PulseNet Post WGS: Utah Public Health Lab’s Perspective

APHL Annual Meeting
Laboratory Impacts Post PulseNet Transition to WGS
June 6 2019
Dr. Kelly F. Oakeson
Total Number of Isolates Sequenced per Year

- 2016: 345
- 2017: 1142
- 2018: 1020
- 2019: 417
Totals by Organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>506</td>
<td>174</td>
<td>163</td>
<td>212</td>
</tr>
<tr>
<td>E. coli &amp; Shigella</td>
<td>158</td>
<td>158</td>
<td>160</td>
<td>212</td>
</tr>
<tr>
<td>Listeria</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Salmonella</td>
<td>402</td>
<td>78</td>
<td>108</td>
<td>261</td>
</tr>
<tr>
<td>Vibrio</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Non-PulseNet</td>
<td>64</td>
<td>2</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

Years: 2016, 2017, 2018, 2019
UPHL's Workflow
Reference Free WGS Analysis

1. Fastq Files
   - Raw fastq file
   - Transferred to our local Linux box using BaseMount

2. Sequence QC
   - FastQC
   - Quick QC check
   - Shovill
   - Draft Genome Assemblies

3. Assembly QC
   - Quast
   - QC check for Assemblies

4. Organism ID
   - MASH
   - Verification of expected organism and QC check
   - SeqSero
   - Run Salmonella isolates only

5. Salmonella Serotyping
   - SerotypeFinder
   - Using Abricate
   - ResFinder
   - Using Abricate

6. QC Report
   - MultiQC
   - Results end up in one unified QC report

7. Phylogenetic Analysis
   - Roary, IQ-Tree & R
   - Phylogenetic Relationships

8. Genome Annotation
   - Prokka
   - Gene Inventories

9. QC Report
   - MultiQC
   - Results end up in one unified QC report
Whole-Genome Sequencing and Bioinformatic Analysis of Isolates from Foodborne Illness Outbreaks of *Campylobacter jejuni* and *Salmonella enterica*

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**ABSTRACT**  Whole-genome sequencing (WGS) via next-generation sequencing (NGS) technologies is a powerful tool for determining the relatedness of bacterial isolates in foodborne illness detection and outbreak investigations. WGS has been applied to national outbreaks (for example, *Listeria monocytogenes*); however, WGS has rarely been used in smaller local outbreaks. The current study demonstrates the superior resolution of genetic and evolutionary relatedness generated by WGS data analysis, compared to pulsed-field gel electrophoresis (PFGE). The current study retrospectively applies WGS and a reference-free bioinformatic analysis to a Utah-specific outbreak of *Campylobacter jejuni* associated with raw milk and to a national multistate outbreak of *Salmonella enterica* subsp. *enterica* serovar Typhimurium associated with rotisserie chicken, both of which were characterized previously by PFGE. Together, these analyses demonstrate how a reference-free WGS workflow is not reliant on determination of a reference sequence, like WGS workflows that are based on single-nucleotide polymorphisms, or the need for curated allele databases, like multilocus sequence typing workflows.
Reference Free WGS Analysis & PFGE

Maximum Likelihood Tree of 4,467 Concatenated Protein Coding Genes

PFGE First Enzyme Pattern: UTEXHX01.678
PFGE Second Enzyme Pattern: UTEXHA26.317

Isolates in blue are from environmental sources
Isolates in black are from clinical sources
Isolates in red are reference sequences

E. coli O157:H7 str. Sakai

0.00016 Substitutions / Site
Reference Free WGS Analysis

Patient B: Platelet Bag Isolate 2
Patient B: Platelet Bag Isolate 1
Patient A: Blood Sample Isolate
Donor: Left Antecubital Skin Isolate
    Donor: Axilla Skin Isolate
    Donor: Right Antecubital Skin Isolate

Control Blood Sample Isolate
    C. perfringens E str. JGS1987
    C. perfringens NCTC 8239
    C. perfringens WAL 14572
    C. perfringens ATCC 13124

0.014 Substitutions / Site
Antimicrobial Resistance Prediction
# Salmonella Serotyping

<table>
<thead>
<tr>
<th>Sample</th>
<th>H1 antigen prediction (fliC)</th>
<th>H2 antigen prediction (fljB)</th>
<th>O antigen prediction</th>
<th>Predicted antigenic profile</th>
<th>Predicted serotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNUSAS065211-UT-M03999-181228</td>
<td>12O-4</td>
<td>4:eh:12</td>
<td>Saint Paul</td>
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</tr>
<tr>
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<td>17O-28</td>
<td>28:y:17</td>
<td>Pomona</td>
<td></td>
<td></td>
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<tr>
<td>PNUSAS065208-UT-M03999-181228</td>
<td>O-4</td>
<td>4:i-</td>
<td>potential monophasic variant of Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>12O-4</td>
<td>4:r:12</td>
<td>Heidelberg</td>
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<tr>
<td>PNUSAS065205-UT-M03999-181228</td>
<td>12O-8</td>
<td>8:eh:12</td>
<td>Newport*</td>
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</tr>
</tbody>
</table>
Fee For Service

Services Offered

- Bacterial Genome Sequencing
  Whole genome sequencing (WGS) is the process of determining the complete DNA sequence of an organism's genome.
  The fee for this service is $107 per samples submitted.

- Bacterial Genome Sequencing & Species Identification

- Bacterial Genome Sequencing, Species Identification & Relatedness Analysis

- Microbial Source Tracking via Shotgun Metagenomic Sequencing

- Microbial Source Tracking via Traditional Culturing
Thank You

Utah Public Health Laboratory

Robyn Atkinson-Dunn

Andy Rohrwasser

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