



United States Department of Agriculture

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Food Safety and Inspection Service

Protecting Public Health and Preventing Foodborne Illness



Food Safety and Inspection Service

USDA FSIS Updates on Whole Genome Sequencing

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GenomeTrakr Meeting

Arlington, Virginia

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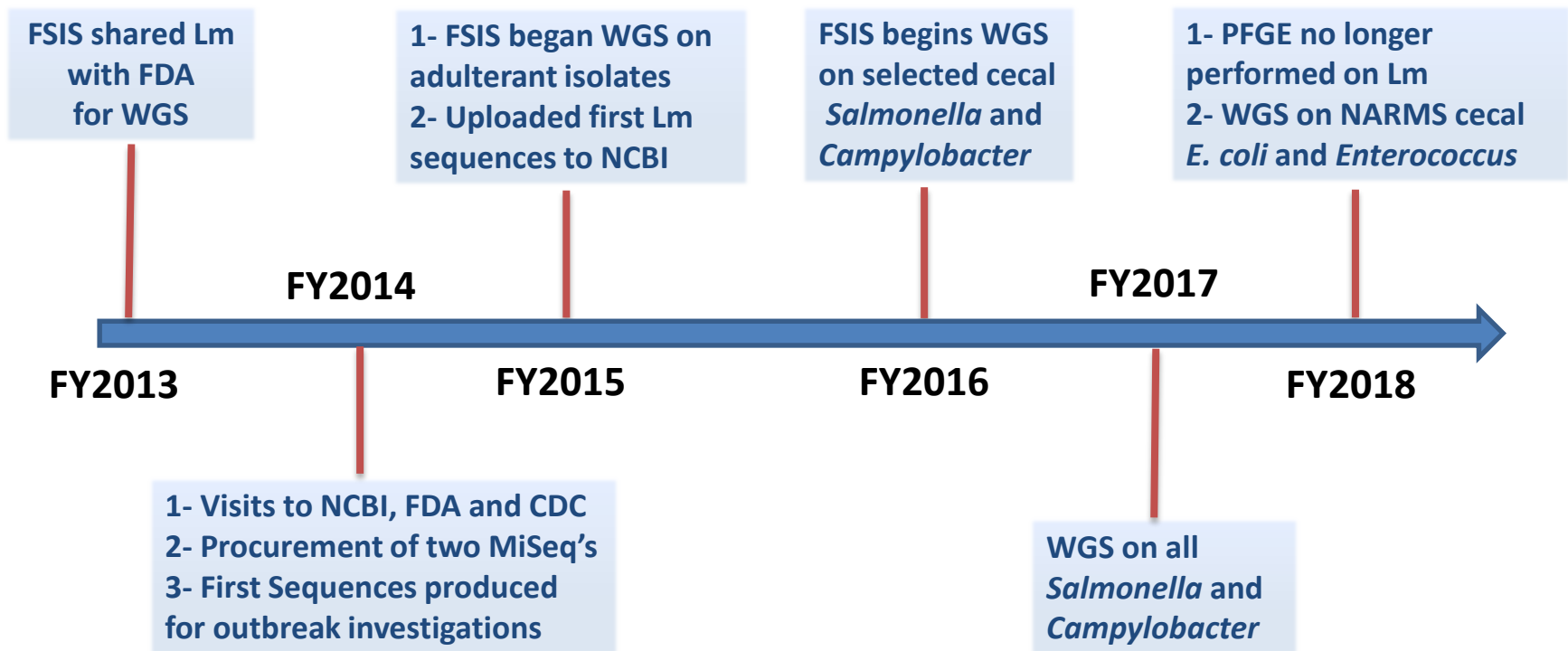
Food Safety and Inspection Service:

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

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WGS at FSIS: Milestones by Year

