Advancing Food Safety: Integration of Environmental Monitoring and Genomics

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FDA/CFSAN/ORS
JIFSAN Global Water Food Safety Summit
November 2019
Impact

- Ecology, Distribution, Persistence
- Combine with genomics (traditional/functional)
  - better source tracking
  - development of preventative controls
### Previous Tomato Outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Species</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>S. Newport*</td>
<td>512 cases</td>
</tr>
<tr>
<td></td>
<td>S. Newport</td>
<td>12 cases</td>
</tr>
<tr>
<td></td>
<td>S. Javiana</td>
<td>90 cases</td>
</tr>
<tr>
<td>2005</td>
<td>S. Newport*</td>
<td>71 cases</td>
</tr>
<tr>
<td></td>
<td>S. Braenderup</td>
<td>76 cases</td>
</tr>
<tr>
<td></td>
<td>S. Enteritidis</td>
<td>77 cases</td>
</tr>
<tr>
<td>2006</td>
<td>S. Newport*</td>
<td>107 cases</td>
</tr>
<tr>
<td></td>
<td>S. Typhimurium</td>
<td>186 cases</td>
</tr>
<tr>
<td>2007</td>
<td>S. Newport*</td>
<td>57 cases</td>
</tr>
<tr>
<td>2010</td>
<td>S. Newport</td>
<td>46 cases</td>
</tr>
<tr>
<td></td>
<td>(retrospective traceback confirmed)</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>S. Newport*</td>
<td>99 cases</td>
</tr>
<tr>
<td>2014</td>
<td>S. Newport *</td>
<td>283 cases</td>
</tr>
<tr>
<td></td>
<td>S. Javiana</td>
<td>78 cases</td>
</tr>
</tbody>
</table>

* Same PFGE “pattern 61”

First Incident Associated with Maryland Produce
Delmarva Peninsula
Virginia Tech Eastern Shore Agricultural Research Extension (AREC), Painter, VA
Summary Findings From VA

Local Surface Waters Were Consistently Contaminated Throughout The 3 YR Period
Environmental/Surface water sampling

- Collected samples from 11 different states on the East Coast
- Some studies were short (*5 sites per state, 4 samplings over 1 summer)
- Some studies were one year or more, longitudinal efforts to the same sites at regular intervals
S. Newport: 547 isolates from 11 states

Lineage III

STs for 530 Lineage III Newport

- ST118
- ST350
- ST5
- TBD

NJ | OH | PA | MD | DE | VA | WV | SC | NC | GA | FL
---|----|----|----|----|----|----|----|----|----|----
16 | 7  | 1  | 16 | 119| 279| 21 | 2  | 37 | 6  | 8  |
16 | 7  | 1  | 16 | 35 | 288| 21 | 3  | 38 | 6  | 13 |
Pattern61 DELMARVA Clade (344) 0-63 SNPs

Non Pattern61 DELMARVA Clade (189) 0-134 SNPs

* Two were from SC
PFGE Xbal-JJPX01.0061 (30 isolates) shown in red
Grouped into 8 clusters
Less than 30 SNPs difference between the clusters

11SNPs
9SNPs
12SNPs
26SNPs
25SNPs
26SNPs
FDA’s GenomeTrakr

• Distributed network of labs using WGS for foodborne pathogens

• Contributing members:
  – 13 U.S. FDA Labs
  – 11 PulseNet Labs (State Public Health Labs)
  – 5 U.S. Department of Agriculture Labs
  – 7 University Labs
  – 3 Private Labs
  – 2 International Labs (Argentina, Mexico)
  – 1 U.S. Hospital Lab

• All data is submitted to National Center for Biotechnology Information (NCBI)
  • Data curation and bioinformatic support
  • Pathogen Detection
    • Produces daily trees from current SNP clusters
Pathogen Detection BETA

View the recent webinar: *Introducing the Pathogen Detection Isolates Browser*.

