OneUSDA  “Do right and feed everyone”

Food Safety and Inspection Service
Protecting Public Health and Preventing Foodborne Illness
USDA FSIS Updates on Whole Genome Sequencing

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Food Safety and Inspection Service, USDA

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Arlington, Virginia
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Why Whole Genome Sequencing (WGS)?

- **Improved resolution for foodborne illness investigations**
  - Improved strain discrimination, illness cluster detection, and case classification

- **Supports FSIS mission goals**
  - Effectively use science to understand foodborne illness and emerging microbiological trends
  - Identification of environmental harborage or recurrences of pathogens in FSIS-regulated establishments/products to further support the inspection and verification process

- **Alignment of pathogen surveillance with our domestic public health and regulatory partners**
  - Collaborative efforts with US Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA-CFSAN), the US Centers for Disease Control and Prevention (CDC), the US National Institutes of Health National Center for Biotechnology Information (NCBI), and also state/local health partners/laboratories
Food Safety and Inspection Service

WGS at FSIS: Milestones by Year

FY2013
- Visits to NCBI, FDA and CDC
- Procurement of two MiSeq’s
- First Sequences produced for outbreak investigations

FY2014
- FSIS shared Lm with FDA for WGS
- 1- FSIS began WGS on adulterant isolates
- 2- Uploaded first Lm sequences to NCBI

FY2015
- FSIS begins WGS on selected cecal Salmonella and Campylobacter

FY2016
- PFGE no longer performed on Lm
- WGS on NARMS cecal E. coli and Enterococcus

FY2017
- WGS on all Salmonella and Campylobacter

FY2018
Food Safety and Inspection Service:
WGS at FSIS: Current Status

- FSIS built capacity for conducting WGS on all isolates obtained from FSIS sampling programs
  - Currently 12 sequencers in FSIS Field Service Laboratories,
  - In FY17, FSIS sequenced 7,282 isolates; In FY18, FSIS sequenced ~11,000

- In collaboration with our public health and regulatory partners, FSIS currently considers available WGS analyses in addition to PFGE, epidemiological and trace-back information to further understand the relationship between clinical and food isolates

- FSIS works with National Antimicrobial Resistance Monitoring System (NARMS) partners (FDA, CDC) to understand the occurrence or introduction of antimicrobial resistance genes in pathogens of interest
How can you locate FSIS data on NCBI?

**FSIS Submissions to NCBI Bioprojects**

- PRJNA242847
  - GenomeTrakr Project: USDA-FSIS (*Salmonella*)
- PRJNA215355
  - GenomeTrakr Project: FDA (*Listeria monocytogenes*)
- PRJNA287430
  - USDA-FSIS: *Campylobacter*
- PRJNA268206
  - GenomeTrakr Project: USDA-FSIS (STEC)
- PRJNA292666
  - FSIS NARMS *Salmonella*
- PRJNA292668
  - FSIS NARMS *Campylobacter*
- PRJNA292669
  - FSIS NARMS *Enterococcus*
Food Safety and Inspection Service:
**WGS workflow timeline: 16 isolates**

**Day 1**
- DNA extraction: 3 hours
- DNA quantity assessment (Qubit): ~1 hour

**Day 2**
- Dilution of DNA, tagmentation and indexing: ~2 hours

**Day 3**
- Quantify DNA and library prep
- Combine indexed DNA and load into cartridge: ~2 hours

**Day 3-5**
- MiSeq run times:
  - 300 cycle kit: 26 hours
  - 500 cycle kit: 42 hours

**Day 6**
- Data transfer and analyses: ~2-4 hours
Three quality metrics we can determine to assess if the FASTQ files are of sufficient quality.

The last metric listed is an assembly-based metric.
Food Safety and Inspection Service:

Usage of WGS: Single Characterization Workflow

- **Source attribution tracking**
- **Campylobacter** speciation
  - Real-time PCR
- **Serotyping/serogrouping**
  - *Salmonella*
  - Adulterant STECs
- **Alternative to PFGE for comparison of genotypes**
  - wgMLST analyses
  - SNP analyses
- **Antimicrobial Resistance (Phenotype prediction)**
  - *Salmonella*
  - *Campylobacter*
  - *E. coli*
  - *Enterococcus*
- **Identify characterized genes of interest**
  - Resistance to environmental factors (heat, acid, metals, etc)
  - Plasmid typing
  - Virulence factors
    - *stx/eae* sub-types (STEC)

A single workflow for many characterization approaches *via* informatics
Assembly: We have a FASTA what can we do with it?

