Listeria Sequencing in Real Time

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Sequencing Application Evolution

Real-time sequencing for outbreak surveillance

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Laboratory Strategy to Meet The Challenge of Culture
Independent Diagnostic Methods (CIDT)

1. **Preserve cultures**
   - Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)

2. **Prepare for the future working on pure cultures**
   - Real-time WGS surveillance of listeriosis
   - Global Microbial Identifier (GMI)
   - Communication: White papers, Meetings, Presentations, Publications

3. **Metagenomics No cultures**
   - 250 STEC genomes study
   - 100k Foodborne pathogens sequencing study
   - LRN metagenomics study
   - 250 STEC genomes study
   - Bigs.db
   - Applied Maths, DNASTAR

**Surveillance by whole genome sequencing (WGS)**

**Surveillance and diagnostics by metagenomics**

**CIDT Working groups:**
1. Overarching WG
2. Regulatory WG
3. PHL/Funding/Best Practices WG
4. CIDT panel evaluation WG
5. Industry WG
Proof-of-Concept on the Use of Real-Time Whole Genome Sequencing in Conjunction with Enhanced Surveillance for Listeriosis

• Collaboration among the public health departments in the states, CDC, FDA, USDA, and NCBI

• International component: Developing and refining bioinformatics ‘pipelines’ with partners in Canada, England, France, Denmark and Australia
WGS For Surveillance

- WGS has been used retrospectively but rarely to guide public health action in an outbreak investigation and never for routine surveillance
- Need to prove the public health impact of WGS to move the technology forward
  - Can outbreaks be detected and solved faster, with fewer cases?
Why *Listeria monocytogenes*?

- Listerialosis is a foodborne disease that is serious, fairly rare, and commonly associated with outbreaks
  - Low cost and potentially high public health impact
- Current subtyping methods are not ideal
  - No high-discriminatory alternative to PFGE
  - WGS provides information about the evolutionary relationship between isolates
- The epidemiological surveillance component is very strong with the *Listeria* Initiative
- The food regulatory component of *Listeria* control is strong
- The *Listeria* genome is fairly small and relatively easy to sequence and analyze
What do we want to do?

- Sequence all clinical isolates in the U.S. during one year as close to real-time as possible in parallel with current surveillance
  - PulseNet PFGE, strain characterization at CDC, interview of case-patients
- Upload sequences to NCBI (Genbank), a public database, as the sequences are generated
  - With metadata that do NOT identify state or isolation date but with link to the PulseNet database
- Evaluate data on a weekly basis
- Follow-up on clusters detected
  - Both PFGE and WGS defined clusters
Technical Questions

- Is it possible to sequence and analyze the data in real-time?
- What problems will we encounter?
- Which targets has the strongest epidemiological concordance?
  - Core SNPs
  - Kmer SNPs
  - wgMLST
- How do we define clusters?
- Do we need to define clusters?
- How do we track/monitor clusters in real time?
- How can we compare strains/isolates over time?
- What bioinformatics tools and approaches works most efficiently for outbreak detection, delineation and control?
  - Speed ~ accuracy
- How does it work compared to PFGE?
Experience So Far

- Exciting technology
- All PFGE clusters do also cluster by WGS
  - Some are split
  - More accurate case definition
- One cluster identified by WGS that was not recognized by PFGE
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- All PFGE clusters do also cluster by WGS
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- One cluster identified by WGS that was not recognized by PFGE
- Resource intensive
  - Not been able to test the performance of different clustering tools in real-time
Whole Genome Sequencing of *Listeria monocytogenes* during the Crave Brothers Cheese outbreak

High confidence core SNP

Excluded CO case
Retrospective controls

Epi-related
PFGE match - from outbreak period

NY – no soft cheese exposure

TX - no exposure info
Whole Genome Sequencing of *Listeria monocytogenes* during the Crave Brothers Cheese outbreak

**kSNP**

- **Red** = epi-related
- **Orange** = PFGE match- from outbreak period
- **Blue** = excluded CO case
- **Light blue** = retrospective controls

NY – no soft cheese exposure

TX - no exposure info

**0.87**
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Disclaimers:
“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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