NCBI Pathogen Detection integrates bacterial pathogen genomic sequences originating in food, environmental sources, and patients. It quickly clusters and identifies related sequences to uncover potential food contamination sources, helping public health scientists investigate foodborne disease outbreaks.

Find isolates now!

Examples:
1. Search for isolates encoding a mobile colistin resistance gene and a KPC beta-lactamase search: AMR_genotypes.mcr* AND AMR_genotypes.blaKPC*.
2. Search for Salmonella isolates from the USA search: ppo_loc.name:USA AND taxgroup_name:"Salmonella enterica"

Explore the Data

<table>
<thead>
<tr>
<th>Species</th>
<th>New Isolates</th>
<th>Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterica</td>
<td>18</td>
<td>235,224</td>
</tr>
</tbody>
</table>

Learn More

About
FAQ
Browser Factsheet
Antimicrobial Resistance Factsheet
Antimicrobial Resistance
Contributors
Help

Data Resources

Isolates Browser
Pathogen Detection Reference Gene Catalog
Isolates with antibiotic resistant phenotypes
Download analysis results (FTP)
Antimicrobial Resistance Genes

ResFinder Analysis

- Threshold of Identity 90%
- Minimum Length 60%

All 530 isolates have the identical Acquired antimicrobial resistance gene profile

All but six isolates share the identical Known Mutation profile

<table>
<thead>
<tr>
<th>Acquired antimicrobial resistance gene</th>
<th>Predicted phenotype</th>
<th>Identity</th>
<th>Query/Template length</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aac(6’)-Ia</strong></td>
<td>Aminoglycoside resistance</td>
<td>98.63%</td>
<td>438/438</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Known Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
</tr>
<tr>
<td>parC</td>
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</table>

SPI Presence

SPIFinder Analysis

- Threshold of Identity 90%
- Minimum Length 60%

Due to the usage of draft genome, false negative are possible
## Functions of SPIs identified in Lineage III S. Newport

<table>
<thead>
<tr>
<th>SPI</th>
<th>Size (kB)</th>
<th>GC%</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI-1</td>
<td>38.8</td>
<td>45.9</td>
<td>TTSS-1, invasion and proinflammatory responses, Fe2+ and Mn 2+ uptake</td>
</tr>
<tr>
<td>SPI-2</td>
<td>39.8</td>
<td>47.4</td>
<td>TTSS-2, intracellular survival; tetrathionate respiration</td>
</tr>
<tr>
<td>SPI-3</td>
<td>17.3</td>
<td>47.6</td>
<td>Colonization of GI tract, Mg2+ uptake</td>
</tr>
<tr>
<td>SPI-4</td>
<td>23.4</td>
<td>44.8</td>
<td>TISS, colonization of cattle GI tract</td>
</tr>
<tr>
<td>SPI-5</td>
<td>7.6</td>
<td>43.6</td>
<td>TTSS-1 and TTSS-2 effector proteins, enteropathogenic responses</td>
</tr>
<tr>
<td>SPI-12</td>
<td>6.3</td>
<td>49.92</td>
<td>TTSS-2 Effector</td>
</tr>
<tr>
<td>SPI-13</td>
<td>19.5</td>
<td>48.1</td>
<td>Virulence in chicks</td>
</tr>
<tr>
<td>SPI-14</td>
<td>8.7</td>
<td>41.4</td>
<td>Virulence in chicks</td>
</tr>
<tr>
<td>C63PI</td>
<td>SPI locates at centisomal 63</td>
<td></td>
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# Clade defining SNPs