- In most cases, characterization data is obtained from assembly data (FASTA file)

- Using local BLAST databases we are able to determine MLST genes, serotype determining genes, virulence genes, and antimicrobial resistance genes

- Utilize research partners’ custom databases to identify genes involved in sanitizer resistance, metal resistance, and plasmid-associated replicons
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Assembly: We have a FASTA what can we do with it?

### Campylobacter Characterization

<table>
<thead>
<tr>
<th>FSIS_Number</th>
<th>Species</th>
<th>Sequence_type</th>
<th>File_size</th>
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<tbody>
<tr>
<td>FSIS1608480</td>
<td>C. coli</td>
<td>ST-7426</td>
<td>1940103</td>
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<tr>
<td>FSIS1608481</td>
<td>C. jejuni</td>
<td>ST-6238</td>
<td>1928269</td>
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<tr>
<td>GMI16-1</td>
<td>C. coli</td>
<td>ST-7426</td>
<td>1938894</td>
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<td>GMI16-2</td>
<td>C. jejuni</td>
<td>ST-6238</td>
<td>1930415</td>
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### Salmonella Characterization

<table>
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<th>FSIS_Number</th>
<th>Factors</th>
<th>Serotype</th>
<th>Sequence_type</th>
<th>File_size</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSIS1608370</td>
<td>C1,z38</td>
<td>Lille</td>
<td>ST-297</td>
<td>5241302</td>
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<tr>
<td>FSIS1608559</td>
<td>C2,i,z6</td>
<td>Kentucky</td>
<td>ST-152</td>
<td>5179970</td>
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<tr>
<td>FSIS1608560</td>
<td>1,2,B,i</td>
<td>Typhimurium</td>
<td>ST-19</td>
<td>5169607</td>
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<tr>
<td>FSIS1608562</td>
<td>1,6,E1,l,v</td>
<td>London</td>
<td>ST-155</td>
<td>5098914</td>
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### Listeria Characterization

<table>
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<tr>
<th>FSIS_Number</th>
<th>GENE</th>
<th>sequence_type</th>
<th>Lineage</th>
<th>File_size</th>
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<tbody>
<tr>
<td>FSIS1608509</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-87</td>
<td>Lineage-I</td>
<td>3027617</td>
</tr>
<tr>
<td>FSIS1608510</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-1</td>
<td>Lineage-I</td>
<td>3254280</td>
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<td>FSIS1608511</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-5</td>
<td>Lineage-I</td>
<td>3145677</td>
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<tr>
<td>FSIS1608623</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-321</td>
<td>Lineage-II</td>
<td>3096560</td>
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<tr>
<td>GMI16-3</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-2</td>
<td>Lineage-I</td>
<td>3060032</td>
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<tr>
<td>GMI16-4</td>
<td>actA hly inlA inlB plcA plcB prfA</td>
<td>ST-121</td>
<td>Lineage-II</td>
<td>3197949</td>
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Food Safety and Inspection Service:

Single Characterization Workflow: Serotype Determination

- FSIS sequenced 7665 *Salmonella* isolates from various sampling programs from Jan 2015 through Jun 2017.

- Compared serotype reported by routine methods (molecular serotyping or traditional serology) with serotype determined using SeqSero to query WGS data:
  - For 94.34% (7231/7665) of isolates, WGS that matched reported serology result.
  - For 5.66% (434/7665) of isolates, WGS did not match reported serology result.
    - Includes isolates with incomplete genetic factor set (cannot call/identify serotype).
Single Characterization Workflow: Antimicrobial Resistance

- Analyzed ~1791 *Salmonella* isolates from FY2016 HACCP and NARMS cecal sampling
- Compared genotypic prediction for resistance to reported phenotype using NARMS panel

