other laboratories’ responses

* If local epis, supervisors, etc. wish to see if there are matches at the national level/neighborhood states
Local Cluster Detection and Follow-Up

Existing Clusters on CDC Team

- Once a week save cluster bundle files provided by CDC in “Important PulseNet Documents” in local databases
  - See if anything new matches a cluster
  - If matches exist, refer to the Active Cluster Report to see if the cluster is still active

- Sign up to receive email notifications for all organisms on CDC Team

- When you receive new notification, wait for CDC to rename posting and post line list
  - If your local patterns are in list, download outbreak code
  - Continue to compare new patterns to posted bundle
**Local Cluster Detection**

**Routine PFGE typing**

→ **Upload patterns to Online server***

- **Vibrio spp.**
- **STEC E. coli, Salmonella, Shigella, Campylobacter**
- **Listeria monocytogenes**

**30-day “Hot List” search**

**60-day “Hot List” search, by serotype**

**120-day “Hot List” search**

**Perform cluster analysis**
- verify patterns
- look at pattern frequency over time

**Report internally using “in-house” procedures**

**Report epi-related clusters or those w/ increase in pattern freq to CDC Team**

*If no laboratorian is certified for analysis, must email TIFFs and demographic information to CDC (pfge@cdc.gov)
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
National Cluster Detection and Response

- Perform cluster search within national database or respond to local CDC Team posting or other request
- Assign cluster codes to clusters that indicate an increase in a particular PFGE pattern at the national level
- Create: line lists, frequency graphs, pie charts, cluster reports
- Report multi-state clusters that indicate an increase in a particular PFGE pattern
- Respond to epi requests
What is a Cluster Search?

- Patterns submitted
- Cluster searches performed
- Visually compare indistinguishable patterns with 1st enzyme, then 2nd (always with *E. coli* and *Listeria*)
- Patterns/clusters named by CDC

Cluster of indistinguishable patterns by primary enzyme
Does the cluster warrant further investigation?

- Pattern Frequencies (national and regional)
  - If frequency is more than twice what is expected in a month’s time
    - Take into consideration seasonal increases

- Non-human matches
  - If a pattern from a non-human isolate matches 3+ patterns from human isolates
Pattern Frequencies

Seasonal Peaks in *Salmonella* Berta
Pattern Frequencies

An obvious cluster
Pattern Frequencies

Example

- A local PulseNet participant has detected a cluster of 8 Salmonella Enteritidis isolates that are pattern JEGX01.0004
  - JEGX01.0004 is the MOST common pattern for Salmonella Enteritidis, representing 45% (~14,120 patterns) of all Salmonella Enteritidis in the database

- More common patterns tend to have less obvious clusters, and are often defined by an increase above baseline
Common Patterns: Detecting true increases can be difficult
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
Communication: Cluster Reports

Sample cluster report that is sent to the CDC epidemiologists

Salmonella
JKA
0808OHJPX-1c, S. Typhimurium posted by OH
XbaI: JPPX01.1126 (0.13%)
BlnI: JPPX26.0029 (0.13%)
60 Days: AK, ME, NM(2), NYC, OH(5), PA(4), VT
Source:
Notes: no recent matches in PNC
USDA/FDA: Nothing recent in VetNet
MLVA Info:
Epi Update: Sent to for follow-up

- Outbreak Code, serotype, posting lab
- Patterns involved and %
- States involved
- Notes, USDA/FDA/MLVA info
- Epi Update

Frequency graph of pattern

Pie Chart of pattern %
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
Collaboration with Other Agencies

- Patterns submitted directly to PulseNet USA
  - United States Department of Agriculture (USDA)
    - Food Safety and Inspection Service (FSIS)
    - Agricultural Marketing Service (AMS)
    - Animal and Plant Health Inspection Service (APHIS)
  - U.S. Food and Drug Administration (FDA)
    - Center for Food Safety and Nutrition (CFSAN)
    - Center for Veterinary Medicine (CVM)
- Agricultural Labs
  - Ohio, New York, Florida
- Veterinary Labs
  - Michigan State University
Collaboration with other Networks

- Direct connection to other databases
  - USDA Agricultural Research Service (VetNet)
  - PulseNet Canada
  - Checked against all clusters reported to CDC epidemiologists

- Other International Networks
  - PulseNet Latin America, Europe, Asia Pacific, and Middle East
  - Postings on PulseNet International web-based discussion forum
  - Emails to contacts in each network during outbreak investigation
Long-term surveillance of PFGE patterns allows us to follow trends in pattern frequencies.
Long-term surveillance—does this cluster represent a true increase in the baseline level of this particular pattern, for this time and place?
Database Uses: Attribution Analysis

What is the relative contribution of each food source to the burden of foodborne illness in humans?
Database Uses: Trends in Emerging Pathogens

Annual submissions of non-O157 STEC to PulseNet
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

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