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Clade VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>228329</td>
<td>SEENLE01_001045</td>
<td>hypothetical protein</td>
<td>G</td>
<td>CTG</td>
<td>L</td>
<td>synonymous A</td>
<td>CTA</td>
<td>L</td>
<td></td>
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<tr>
<td>671544</td>
<td>SEENLE01_003170</td>
<td>flotilin</td>
<td>T</td>
<td>GTA</td>
<td>V</td>
<td>nonsynonym A</td>
<td>GAA</td>
<td>E</td>
<td></td>
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<tr>
<td>1068222</td>
<td>SEENLE01_005140</td>
<td>multidrug export protein EmrA</td>
<td>G</td>
<td>GCC</td>
<td>A</td>
<td>nonsynonym A</td>
<td>ACC</td>
<td>T</td>
<td></td>
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<tr>
<td>1252656</td>
<td>SEENLE01_006025</td>
<td>pyridoxine 5'-phosphate synthase</td>
<td>C</td>
<td>ACC</td>
<td>T</td>
<td>synonymous T</td>
<td>ACT</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>2335611</td>
<td>SEENLE01_011225</td>
<td>methyl viologen resistance protein SmvA</td>
<td>C</td>
<td>GCC</td>
<td>A</td>
<td>synonymous T</td>
<td>GCT</td>
<td>A</td>
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<tr>
<td>2337186</td>
<td>SEENLE01_011235</td>
<td>porin</td>
<td>G</td>
<td>CTG</td>
<td>L</td>
<td>synonymous A</td>
<td>CTA</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>2577044</td>
<td>SEENLE01_012435</td>
<td>threonine-tRNA ligase</td>
<td>C</td>
<td>CGC</td>
<td>R</td>
<td>nonsynonym T</td>
<td>TGC</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3217918</td>
<td>SEENLE01_015790</td>
<td>glycosyl transferase</td>
<td>C</td>
<td>CCT</td>
<td>P</td>
<td>nonsynonym T</td>
<td>TCT</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>3517731</td>
<td>SEENLE01_017335</td>
<td>multidrug ABC transporter permease/ATP-binding protein</td>
<td>C</td>
<td>GCG</td>
<td>A</td>
<td>nonsynonym T</td>
<td>GTG</td>
<td>V</td>
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<tr>
<td>3517802</td>
<td>SEENLE01_017335</td>
<td>multidrug ABC transporter permease/ATP-binding protein</td>
<td>G</td>
<td>GAG</td>
<td>E</td>
<td>synonymous A</td>
<td>GAA</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>4106327</td>
<td>SEENLE01_020075</td>
<td>PTS sugar transporter</td>
<td>C</td>
<td>TCA</td>
<td>S</td>
<td>nonsynonym T</td>
<td>TTA</td>
<td>L</td>
<td></td>
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<tr>
<td>4106947</td>
<td>SEENLE01_020080</td>
<td>Fis family transcriptional regulator</td>
<td>G</td>
<td>TGG</td>
<td>W</td>
<td>nonsense A</td>
<td>TAG</td>
<td>*</td>
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<tr>
<td>4457460</td>
<td>SEENLE01_021735</td>
<td>chemotaxis protein</td>
<td>G</td>
<td>GAT</td>
<td>D</td>
<td>nonsynonym T</td>
<td>TAT</td>
<td>Y</td>
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<td>4457520</td>
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<td>chemotaxis protein</td>
<td>G</td>
<td>GTG</td>
<td>V</td>
<td>nonsynonym A</td>
<td>ATG</td>
<td>M</td>
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<tr>
<td>Clade IV</td>
<td></td>
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<tr>
<td>1321245</td>
<td>SEENLE01_006320</td>
<td>outer membrane protein assembly factor BamB</td>
<td>A</td>
<td>ACC</td>
<td>T</td>
<td>nonsynonym g</td>
<td>gCC</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>
70% of inoculated blossoms resulted in *Salmonella* isolated from within or on the developing fruit
Newport, Javiana, Montevideo, and Saintpaul were all recovered from within developing tomatoes
*Salmonella* Newport internalization through root system was internalized to stem (20%) and fruit (5%)

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**Cocktail**: Newport Javiana Montevideo Saintpaul

