Listeria: How I Learned to Stop Worrying and Love the Sequencer

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LCDR US Public Health Service
Division of Foodborne, Waterborne, and Environmental Diseases
Centers for Disease Control and Prevention

With apologies to Mr. Kubrick
Do Cases Represent an Outbreak?
Find Related Cases by Pulsed-Field Gel Electrophoresis (PFGE)
Limitation: Genetically Unrelated Isolate Might Appear Same by PFGE
Limitation: Genetically Related Isolate Might Appear Different By PFGE
Listeria WGS Pilot Project

- Started September 2013
- Goal: Sequence all *Listeria monocytogenes* isolates
- Near real-time (<1 week for patient isolates)
Why *Listeria monocytogenes*?

- Illness is rare but serious, costly, and commonly outbreak associated
- Current subtyping methods (e.g., pulsed-field gel electrophoresis) are not ideal
- *Listeria* genome is fairly small and relatively easy to sequence and analyze
- Strong epidemiologic surveillance (*Listeria Initiative*)
- Strong regulatory component
Listeria WGS Analysis

- Kmer
- High-quality single nucleotide polymorphism (hqSNP)
- Whole-genome multilocus sequence typing (wgMLST)
Relationship Between SNPs and wgMLST Alleles

SNPs

<table>
<thead>
<tr>
<th>gene 1</th>
<th>gene 2</th>
<th>gene 3</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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wgMLST allele differences

1
Relationship Between SNPs and wgMLST Alleles

<table>
<thead>
<tr>
<th>SNP</th>
<th>gene 1</th>
<th>gene 2</th>
<th>gene 3</th>
<th>wgMLST allele differences</th>
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<tr>
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</table>
Relationship Between SNPs and wgMLST Alleles

<table>
<thead>
<tr>
<th>SNPs</th>
<th>gene 1</th>
<th>gene 2</th>
<th>gene 3</th>
<th>wgMLST allele differences</th>
</tr>
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<td>1</td>
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<tr>
<td>5</td>
<td></td>
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</table>
Listeria Cluster Metrics Before and After WGS

- Pre-WGS (Sept 2012–Aug 2013)
- WGS Year 1 (Sept 2013–Aug 2014)
- WGS Year 2 (Sept 2014–Aug 2015)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Pre-WGS</th>
<th>WGS Yr 1</th>
<th>WGS Yr 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of clusters detected</td>
<td>14</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>No. of clusters detected sooner or only by WGS</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of outbreaks solved (food source identified)</td>
<td>2</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Median no. of cases per cluster</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>No. of cases linked to food source</td>
<td>13</td>
<td>20</td>
<td>98</td>
</tr>
</tbody>
</table>
How WGS Influenced Investigations

- Show that apparent PFGE clusters are not single-source outbreaks or are pseudo-clusters
- Refine outbreak case definitions by excluding cases
- Show that isolates with different PFGE patterns are related
- Increase confidence in the link between human and product isolates
- Characterize the ecology of long-term pathogen reservoirs in the food chain
BREAKING UP PFGE CLUSTERS
PFGE Cluster Possibly Associated with Uncommon Deli Meats, 2013

92% (12/13) reported eating deli meat (incl. liverwurst, head cheese)
WGS for PFGE Cluster

wgMLST (<All Characters>)

Allele median[min-max] differences

Id
PNUSAL000156
PNUSAL000250
PNUSAL000200
PNUSAL000146
PNUSAL000428
PNUSAL000079
PNUSAL000029
PNUSAL000309
PNUSAL000350

251[26-525]

240[26-525]

242[26-274]

46[26-59]

63[50-73]
wgMLST (<All Characters>)
Allele median[min-max] differences

CLUSTER
REFINING CASE DEFINITIONS
2013 Outbreak Linked to French-Style Soft Cheese

At a Glance:
- Case Count: 6
- States: 5
- Deaths: 1
- Hospitalizations: 6
- Recall: Yes

Current isolates
- 2013L-5121 (excluded)
- 2013L-5284
- 2012L-5105
- 2012L-5274
- 2013L-5374 (excluded)
- 2012L-5487

Retrospective solates
- 2011L-2809
- 2010L-1790
- 2013L-5337
- 2013L-5298
- 2013L-5283
- 2013L-5275
- 2013L-5214
- 2013L-5223

Cases with exposure to implicated product
WGS Refines Case Definition (Same PFGE) “How Close is Close?”