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug(s)</th>
<th>Isolates with R phenotype</th>
<th>Resistance gene(s) or mutation(s)</th>
<th>Geno/Pheno Correlation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-lactam</td>
<td>Aug</td>
<td>R = 89</td>
<td>blaCMY(n=88)</td>
<td>98.88</td>
</tr>
<tr>
<td></td>
<td>Amp</td>
<td>R = 217</td>
<td>blaTEM(n=73),blaCMY(n=88),blaCARB(n=6),blaCTX(n=54),blaSHV(n=1),blaHERA(n=3),blaOXA(n=0)</td>
<td>99.08</td>
</tr>
<tr>
<td></td>
<td>Axo</td>
<td>R = 145</td>
<td>blaCMY(n=88),blaCTX(n=54),blaSHV(n=1)</td>
<td>98.62</td>
</tr>
<tr>
<td></td>
<td>Fox</td>
<td>R = 91</td>
<td>blaCMY(n=86)</td>
<td>94.51</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Gen</td>
<td>R = 58</td>
<td>aac(n=49),aadB(n=0)</td>
<td>84.48</td>
</tr>
<tr>
<td></td>
<td>Str</td>
<td>R = 476</td>
<td>str(n=342),aadA(n=142),aph(6'-lc(n=8)</td>
<td>95.80</td>
</tr>
<tr>
<td>Phenicols</td>
<td>Chl</td>
<td>R = 102</td>
<td>flor(n=97),cmlA(n=3),catA(n=2),oqxAB(n=0)</td>
<td>97.06</td>
</tr>
<tr>
<td>Macrolide</td>
<td>Azi</td>
<td>R = 7</td>
<td>mph(n=3),erm(n=1)</td>
<td>57.14</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>Fis</td>
<td>R = 313</td>
<td>sul1(n=144),sul2(n=195),sul3(n=1)</td>
<td>99.36</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Cot</td>
<td>R = 64</td>
<td>dfrA(n=62)</td>
<td>96.88</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Tet</td>
<td>R = 587</td>
<td>tetA(n=276),tetB(n=237),tetC(n=6),tetD(n=1),tetG(n=6),tetM(n=3)</td>
<td>99.15</td>
</tr>
<tr>
<td>Quinolone</td>
<td>Cip</td>
<td>R = 92</td>
<td>qnrB(n=25),qnrS(n=2),oqxAB(n=0),aac(6')lb(n=0),gyrA(n=0),parC(n=0)</td>
<td>29.35</td>
</tr>
<tr>
<td></td>
<td>Nal</td>
<td>R = 76</td>
<td>qnrB(n=12),qnrS(n=0),oqxAB(n=0),aac(6')lb(n=0),gyrA(n=62),parC(n=0)</td>
<td>97.37</td>
</tr>
</tbody>
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## Single Characterization Workflow: Virulence Factor Typing in STEC

<table>
<thead>
<tr>
<th>Serogroup (no. sequenced)</th>
<th>Top 7 gene MLST sequence types</th>
<th>Top stx types</th>
<th>eae types</th>
<th>Top Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26 (50)</td>
<td>ST-21 (82%)</td>
<td>stx1a (80%)</td>
<td>Beta1 (100%)</td>
<td>O26:H11 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2a (16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O45 (20)</td>
<td>ST-17 (95%)</td>
<td>stx1a (100%)</td>
<td>Epsilon (95%)</td>
<td>O111:H8 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O103 (147)</td>
<td>ST-17 (67.8%)</td>
<td>stx1a (98.0%)</td>
<td>Epsilon (84.9%)</td>
<td>O103:H2 (84.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O111 (44)</td>
<td>ST-16 (93.0%)</td>
<td>stx1a (86.0%)</td>
<td>Theta (100%)</td>
<td>O111:H8 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O121 (15)</td>
<td>ST-655 (93.3%)</td>
<td>stx2a (100%)</td>
<td>Epsilon (100%)</td>
<td>O121:H19 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O145 (18)</td>
<td>ST-32 (100%)</td>
<td>stx1a stx2d (38.9%)</td>
<td>Gamma-1 (100%)</td>
<td>O145:H28 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2a (27.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2c (11.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O157 (149)</td>
<td>ST-11 (96.00%)</td>
<td>stx1a stx2a (28.2%)</td>
<td>Gamma-1 (100%)</td>
<td>O157:H7 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2c (22.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2a (20.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- *E. coli* serotypes, O157:H11 & O157:H29 identified to be lacking *eaestx* through WGS
- *stx*-negative strains can be identified as serotype *E. coli* O157:H7 based on WGS
Food Safety and Inspection Service:
WGS and Antimicrobial Resistance (AMR): $bla_{CTX-M-65}$ S. Infantis