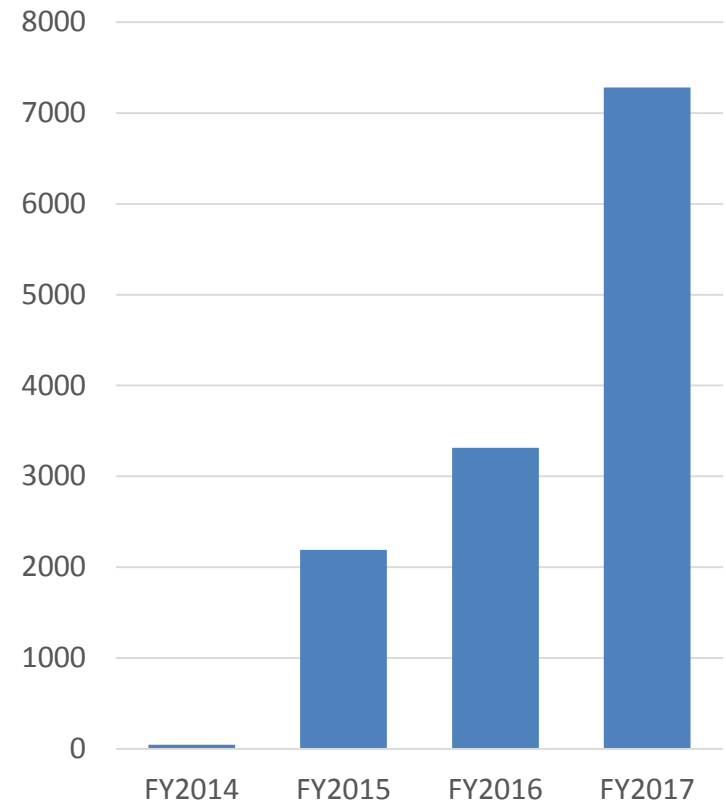


Food Safety and Inspection Service:

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

Number of Isolates Sequenced by Fiscal Year



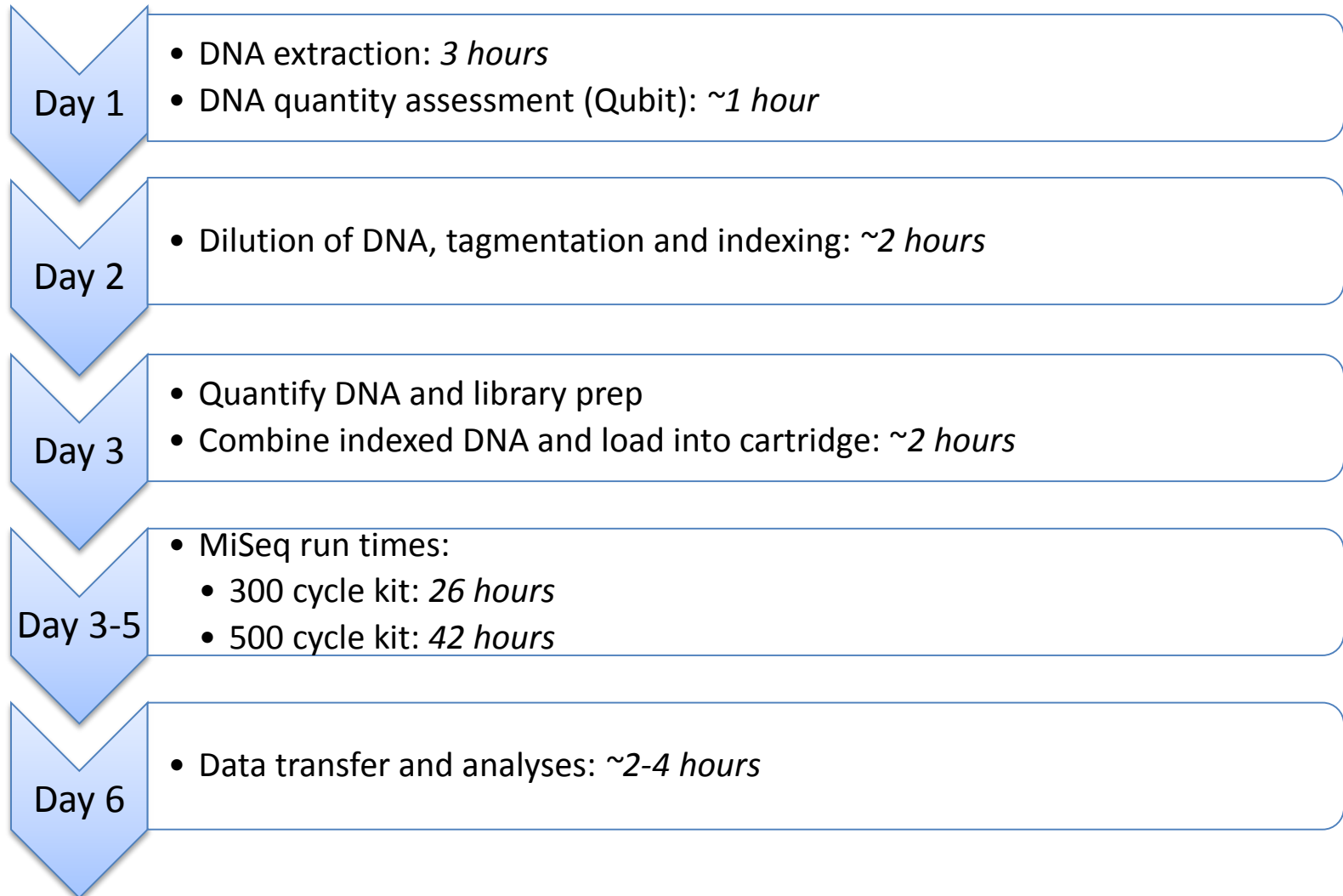
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How can you locate FSIS data on NCBI?