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Transcriptomics examination of adaptation of lineage III Newport in tomato
Salmonella Adaptations of particular interest to food safety specialists

<table>
<thead>
<tr>
<th></th>
<th>Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thermal tolerance</td>
</tr>
<tr>
<td>2</td>
<td>Dessication resistance</td>
</tr>
<tr>
<td>3</td>
<td>Osmotic/Ionic tolerance</td>
</tr>
<tr>
<td>4</td>
<td>Quat resistance</td>
</tr>
<tr>
<td>5</td>
<td>Chlorine resistance</td>
</tr>
<tr>
<td>6</td>
<td>Biofilm persistence</td>
</tr>
<tr>
<td>7</td>
<td>Surface adherence</td>
</tr>
<tr>
<td>8</td>
<td>Antibiotic resistance</td>
</tr>
<tr>
<td>9</td>
<td>Antimicrobial resistance</td>
</tr>
<tr>
<td>10</td>
<td>Ecological fitness</td>
</tr>
<tr>
<td>11</td>
<td>Heavy metal resistance</td>
</tr>
<tr>
<td>12</td>
<td>Metabolic persistence</td>
</tr>
<tr>
<td>13</td>
<td>Enhanced hydrophobic fitness</td>
</tr>
<tr>
<td>14</td>
<td>Produce invasiveness</td>
</tr>
<tr>
<td>15</td>
<td>Flower invasiveness</td>
</tr>
<tr>
<td>16</td>
<td>Root system invasiveness</td>
</tr>
<tr>
<td>17</td>
<td>Acid resistance</td>
</tr>
<tr>
<td>18</td>
<td>Surface water fitness</td>
</tr>
<tr>
<td>19</td>
<td>In vivo plant migratory fitness</td>
</tr>
<tr>
<td>20</td>
<td>Soil fitness</td>
</tr>
<tr>
<td>21</td>
<td>Capsaicin resistance</td>
</tr>
<tr>
<td>22</td>
<td>Swarming</td>
</tr>
<tr>
<td>23</td>
<td>Trans-ovarian poultry colonization</td>
</tr>
<tr>
<td>24</td>
<td>Fecal persistence (poultry)</td>
</tr>
<tr>
<td>25</td>
<td>Yolk content invasion</td>
</tr>
<tr>
<td>26</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>27</td>
<td>External amoeba harborage</td>
</tr>
<tr>
<td>28</td>
<td>Internal amoeba harborage</td>
</tr>
<tr>
<td>29</td>
<td>Acyl-homoserine lactone (AHL)</td>
</tr>
<tr>
<td>30</td>
<td>KatE stationary-phase catalase</td>
</tr>
<tr>
<td>31</td>
<td>In vivo migratory fitness</td>
</tr>
<tr>
<td>32</td>
<td>RDAR phenotype</td>
</tr>
<tr>
<td>33</td>
<td>The ‘Weltevreden’ type</td>
</tr>
<tr>
<td>34</td>
<td>Persistence within the tomato**</td>
</tr>
</tbody>
</table>
Key Conclusions

• *Salmonella* Pattern 61 and other clinically relevant isolates found consistently in waters and sediments

• Environmental waters and sediments are potential reservoirs for *Salmonella*

• Agricultural practices related to the use of contaminated surface waters are highly likely to introduce *Salmonella* onto crop plants
Water Sampling Methodologies

Traditional Grab Sample 1 L

Modified Moore Swab (mMMS) 10 L

Hollow Fiber Ultrafiltration 100 L

Pros/Cons:
- Ease of collection/processing
- Cost
- Sensitivity of detection
- Equivalent sample volume
Acknowledgements

Dr. Guojie Cao
CFSAN HPOP group
  Dr. Jie Zheng
  Christina Ferreira
  Elizabeth Reed
CFSAN Core Sequencing group
CFSAN Bioinformatics group
If tomatoes are classified as a fruit...

Isn't ketchup technically a smoothie?