- All isolates shown part of a PFGE-defined *Listeria* cluster investigation
- Varying levels of relatedness within the PFGE cluster
- Can fine-tune analyses by focusing on cases at varying levels of genetic relatedness

Median: 80 SNPs
Median: 9.5 SNPs
Median: 33 SNPs
Median: 80 SNPs
Epidemiology and Exposure Data Still Needed

- Isolate from kale fairly closely related to other isolates
- None of the patients had eaten kale, however
- Although more specific, WGS data still need to be interpreted in the context of all the evidence

WGS analysis by Enteric Diseases Laboratory Branch, CDC
TYING PFGE PATTERNS TOGETHER
Identified Listeriosis Cluster Not Evident by PFGE

- 5 cases, 3 PFGE patterns, 3 states
- All patients originally from Eastern Europe

WGS analysis by Enteric Diseases Laboratory Branch, CDC
Examples from Outbreaks Linked to Ice Cream and Middle Eastern-Style Soft Cheese

Ice Cream

<table>
<thead>
<tr>
<th>cdc_id</th>
<th>SourceSite</th>
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<tbody>
<tr>
<td>2015L-6287</td>
<td>Blood</td>
</tr>
<tr>
<td>2015L-6628</td>
<td>Blood</td>
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<tr>
<td>2014L-6388</td>
<td>Blood</td>
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<tr>
<td>2014L-6417</td>
<td>Blood</td>
</tr>
<tr>
<td>2015L-6434</td>
<td>Blood</td>
</tr>
<tr>
<td>2013L-5577</td>
<td>Blood</td>
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<tr>
<td>2013L-5718</td>
<td>Blood</td>
</tr>
<tr>
<td>2014L-6632</td>
<td>Blood</td>
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<td>2013L-5422</td>
<td>Blood</td>
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<tr>
<td>2013L-5748</td>
<td>Blood</td>
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<tr>
<td>2013L-5186</td>
<td>CSF</td>
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<tr>
<td>2015L-6103</td>
<td>Blood</td>
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<td>2015L-6328</td>
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<tr>
<td>2014L-6608</td>
<td>Fluid</td>
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<td>2013L-5346</td>
<td>Blood</td>
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<tr>
<td>2012L-5510</td>
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</tr>
<tr>
<td>2013L-5153</td>
<td>Blood</td>
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<td>2014L-6182</td>
<td>Blood</td>
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<td>2012L-5191</td>
<td>Blood</td>
</tr>
<tr>
<td>2010L-2156</td>
<td>Blood, NOS</td>
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<td>2014L-6239</td>
<td>Blood</td>
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<td>2015L-6458</td>
<td>Blood</td>
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<tr>
<td>2015L-6587</td>
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<td>2013L-5195</td>
<td>CSF</td>
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<table>
<thead>
<tr>
<th>Allele median[min-max] differences at node</th>
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<tbody>
<tr>
<td>12.5[0-18]</td>
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<table>
<thead>
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<th>wgMLST</th>
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<tbody>
<tr>
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<table>
<thead>
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<th>PFGE-AscI-pattern</th>
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<tbody>
<tr>
<td>GX6A16.0026</td>
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<tr>
<td>GX6A16.0026</td>
</tr>
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<td>GX6A12.0355</td>
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<table>
<thead>
<tr>
<th>Ice Cream</th>
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<tbody>
<tr>
<td>Soft Cheese</td>
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<table>
<thead>
<tr>
<th>Queso Fresco</th>
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<tbody>
<tr>
<td>14[4-26]</td>
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<table>
<thead>
<tr>
<th>BLOOD</th>
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<tbody>
<tr>
<td>15[4-101]</td>
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<table>
<thead>
<tr>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>15[4-39]</td>
</tr>
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</table>

LINK BETWEEN HUMAN AND PRODUCT ISOLATES
### July 2014: PFGE Cluster of Listeriosis Found by PulseNet

**States:** AL, IL (4), MA, TN

### Cluster Epi
- 6/7 (86%) male
- Ages 40-85 years
- Two deaths
- LI forms for 2 patients
- Both ate queso fresco
- Matching cheese and environmental isolates noted from Indiana from 2011

#### Number of Cases

<table>
<thead>
<tr>
<th>Week of isolation in 2014</th>
<th>June</th>
<th>July</th>
<th>August</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>22</td>
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<td>17</td>
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<td></td>
<td>29</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>31</td>
</tr>
</tbody>
</table>

- **Number of Cases:**
  - June: 2 people
  - July: 6 people
  - August: 3 people
hqSNP Phylogeny
Aug 13, 2014

Phylogeny by EDLB

38 [29-47]
Excluded clade (mostly IL)

46.5 [34-58]
New England clade

74
PA clade

2.5 [0-7]
IL cluster clade

Isolate before June 2014 (GX6A16.0319|GX6A12.0244)
Isolate before June 2014 (GX6A16.0319|GX6A12.0246)
Cluster 1407MLGX6-1 (GX6A16.0319|GX6A12.0246)
Cluster Epidemic Curve