FSIS Submissions to NCBI Bioprojects

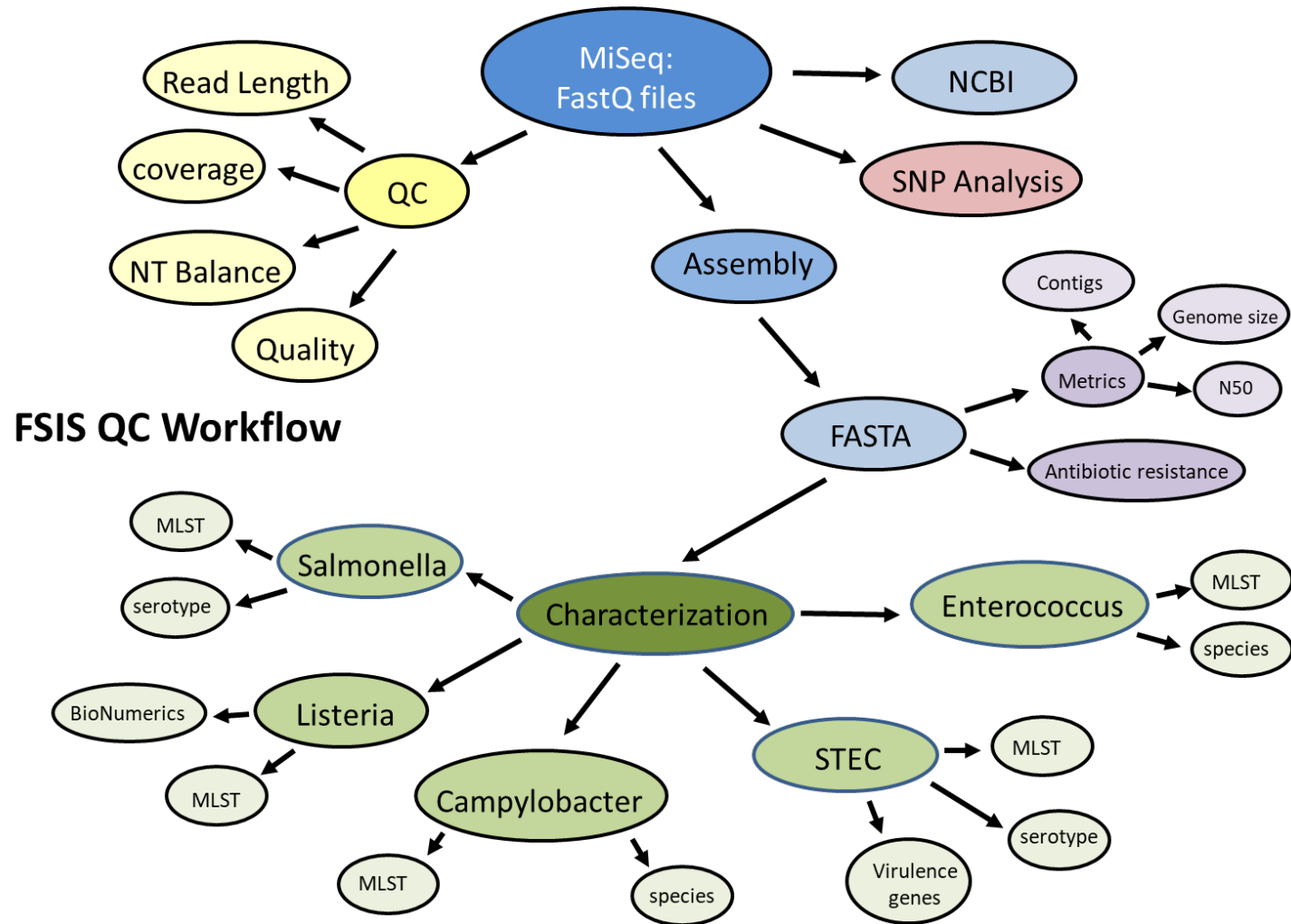
- PRJNA242847
 - GenomeTrakr Project: USDA-FSIS (*Salmonella*)
- PRJNA215355
 - GenomeTrakr Project: FDA (*Listeria monocytogenes*)
- PRJNA287430
 - USDA-FSIS: *Campylobacter*
- PRJNA268206
 - GenomeTrakr Project: USDA-FSIS (STEC)
- PRJNA292666
 - FSIS NARMS *Salmonella*
- PRJNA292668
 - FSIS NARMS *Campylobacter*
- PRJNA292669
 - FSIS NARMS *Enterococcus*

Food Safety and Inspection Service: WGS workflow timeline: 16 isolates



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WGS Analyses: Quality Control and Characterization Workflow



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Raw FASTQ data: Is the data good?

