Whole Genome Sequencing of *Listeria monocytogenes*: A Public Health Revolution

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National Center for Emerging and Zoonotic Infectious Diseases
Division of Foodborne, Waterborne, and Environmental Diseases
“Transforming Public Health Microbiology – PulseNet and Beyond”

- Replacing traditional microbiology with WGS
  - For Food Safety Reference Labs:
    - Cost-efficient consolidation of multiple workflows: Identification – serotyping – virulence profiling – antimicrobial resistance characterization – subtyping
Listeria Outbreaks and Incidence, 1983-2014

No. outbreaks

Incidence (per million pop)

Era
Outbreaks per year
Median cases per outbreak

Pre-PulseNet
0.3
69

Early PulseNet
2.3
11

Listeria Initiative
2.9
5.5

WGS
9
4

Data are preliminary and subject to change
Listeria Cluster Metrics Before and After WGS

Data are preliminary and subject to change
Listeria and Caramel Apples

- 35 cases, 12 states
- Nov 20: Small multistate cluster detected by Whole Genome Sequencing
  - Dec 19: First recalls, public warned
  - Investigation continues
- Whole Genome Sequencing
  - Outbreak identified a week faster than would have been by PFGE
- Novel food vehicle
  - Food microbiology research needed to find how best to prevent in future
wgMLST and PFGE in the 2014 Caramel Apples *Listeria* Outbreak

Allele differences at node: median (min–max)
(>5,800 loci analyzed by BioNumerics software)

Cluster 1 (≤6 allele differences)
- **Unrelated isolates (hot dog and patient)**
- **Highly-related patient isolate; different PFGE pattern**

Cluster 2 (≤10 allele differences)
- **Unrelated patient isolate (Sept. 2014)**

Not closely related (minimum 1,628 allele differences)
LISTERIA AND BLUE BELL ICE CREAM
Contaminated Production Facilities and Illnesses Linked to Blue Bell Creameries

CDC recommends to not eat, serve, or sell any Blue Bell brand products. This complicated investigation of a Listeriosis outbreak involves serious illnesses from 2010 through 2015 linked to two Blue Bell production facilities.

Arizona
- 1 case linked to ice cream made in Oklahoma facility

Texas
- 3 cases in separate hospitals linked to ice cream made in Oklahoma facility
- *Listeria* found in ice cream products made in Texas facility

Kansas
- 5 cases in one hospital linked to ice cream made in Texas facility, resulting in 3 deaths

Oklahoma
- 1 case linked to ice cream made in Oklahoma facility
- *Listeria* found in ice cream products and in Oklahoma facility where they were made
- Facility closed indefinitely

Human illness(es)

Texas Blue Bell production facility

Oklahoma Blue Bell production facility

Illness(es) linked to Texas facility

Illness(es) linked to Oklahoma facility

Learn more: [www.cdc.gov/listeria/bluebell](http://www.cdc.gov/listeria/bluebell)
**PFGE Patterns Seen In The Blue Bell ( ) and Jeni’s ( )* Ice Cream Listeria Recalls**

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<th>PFGE-ApaI</th>
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</table>
wgMLST Tree Of Isolates From The Blue Bell (*) and Jeni’s (*) Ice Cream Listeria Recalls
**WGS Of *Listeria monocytogenes* From The Crave Brothers Cheese outbreak (2013)**

hqSNP

Historical isolates from the plant environment added to the comparison (courtesy FDA/CFSAN)

- **Red** = epi-related clinical isolates
- **Blue** = retrospective clinical controls or not outbreak related
- **Green** = historical environmental isolates from the plant
- **Black** = unrelated isolate used as an outlier to root the tree
Listeria Initiative

- Nationwide initiative to collect standardized exposure information from listeria patients (since 2005)
- Coordinated by CDC

**Proportion of listeriosis cases* with epidemiological data reported to the Listeria Initiative linked to molecular subtyping data, by CY**

- 2011–2012 Baseline: 0.55
- 2013 Actual: 0.64
- 2014 Target: 0.7
- 2015 Target: 0.8
- 2016 Target: 0.85
- 2017 Target: 0.9

*Reported to the National Notifiable Disease Surveillance System (NNDSS)

**Note: Data for 2014 will be available fall 2015 after NNDSS data available

Data are preliminary and subject to change
Click-of-a-Button Tool Linking Nodes in a WGS Tree to Exposure Information
ANI of *Listeria*

- **Average Nucleotide Identity (ANI)** – similarity measure of the nucleotide content in homologous regions of two isolates
- **Suggested as a robust way to identify the species of an isolate with well characterized reference strains by WGS**
- **Species identity ANI >0.95**

Gladney, Huang, Kucerova, Katz, Roache, Carleton, Tarr: Validation of Whole Genome Average Nucleotide Identity for Identification of *Listeria monocytogenes* and related species, SFAF 2015
ANI of *Listeria*

Box-plots of ANI-values for different within and between *Listeria* species combinations
ANI of *Listeria*

*L. monocytogenes*~*L. monocytogenes*~*L. non-monocytogenes*
ANI of *Listeria*

*L. monocytogenes* lineages ~ other *L. monocytogenes* lineages ~ *L. non-monocytogenes*

Lineage I  
Lineage II  
Lineage III & IV
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“The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention”
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