- Highly-related by WGS
- Less related by WGS

Number of Cases

<table>
<thead>
<tr>
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<tr>
<td>22</td>
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<tr>
<td>29</td>
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<td>10</td>
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<td>17</td>
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<tr>
<td>17</td>
<td>0</td>
<td>1</td>
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States:
- IL: Illinois
- MI: Michigan
Counties where cases are located

Suspicious, but running out of leads
September 4: A Crack in the Case

- Isolates uploaded with matching PFGE pattern from mung bean sprouts and sprout irrigation water
- Collected as part of routine FDA inspection
- Where was the facility?
Exposure Data

- **LI forms**
  - 1 Illinois patient ate sprouts
  - Other patient interviewed with version of questionnaire that didn’t include sprouts; now unreachable

- **1/1 sprout consumption vs. 35/760 (5%) historical cases**

- OR = 20.1 (95% CI, 1.06–infinity)

- But one case?
Information from Michigan

- Patient denies sprout consumption, but reports eating pad thai at several Asian-style restaurants
- At least two serve mung bean sprouts
Patient Isolates Virtually Identical to Sprout Isolates by WGS

Whole-genome Multilocus Sequence Typing (wgMLST)

Allele Median [min-max]

Tennessee
Pennsylvania

Illinois
Illinois
Environmental Isolate
Sprout
Illinois
Illinois

Pennsylvania
Massachusetts
Alabama
Illinois

Isolate from MI also highly-related (not shown)

hqSNP Analysis

Tennessee
Pennsylvania

Illinois
Illinois
Environmental Isolate
Sprout
Illinois
Illinois

Pennsylvania
Massachusetts
Alabama
Illinois

68 hqSNPs
275 hqSNPs
65.5 hqSNPs [54-72]

0.02

1 ± 1 hqSNPs [0-3]

58 hqSNPs [0-72]

WGS analysis by Enteric Diseases Laboratory Branch, CDC
*Listeria monocytogenes* Outbreak Linked to Mung Bean Sprouts

- Despite limited epi data (2/2 consuming sprouts), WGS results and inspection findings enough to take action
- CDC and states recommended that consumers not eat and retailers not buy sprouts from the producer

- Although limited information is available about the specific sprout products that ill people consumed, the whole genome sequencing findings, together with the sprout consumption history of two patients and inspection findings at the firm, suggest that these illnesses could be related to products from

- Ultimately the company agreed to permanently cease operations
- Federal government later filed lawsuit to prevent firm from resuming production
Caution: Isolates in an Outbreak Are Not Always Highly Related

Example of polyclonality

Allele differences at node: median [min–max]

Cluster 1

Cluster 2

Patient isolates

Apple and environmental isolates

Deadly Outbreak Linked to Caramel Apples; CDC Warns Americans 'Not To Eat ANY'

WGS analysis by Enteric Diseases Laboratory Branch, CDC
ECOLOGY OF PATHOGEN RESERVOIRS
2013 Outbreak Linked to Farmstead Cheese

- Environmental and food samples from cheese-making facility, 2010–2013
- Outbreak cases, 2013
- Patient isolate, 2010

Isolates from 2010 virtually identical to isolates from 2013

WGS analysis by Enteric Diseases Laboratory Branch, CDC
2013 Outbreak Linked to Mexican-Style Soft Cheese

WGS analysis by Enteric Diseases Laboratory Branch, CDC
2013–2014 Outbreak Linked to Central and South American-Style Soft Cheeses

wgMLST (<All Characters>)

Allele median[min-max] differences at node

Source Site

Environmental Swabs
Environmental Swabs
BLOOD
Cheese
Cheese
placenta
CSF
Cheese
Blood
Blood
placenta
Environmental Swabs
Environmental Swabs
CSF
Urine
Blood

Different PFGE pattern from others

WGS analysis by Enteric Diseases Laboratory Branch, CDC
Listeriosis Linked to Recalled Stone Fruits

- July 2014 recall receives extensive media coverage
- Hundreds of inquiries from concerned clinicians and public
- Stone fruit isolates obtained from company
- 4 human isolates in 2014 with PFGE match
- Was it an outbreak?
Stone fruit recall widens on possible Listeria outbreak

Karma Allen | @iam_karma
Monday, 4 Aug 2314 | 4:31 PM ET

Median no. of allele differences (min–max)

- 3 (0–10)
- 6 (0–14)
- 4 (1–7)
- 10 (0–43)
- 11 (0–47)
- 2.5 (0–5)
- 3 (0–8)
- 12 (0–69)

- Isolates from recalled stone fruits
- Isolates from patients
- Isolates from nectarines
- Isolates from peaches
- Massachusetts isolate
- Minnesota isolate
- Illinois isolate
- South Carolina isolate

