Pulsed-Field Gel Electrophoresis
PFGE: What does it do for you?

ERRT Conference
Laura Iwig
July 31, 2012
• Patient seeks medical attention and specimen collection
• Testing at hospital or reference lab
• Positive for Salmonella, Shigella, E. coli, Campylobacter
• Isolate is sent to KY Division of Laboratory Services for serotyping and PFGE
At DLS

- Bacteriology lab will grow isolated colony:
  - If not isolated PFGE will not yield viable results
- PFGE lab after BaCT confirmation

Bacterial Suspension

Plug Mold

Mix with Agarose

Chemical Lysis and Washing

DNA in Plugs

Restriction Enzyme

Electrophoresis (PFGE)

Documentation (capture gel image)

Data Analysis

http://www.cdc.gov
Steps of PFGE

• Isolate Colony
• Prepare Buffers and Agarose
• Make Plugs
• Lysis of cells
• Wash Plugs
• Prepare and load gel

http://www.cdc.gov/features/dspulseNetFoodborneIllness/
PFGE Steps Continued

• Run Electrophoresis (18-19 hours)
• Imaging
• Analysis of image
• Send to CDC for pattern allocation

http://www.pulsenetinternational.org/networks/Pages/newzealand.aspx
**Time Line**

- **Receive Isolate from BacT**
- **Prep Work 30 minutes**
- **Washing 1.25 - 1.75 hours**
- **Casting Agarose Gel 1-1.25 hours**
- **Electrophoresis 18-20 hours**
- **14-18 Hours Streak plates and incubate**
- **Restriction 2-2.5 hours**
- **Loading restricted plugs 30-40 minutes**
- **Marking and sending to Bionumerics 15-25 minutes**
- **Lysing 2-2.5 hours**
- **Staining and destaining 1.5-2 hours**

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*Kentucky UNBRIDLED SPIRIT*
Communication with CDC: Assigning Pattern Names

- Marked images are sent to CDC electronically
- Patterns retrieved by connecting to CDC server
- Can take several days for CDC to assign pattern to isolate
### Database entries

<table>
<thead>
<tr>
<th>Key</th>
<th>Index</th>
<th>LabID</th>
<th>Serotype</th>
<th>Ogroup</th>
<th>PFGE-XbaI-p</th>
<th>PFGE-Bln1-p</th>
<th>Outbreak</th>
<th>Source</th>
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<tbody>
<tr>
<td>12-2220624-0046</td>
<td>4143</td>
<td>KY</td>
<td>I4 [6]+[6]-</td>
<td>B (4)</td>
<td>JF001.0004</td>
<td>JF001.0004</td>
<td>1008YTJFPX-1</td>
<td>KY</td>
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<td>12-2225025-0150</td>
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<td>KY</td>
<td>Entertidie</td>
<td>B1 (9)</td>
<td>JF001.0004</td>
<td>JF001.0004</td>
<td>KY</td>
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<td>Entertidie</td>
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<td>JF001.0004</td>
<td>JF001.0004</td>
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<td>KY</td>
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<td>JF001.0004</td>
<td>JF001.0004</td>
<td>KY</td>
<td></td>
</tr>
</tbody>
</table>

### Bionumerics

- **Database**: Salmonella
- **Entries**: 4807 entries
- **Experiments**: 20 experiments
- **Programs**:
  - BioNumerics

**Cabinet for Health and Family Services**

**Kentucky**

**UNBRIDLED SPIRIT**
Surveillance Activities

- Weekly surveillance: 60 day counts in BioNumerics
- Constant communication with Reportable Diseases Section
- Monthly meetings with Reportable Diseases
60 Day Salmonella Count: July, 2012
Reporting a Cluster

• Salmonella: 3 or more isolates with matching PFGE patterns or 2x baseline for more common PFGE patterns

• E. Coli: report 2 or more isolates with matching PFGE pattern to PulseNet

• Shigella Sonnei: more than 3 isolates with matching PFGE patterns with additional investigation

• All other Shigella Serotypes: report all clusters with 2 more matching PFGE patterns
**Discussion board for Salmonella**

**Actions**

<table>
<thead>
<tr>
<th>ID</th>
<th>Post</th>
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<tbody>
<tr>
<td></td>
<td>Started: 7/16/2012 3:19 PM</td>
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1650. **(JEGX01.0021)_KY_Enteritidis**

KY appears to have a cluster of 7 S. enteridis that match by XbaI. Key ID are as follows:

<table>
<thead>
<tr>
<th>Key Id</th>
<th>County</th>
<th>Age</th>
<th>Sex</th>
<th>DOC</th>
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<tbody>
<tr>
<td>12-24035897-0310</td>
<td>Nelson</td>
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<td>6/5/2012</td>
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<tr>
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<td>F</td>
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<td>6/5/2012</td>
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<tr>
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<td>Grant</td>
<td>7</td>
<td>M</td>
<td>6/11/2012</td>
</tr>
</tbody>
</table>

Bundle file is attached.

Epi has been informed.

Epi Contact:
Laura Iwig
laura.iwig@ky.gov
Hello,

These Enteritidis isolates posted by KY have been assigned pattern JEGX01.0021. This pattern has been seen 229 times in the past 60 days. Currently this is not an increase at the national level; therefore, a cluster code will not be assigned at this time. We will continue to monitor this pattern for future uploads. Please continue to update this posting with any additional information.

Thank you,

Beth McGlinchey
Good Afternoon,

KY appears to have a cluster of S. Braenderup. This pattern is new to the KY database. Key ID and collection date are as follows:

<table>
<thead>
<tr>
<th>Key ID</th>
<th>County</th>
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<th>Sex</th>
<th>DOC</th>
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<tbody>
<tr>
<td>12-25349231-0491</td>
<td>Boyle</td>
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<td>05/31/2012</td>
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<tr>
<td>12-25527771-0527</td>
<td>Jefferson</td>
<td>5</td>
<td>M</td>
<td>06/03/2012</td>
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<td>23</td>
<td>M</td>
<td>06/04/2012</td>
</tr>
<tr>
<td>12-25639570-0548</td>
<td>Hardin</td>
<td>0-21</td>
<td>M</td>
<td>06/08/2012</td>
</tr>
</tbody>
</table>

The bundle file is attached.

Epi Contact:
Laura Iwig
Laura.iwig@ky.gov
502-564-4446 x 4454
Hello Everyone,

Kentucky initially posted their original message on 6/28/2012 and the cluster was observed for a week before assigning a cluster code.

This cluster of Salmonella Braenderup has been given the cluster code 1207KYJBP-1. The PFGE XbaI Pattern associated with this cluster is JBPX01.0874, comprising 0%(0/6537) of S. Braenderup isolates in the database.

In the last 60 days, this pattern has been seen 8 times from the following states:

SourceStates: AK, KY (4), NC, VA, WV;
LabIDs: AK, KY (4), NC, VA, WV.

Please find attached a line list and bundle file.

The pattern associated with this cluster (JBPX01.0874) is a new pattern in the Salmonella database. Please continue to post updates and epi information.

Thank You,

Sung Im
PulseNet Database Administration Team
Phone: (404) 639-4558
PulseNet: (404) 639-4558
Special Requests

- In outbreak situations, isolates can be prioritized
- PFGE process cannot be sped up (3-4 day process)
Thanks to:

• Joshua Tobias, PhD
• William Grooms
Questions?