Integration of CID-Related Microbiological Test Methods in FDA's Food Safety Laboratory: Current and Future Perspectives

Yi Chen Ph.D.
yi.chen@fda.hhs.gov

Division of Microbiology
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
U.S. Food and Drug Administration

- One of the oldest regulatory agencies in U.S.
- Food and Drugs Act of 1906
- Federal Food, Drug and Cosmetic Act, 1938
- Approximately 10% of every consumer dollar spent on food
Regulatory Authority

• Responsible for the safety of 80% of all food consumed in the United States
  – Entire domestic and imported food supply
  – Except
    • Meat
    • Poultry
    • Frozen, dried and liquid eggs

Regulated by USDA
Examples of Products Regulated by FDA

- Beverages (including bottled water, alcoholic beverages and soft drinks)
- Colors Added to Food, Drugs, Cosmetics, and Medical Devices
- Cosmetics
- Dairy Products
- Dietary Supplements
- Produce

- Food Ingredients
- Food Packaging
- Game Meat
- Grain-based Products
- Infant Formula
- Medical Foods
- Plant Products
- Seafood
- Spices
• North Americans are eating a greater *variety* of foods and desire a wide variety of foods year round

  – U.S. per capita consumption of fresh fruit and vegetables increased 36% from 1981 to 2000.

  – Total consumption of imported fish increased from less than 50% in 1980 to more than 75% today.

  – Typical grocery store carried 173 produce items in 1987 and is now carrying 345 produce items
Program Goal 1: Establish science-based preventive control standards across the farm-to-table continuum

Obj. 3: Evaluate and improve the effectiveness of preventive control standards – detection methods

Program Goal 6: Improve detection of and response to foodborne outbreaks and contamination incidents

Obj. 1: Improve response to foodborne outbreaks and contamination incidents

Obj. 2: Investigate and adopt innovative technologies and processes to detect and investigate foodborne outbreaks and contamination events
Whole Genome Sequencing Program

- To differentiate sources of contamination, even within the same outbreak
- To determine which ingredient in a multi-ingredient food harbored the pathogen associated with an outbreak
- To narrow the search for the source of a contaminated ingredient, even when the source is halfway around the world
- To determine unexpected vectors for food contamination
- To evaluate persistence of pathogens in the production environment
Metagenomic Identification of *Salmonella* in Tomatoes and Tomato Growing Environrs

*Salmonella* hits from tomato phyllosphere metagenome

S. Newport
Culture Still Needed

• Evidence of viable culture before taking regulatory actions

• False positive/negative results not completely eliminated for complex matrices in spite of improved performance and advanced technology

• FDA / AOAC / ISO - All rapid screening methods validated by culture confirmation. When testing samples, all rapid screening methods followed by culture confirmation
PFGE results for animal feed are analyzed by CVM

CFSAN and CVM provide PFGE data analysis duties for data generated by field labs.

Long term epidemiology and retrospective analysis
Salmonella Serotyping

**SeqSero** – strong sequencing signal needed

*Salmonella* Serotyping by Whole Genome Sequencing

- *The following formats are supported for raw reads input: .fastq.gz (preferred), .fastq, and .sra.*
- Please select your input file:

- *The following formats are supported for raw reads input: .fastq.gz (preferred), .fastq, and .sra.*
- Please select the first reads file:

- Please select the second reads file:

- *The following formats are supported for raw reads input: .fastq.gz (preferred), .fastq, and .sra.*
- Please select your input file:

- *The FASTA format is supported for genome assembly input.*
- Please select your input file:
## Listeria Serotyping