MMWR. Mar 20, 2015.
Patients with Recurrent *Listeria* Infections

- 70 year old man: 3 positive blood cultures, attributed to pacemaker lead infection

- June 2013
- Sept 2013
- May 2014
Patients with Recurrent *Listeria* Infections

- ~70 year old man: 3 positive blood cultures, attributed to pacemaker lead infection

Timeline:
- June 2013
- Sept 2013
- May 2014

- All food removed from home
- 3 hqSNPs
Patients with Recurrent *Listeria* Infections

- ~70 year old man: 3 positive blood cultures, attributed to pacemaker lead infection

- All food removed from home

- June 2013

- Sept 2013

- May 2014

- 3 hqSNPs

- 22 hqSNPs

- 23 hqSNPs
Patients with Recurrent *Listeria* Infections

- ~70 year old man: 3 positive blood cultures, attributed to pacemaker lead infection
  
  All food removed from home

  June 2013 → Sept 2013
  - 3 hqSNPs

  Sept 2013 → May 2014
  - 22 hqSNPs
  - 23 hqSNPs

- ~80 year old man: 2 positive blood cultures 14 months apart; isolates 19 hqSNPs different
Listeria WGS Discussion

- Helped detect more clusters and solve more outbreaks
- Focused epidemiological resources
- Identified new food sources of listeriosis
- Epidemiologic data remain essential
- WGS not just for Listeria anymore
Acknowledgments

State, Local, and Territorial Health Departments

Advanced Molecular Detection Initiative

Food and Drug Administration

US Department of Agriculture’s Food Safety and Inspection Service

Enteric Diseases Laboratory Branch

Enteric Diseases Epidemiology Branch

Outbreak Response and Prevention Branch

Epidemic Intelligence Service

Atlanta Research and Education Foundation

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Minnesota Dept. of Health notes 2 PFGE-matched isolates

PulseNet query identifies 3 more cases in other states

All 5 patients report soft cheeses on Listeria Initiative form

OR = 10.8 (1.8–∞)

3 patients re-interviewed; 2 name Company A cheese

Cheese prelim. positive for Lm; public warning issued

Matching isolates found previously from Company A plant; inspection begins

Company A and FDA announce recall
Pre-Packaged Salad Recall, 2014
WGS Links Sporadic Case

- **March 13**: Lettuce recall based on *Listeria* testing in Canada
- **April 21**: OH inquires about PFGE pattern of lettuce isolate
  - Finds matching case with onset in late March
  - 6th most common pattern in US
- **April 25**: State learns that patient consumed multiple types of pre-packaged lettuce, including recalled brand
- **May 13**: Canada reports clinical and lettuce isolates have 0 SNP difference among ~2.7 million base pairs examined
WGS Analysis by EDLB, CDC

hqSNP

21.5 hqSNPs [0-33]

24 hqSNPs [0-94]

58 hqSNPs [46-58]

43 hqSNPs

Lettuce isolate

Clinical isolate

5 hqSNPs

2013L-5439
2013L-5527
2013L-5545
2013L-5546
2013L-5548
2013L-5556
2013L-5558
2013L-5203
2013L-5204
2013L-5308
2013L-5473
2013L-5085
2013L-5587
2013L-5333
2013L-5314
2013L-5182
2013L-5622
2013L-5623
2013L-5521
2013L-5345
2014L-6017
2013L-5548
2013L-5540
2013L-5359
2013L-5297
2013L-5194
2013L-5560
2014L-6086

55 hqSNPs [5-66]

66 hqSNPs [46-84]

wgMLST

CN lettuce
An Analogy Attempt

- Your manuscript draft returns with edits…

Your Outbreak Manuscript
(5,000 pages)
An Analogy Attempt

- Your manuscript draft returns with edits…

- Lots

Your Outbreak Manuscript
(5,000 pages)
An Analogy Attempt

- Your manuscript draft returns with edits…
- Lots
- From all 25 coauthors
An Analogy Attempt

- Your manuscript draft returns with edits…

- Lots

- From all 25 coauthors
  - (Don’t these CDC people have anything better to do?)

Your Outbreak Manuscript

(5,000 pages)
An Analogy Attempt

- Your manuscript draft returns with edits...

- Lots

- From all 25 coauthors
  - (Don’t these CDC people have anything better to do?)

- No Track Changes and some of the pages are out of order
How Can You Quantify the Changes?

- **Kmer**
  - Same-length confetti

Your Outbreak Manuscript

(5,000 pages)
How Can You Quantify the Changes?

- **Kmer**
  - Same-length confetti

- **SNP**
  - Every letter (SNP)

Your Outbreak Manuscript
(5,000 pages)
How Can You Quantify the Changes?

- **Kmer**
  - Same-length confetti

- **SNP**
  - Every letter (SNP)

- **wgMLST**
  - Every section (gene)