- Sequenced the genomes of 10 S. Infantis isolates containing $bla_{CTX-M-65}$

- Isolates collected through NARMS Chicken, Cattle and Human surveillance and product sampling programs

- First report of the $bla_{CTX-M-65}$ gene and a pESI-like megaplasmid from S. Infantis in the U.S.

- Resistance profiles include ampicillin, chloramphenicol, sulfisoxazole, tetracycline, ceftriaxone, ceftiofur, nalidixic acid, trimethoprim/sulfamethoxazole and decreased susceptibility to ciprofloxacin

- Concerns regarding multi-drug resistant (MDR) S. Infantis have been communicated to industry
Food Safety and Inspection Service

WGS and AMR: *Salmonella* Infantis and $bla_{CTX-M-65}$

- *Salmonella* Infantis with $bla_{CTX-M-65}$ in a regulated product showed an upward trend from 2015-2017
  - A single PFGE type was identified as containing a $bla_{CTX-M-65}$ gene with distribution only six isolates in 2015
  - In 2016 the $bla_{CTX-M-65}$ gene was seen in seven PFGE types and the isolates carrying this gene increased to 51
  - In 2017 the $bla_{CTX-M-65}$ gene was seen in 22 PFGE types and the total isolates carrying this gene increased to 140
- Of 1179 FSIS Infantis isolates sequenced, 748 had IncFIB plasmid present
  - 57.1% of isolates with IncFIB plasmids contain $bla_{CTX-M-65}$
  - 94.1% of isolates with IncFIB plasmid have genes for mercury resistance
First identification of Linezolid resistance in the U.S. in bacteria isolated from food animals

Linezolid resistance gene optrA in 3 Enterococcus isolates from cecal content

An additional linezolid resistance gene cfr identified on the same plasmid for one of the isolates

Other resistance markers on these plasmids may indicate that use of other antimicrobials may co-select for these plasmids

Horizontal transmission into bacterial populations that cause human infections is of concern

Accepted for publication in Journal of Antimicrobial Chemotherapy
Food Safety and Inspection Service:
High Quality SNP analysis: NCBI Pathogen Detection Browser

As member of PulseNet, FSIS will use wgMLST functionality of Bionumerics 7.6 developed by CDC and Applied Maths.

Uses gene by gene approach to assess variations (‘alleles’) within each gene:
- SNP(s), indels, rearrangements

Currently used for *Listeria monocytogenes*, additional schemes will be made available for other pathogens.
WGS: Future

Illness Prevention Focus and Collaborations

- WGS in Risk and Attribution
  - Phenotype to Genotype focus
  - Virulence, Pathogenicity, Adaptation, Gene mobility
- Transience vs Harborage and Safe-Harbor Issue
- Use in routine inspection process
- Pathogen introduction and movement among animal, humans, environment and establishments/factories
- Discussion and clarity on legal issues and ramifications
- Opportunities for collaborations and data sharing
Food Safety and Inspection Service:
WGS at FSIS: Where Do We Go From Here

WGS: Future

Focus on Communication and Training

- Standardize and simplify WGS related communications
- Communicating WGS results with regulated establishments
- Development/Availability of audience specific WGS training modules
- Continued engagement (Meetings, Webinars, FAQs etc.)
Food Safety and Inspection Service

Acknowledgements

- USDA FSIS Offices
- USDA ARS
- CDC PulseNet and NARMS
- FDA CFSAN
- FDA CVM
- NCBI
- State Laboratories
Questions?

One Team, One Purpose
Protecting Public Health and Preventing Foodborne Illness