### Serogroup identification

<table>
<thead>
<tr>
<th>Gene target</th>
<th>Primer sequence (5'-3')&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Product size (bp)</th>
<th>Serovar specificity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Protein encoded by the target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>lmo0737</td>
<td>For: AGGGGTTCAAGGACTTACC Rev: ACGATTCTGCTTGCCATTC</td>
<td>691</td>
<td><em>L. monocytogenes</em> serovars 1/2a, 1/2c, 3a, and 3c</td>
<td>Unknown, no similarity</td>
</tr>
<tr>
<td>lmo1118</td>
<td>For: AGGGGTCTTAAATCTTGGA Rev: CGGCTGGTICGGCATACTTA</td>
<td>906</td>
<td><em>L. monocytogenes</em> serovars 1/2c and 3c</td>
<td>Unknown, no similarity</td>
</tr>
<tr>
<td>ORF2819</td>
<td>For: AGCAAAATGCCAAAATCGT Rev: CTCACAAAGCCTCCCATTTG</td>
<td>471</td>
<td><em>L. monocytogenes</em> serovars 1/2b, 3b, 4b, 4d, and 4e</td>
<td>Putative transcriptional regulator</td>
</tr>
<tr>
<td>ORF2110</td>
<td>For: AGTGGAACAATGGATTTGGTGAA Rev: CATCCATCCITACCTTGAC</td>
<td>597</td>
<td><em>L. monocytogenes</em> serovars 4b, 4d, and 4e</td>
<td>Putative secreted protein</td>
</tr>
<tr>
<td>prs</td>
<td>For: GCTGAAGAGATTGCCAAGAAG Rev: CAAAAGAAACCTTGATTTGCCG</td>
<td>370</td>
<td>All <em>Listeria</em> species</td>
<td>Putative phosphoribosyl pyrophosphate synthetase</td>
</tr>
</tbody>
</table>

Doumith et al., 2004

Antisera agglutination still required.
Prophage Analysis

Prophage sequence type correlate with processing facility, and differentiate closely related strains

Verghez et al., AEM, 2011
Prophage and PFGE

Ice cream: three Ascl-PFGE patterns, one WGS cluster
Mixed Strains in Food and Environment

- Multiple *Salmonella* serotypes from the chicken carcass (Scotland, 1989)
- Multiple *Salmonella* serotypes from the same patient (Arkansas, 2012)
- Stone fruit: evidence of mixed culture on the same fruit, serotype 1/2b and 4b of *L. monocytogenes*
- Caramel apples: two different strains of *L. monocytogenes* serotype 4b traced back to the same packing house
- Ice cream: multiple Ascl PFGE patterns from the same production line
Listeria Enumeration

- Cheese, stone fruit, ice cream outbreaks – valuable data for risk assessment

  - Most Probable Number
  - Direct Plating
  - Alternative rapid enumeration?
Microbiological Methods Development

(1) *Salmonella* Newport – “JJPX01.0061” – 2007 outbreak
- growth and survival studies in tomatoes and tomato juices
- invasion studies in whole tomato plants
- qPCR and qRT-PCR detection methods on tomatoes
- bio-control target surrogate

(2) *Escherichia coli* O104:H4– 2011 outbreak – Germany
- plating method evaluation
- studies in leafy greens
- serotyping methods

(3) *Shigella spp.* – parsley, cilantro, alfalfa sprouts, and lettuce
- plating method and qPCR method

(4) *Listeria monocytogenes* – cantaloupe, milkshake
- plating method and enrichment method
- internalization studies
<table>
<thead>
<tr>
<th>Strain</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>ATCC 19606</td>
<td>Human urine</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>ATCC 7966</td>
<td>Milk</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>ATCC 8750</td>
<td>Institut Pasteur</td>
</tr>
<tr>
<td>Acilyclobacillus acidocaldarius</td>
<td>ATCC 106132</td>
<td>Milk</td>
</tr>
<tr>
<td>Acilyclobacillus acidoterrestris</td>
<td>Ad MB1360</td>
<td>Institut Pasteur</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>ATCC 11778</td>
<td>Milk</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC 6633</td>
<td>Institut Pasteur</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>ATCC 33560</td>
<td>Bovine feces</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>ATCC 10231</td>
<td>Human isolate</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>ATCC 8090</td>
<td>NCTC</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>ATCC 13124</td>
<td>Bovine gangrene</td>
</tr>
<tr>
<td><em>Edwardsiella tarda</em></td>
<td>ATCC 15947</td>
<td>CDC</td>
</tr>
<tr>
<td><em>Enterobacter sakazakii</em></td>
<td>ATCC 29544</td>
<td>Human isolate</td>
</tr>
<tr>
<td>Escherichia blattae</td>
<td>ATCC 29907</td>
<td>Cockroach hindgut</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC 8739</td>
<td>Human feces</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O149:H34</td>
<td>NCTC 11602</td>
<td>Human urine</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O1:K1(L1) :H7</td>
<td>ATCC 11775</td>
<td>Human feces</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O111:K58 (B4) :H12</td>
<td>ATCC 33780</td>
<td>Human isolate</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O128:K67 (B12) :H2</td>
<td>NCTC 9708</td>
<td>CDC</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O141:K85 (B) :H4</td>
<td>NCTC 10674</td>
<td>Swine, edema</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O26:K60 (B6) :H11</td>
<td>ATCC 12795</td>
<td>Human feces</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O55:K7</td>
<td>CIP 105215</td>
<td>Stool sample</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O55:H7</td>
<td>CIP 105216</td>
<td>Stool sample</td>
</tr>
<tr>
<td><em>Escherichia fergusoni</em></td>
<td>ATCC 35469</td>
<td>Human feces</td>
</tr>
<tr>
<td>Escherichia hermanii</td>
<td>RDC 72</td>
<td>Egg white</td>
</tr>
<tr>
<td>Escherichia vulneris</td>
<td>RDC 195</td>
<td>Food</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>CIP 82.83T</td>
<td>Potatoes</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>CIP 103762</td>
<td>Institut Pasteur</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>ATCC 13337</td>
<td>NCTC</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>ATCC 13838</td>
<td>NCTC</td>
</tr>
<tr>
<td>Lactobacillus sakei</td>
<td>ATCC 15521</td>
<td>Starter of sake</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC 15313</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Microbacterium flavescens</td>
<td>ATCC 13348</td>
<td>Field soil</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>ATCC 9341</td>
<td>Soil</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>ATCC 25830</td>
<td>Human isolate</td>
</tr>
</tbody>
</table>

