Everything in Sequence: Listeriosis Outbreak Investigations in the Era of WGS

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Disclaimers

- The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

- No financial conflicts of interest to report.
Listeria Outbreaks and Incidence, 1983-2014

Era
Outbreaks per year 0.3
Median cases per outbreak 69
**Listeria Outbreaks and Incidence, 1983-2014**

<table>
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<td>Pre-PulseNet</td>
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</tr>
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<td>Early PulseNet</td>
<td>2.3</td>
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- **No. outbreaks**
  - 1983: 1
  - 1985: 1
  - 1987: 1
  - 1989: 1
  - 1991: 1
  - 1993: 1
  - 1995: 1
  - 1997: 1
  - 1999: 1
  - 2001: 1
  - 2003: 1
  - 2005: 1
  - 2007: 1
  - 2009: 1
  - 2011: 1
  - 2013: 1

- **Incidence (per million pop)**
  - Pre-PulseNet: 2.3
  - Early PulseNet: 11
Listeria Outbreaks and Incidence, 1983-2014

- **Era**
  - Outbreaks per year: Pre-PulseNet, Early PulseNet, Listeria Initiative
  - Median cases per outbreak:
    - Pre-PulseNet: 0.3, 69
    - Early PulseNet: 2.3, 11
    - Listeria Initiative: 2.6, 5.5

- **Incidence (per million pop)**
  - Outbreak: [chart showing data]
  - Incidence: [chart showing data]
Listeria WGS Pilot Project

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

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

SNPs

gene 1

gene 2

gene 3

wgMLST allele differences

1 1
### Relationship Between SNPs and wgMLST Alleles

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<tr>
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Relationship Between SNPs and wgMLST Alleles

wgMLST allele differences

1

2 2

2
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No. outbreaks vs. Incidence (per million pop)
Listeria Outbreaks and Incidence, 1983-2014

- **Pre-PulseNet**: Outbreaks per year: 0.3, Median cases per outbreak: 69
- **Early PulseNet**: Outbreaks per year: 2.3, Median cases per outbreak: 11
- **Listeria Initiative**: Outbreaks per year: 2.6, Median cases per outbreak: 5.5
- **WGS**: Outbreaks per year: 10, Median cases per outbreak: 3

Incidence (per million pop):
- Pre-PulseNet: 0.3 to 6.9
- Early PulseNet: 2.3 to 11
- Listeria Initiative: 2.6 to 5.5
- WGS: 10 to 3
HOW DID WGS AFFECT INVESTIGATIONS?
PFGE Cluster Possibly Associated with Uncommon Deli Meats, 2012–2013

- 80% (12/15) reported eating deli meat
- Several reported uncommon deli meats (e.g., liverwurst, mortadella, ring bologna, cloth bologna)
wgMLST (<All Characters>)
Allele median[min-max] differences

Id
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
CFSAN01
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL

114[0-154]

72[0-84]

58[1-68]

104[1-122]

4[2-4]

53[1-53]
wgMLST (<All Characters>)
Allele median[min-max] differences

Id
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
CFSAN
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
PNUSAL
58[1-68]
14[0-154]
72[0-84]
4[2-4]

wgMLST analysis by Enteric Diseases Laboratory Branch, CDC
Cluster Investigation
December 2013

- PulseNet identifies PFGE cluster of 4 cases with rare pattern
  - California (2), Illinois (1), Massachusetts (1)
  - Specimen collection dates July–December 2013

- All 4 patients seemed to be of Middle Eastern descent (Armenia, Syria, and Yemen)

- 2 patients interviewed with supplemental form

- Unable to identify common food

- Cluster closed in January 2014
PulseNet identifies cluster of 12 cases with different PFGE pattern, also rare
- California (7), Massachusetts (2), New York (2), Colorado
- Specimen collection dates 2013–2015
- 10 sequenced isolates ≤25 alleles by wgMLST

More WGS analysis shows isolates from 2010–2015 with 4 other PFGE patterns are also highly related, including isolates from 2013 cluster
5 different PFGE patterns

All isolates from patients except “unrelated” food isolate at bottom
Investigation Unfolds

- **With more cases**
  - Middle Eastern connection still present
  - Soft cheese association identified (OR 26, $p < 0.001$), mostly Middle Eastern and Mediterranean style
  - Suspect Company A based on brands reported

- **PulseNet finds matching 5 environmental isolates from Company A cheese production facility in 2010**
One dead, 24 sick from multi-state Listeria outbreak linked to soft cheeses

By Debra Goldschmidt, CNN
Updated 1:39 PM ET, Fri September 18, 2015
wgMLST Phylogeny as of Nov. 13

Allele median [min-max] differences at node

SourceSite
- BLOOD
- Blood
- CSF
- Blood
- BLOOD
- Blood
- Blood
- Blood
- CSF
- Blood
- Blood
- Environmental Sponges
- Environmental Sponges
- Stool
- Environmental Sponges
- Blood
- Blood
- Blood
- Fluid
- Swab
- Swab
- Swab
- Swab
- Blood
- Blood
- Blood
- Swab
- Paracentesis Fluid
- Environmental Sponges
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- Blood
- CSF

Environmental swabs, 2015

Environmental swabs, 2010

WGS analysis by Enteric Diseases Laboratory Branch, CDC
Unlikely to Be Implications of WGS

[Person A] drew the “yes” side of the first debate question [at International Association for Food Protection annual meeting], “Is shoe leather epidemiology dead in the era of whole-genome sequencing?”


“You catch things far earlier” with sequencing, said [Person B]. “It can be two cases. If you see a match, Bam! You've got em.”

- Reuters. August 27, 2015
A New Era

Listeria WGS

- 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

National Center for Biotechnology Information

Enteric Diseases Laboratory Branch

Enteric Diseases Epidemiology Branch

Outbreak Response and Prevention Branch

Epidemic Intelligence Service

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

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Public Interest

U.S. Google Search Trends for “Listeria”

- Cantaloupe outbreak
- WGS project started