Lauer et al., Journal of AOAC International, 2009
Cronobacter Incidents in 2011

- FL case, no food testing

- IL case, opened container PIF negative, opened nursery water positive, but not a match to clinical strain

- MO case, opened container PIF positive, reconstituted PIF positive, opened bottle nursery water from hospital positive, all match to clinical strain. Sealed container PIF and sealed bottle nursery water negative

- OK case, opened container PIF negative, tap water used to reconstitute PIF negative

http://www.cdc.gov/cronobacter/investigation.html

Not all foods available for analysis and not all food analysis successful. Clinical culture needed to study the pathogen.
Veterinary Microbiology

- Antibiotic resistance – Will be difficult to link animal reservoirs to drug resistance in human isolates
- Virulence and pathogenicity – What virulence factors found in clinical isolates were acquired among animal sources? Phenotype study.
- Mixed strain situations
SUMMARY

- Cultures of foodborne pathogens needed for regulatory actions at FDA

- Comparisons of PFGE and serotypes among food and environmental isolates can generate valuable information

- Pure cultures are essential for certain applications – enumeration, analysis of regions involved in HGT, antibiotic resistance, virulence, pathogenicity

- Metagenomic analysis has great potential in identification and subtyping of pathogens directly from complex matrices
With Additional Thanks…. 

**FDA** Darcy Hanes, Karen Jarvis, Chris Grim, Palmer Orlandi, David White  
Steven Musser, Patrick McDermott, Ruth Timme  
Marc Allard, Peter Evans, Eric Brown  
Justin Payne, Charlie Wang, Rebecca Bell  
Christine Keys, Errol Strain, Yan Luo  
James Pettengill, Hugh Rand, Darcy Hanes  
Gopal Gopinathrao, Chis Grim, Palmer Orlandi  
David Melka, Cary Pirone Davies, Justin Payne  
Maria Hoffman, Eric Stevens, Andrea Ottesen  
Tim McGrath, Don Burr, Jie Zheng  
Cong Li, George Kastanis, Tim Muravunda  
Shaohua Zhao, Dumitru Macarisin  

**National Institutes of health (NCBI)**  
David Lipman, Jim Ostell, William Klimke  
Martin Shumway, Richa Agarwala  

**State Health Labs**  
Bill Wolfgang (NY), Dave Boxrud (MN), Anita Wright (FL)  
Elizabeth Driebe (AZ), Angela Fritzinger (VA), Rob Myer (MD), Julie Haendiges (MD)  
Ailin Perez-Osorio (WA), Brian Sauders (NYAG), Many More to come… (TX, NM, CA, SD)  

**CDC** Chris Braden, Cheryl Tarr, John Besser, Patti Fields, Peter Gerner-Smidt  
**FSIS** David Goldman, Emilio Esteban, Kristen Holt, Bill Cray  
**Illumina**  
Susan Knowles, Omayma Al-Awar, Kelly Hoon