- Three quality metrics we can determine to assess if the FASTQ files are of sufficient quality
- The last metric listed in is an assembly-based metric

Quality Metric	Listeria	Campylobacter	STEC	Salmonella
Nucleotide balance	0.9-1.1	0.9-1.1	0.9-1.1	0.9-1.1
Average Quality	>30	>30	>30	>30
Expected Coverage	>20X	>20X	>40X	>30X
Assembly Size	2.7-3.3 million bp	1.6-2.0 million bp	4.5-6.2 million bp	4.4-5.5 million bp

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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/ea* sub-types (STEC)

A single workflow for many characterization approaches *via* informatics

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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?

FSIS_Number	Species	Sequence_type	File_size
FSIS1608480	C. coli	ST-7426	1940103
FSIS1608481	C. jejuni	ST-6238	1928269
GMI16-1	C. coli	ST-7426	1938894
GMI16-2	C. jejuni	ST-6238	1930415

Campylobacter
Characterization



FSIS_Number	Factors	Serotype	Sequence_type	File_size
FSIS1608370	C1,z38	Lille	ST-297	5241302
FSIS1608559	C2,i,z6	Kentucky	ST-152	5179970
FSIS1608560	1,2,B,i	Typhimurium	ST-19	5169607
FSIS1608562	1,6,E1,l,v	London	ST-155	5098914

Salmonella
Characterization



Listeria
Characterization



FSIS_Number	GENE	sequence_type	Lineage	File_size
FSIS1608509	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-87	Lineage-I	3027617
FSIS1608510	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-1	Lineage-I	3254280
FSIS1608511	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-5	Lineage-I	3145677
FSIS1608623	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-321	Lineage-II	3096560
GMI16-3	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-2	Lineage-I	3060032
GMI16-4	<i>actA hly inlA inlB plcA plcB prfA</i>	ST-121	Lineage-II	3197949



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)

Food Safety and Inspection Service:

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

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

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Single Characterization Workflow: Virulence Factor Typing in STEC

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

- *E. coli* serotypes, O157:H11 & O157:H29 identified to be lacking *eae/stx* 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



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Institution: DigiTop -- USDA's Digital Desktop Library

Comparative Analysis of Extended Spectrum Beta-Lactamase CTX-M-65-Producing *Salmonella* *Infantis* Isolates from Humans, Food Animals, and Retail Chickens in the United States

Heather Tate^{a,f}, Jason P. Folster^b, Chih-Hao Hsua, Jessica Chen^{b,c}, Maria Hoffmann^d, Cong Lia, Cesar Morales^e, Gregory H. Tyson^a, Sampa Mukerjee^a, Allison C. Brown^b, Alice Green^e, Wanda Wilson^e, Uday Dessai^e, Jason Abbott^a, Lavin Joseph^b, Jovita Haro^e, Sherry Ayers^a, Patrick F. McDermott^a and Shaohua Zhao^a

Author Affiliations

Food Safety and Inspection Service

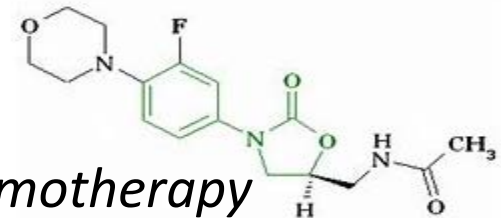
WGS and AMR: *Salmonella* Infantis and *bla*_{CTX-M-65}

- *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

Food Safety and Inspection Service:

WGS and AMR: Linezolid 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 *cftr* 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

#	Strain	Serovar	Isolate	Create Date	Location	Isolation Source	Isolation type	Host	Min-sa	Min-dif	BioSample	Assembly
1	<input checked="" type="checkbox"/> FSIS15010	O103	PDT000072730.1	2015-07-24	USA:NY	Animal-Calf-Formula-fed Veal	environmental/otr		n/a		9 SAMN0392193	
2	<input type="checkbox"/> PNUSAE00	E. coli O103:Phen	PDT000107206.1	2016-02-16	USA	Stool	clinical		11	21	SAMN0448490	
3	<input type="checkbox"/> PNUSAE00	E. coli O103:Phen	PDT000128459.1	2016-05-03	USA	Stool	clinical		8	16	SAMN0493188	
4	<input type="checkbox"/> PNUSAE00	E. coli O103:Phen	PDT000132453.1	2016-05-25	USA	Stool	clinical		10	11	SAMN0517625	
5	<input type="checkbox"/> PNUSAE00		PDT000145400.1	2016-08-24	USA		clinical		8	18	SAMN0554491	

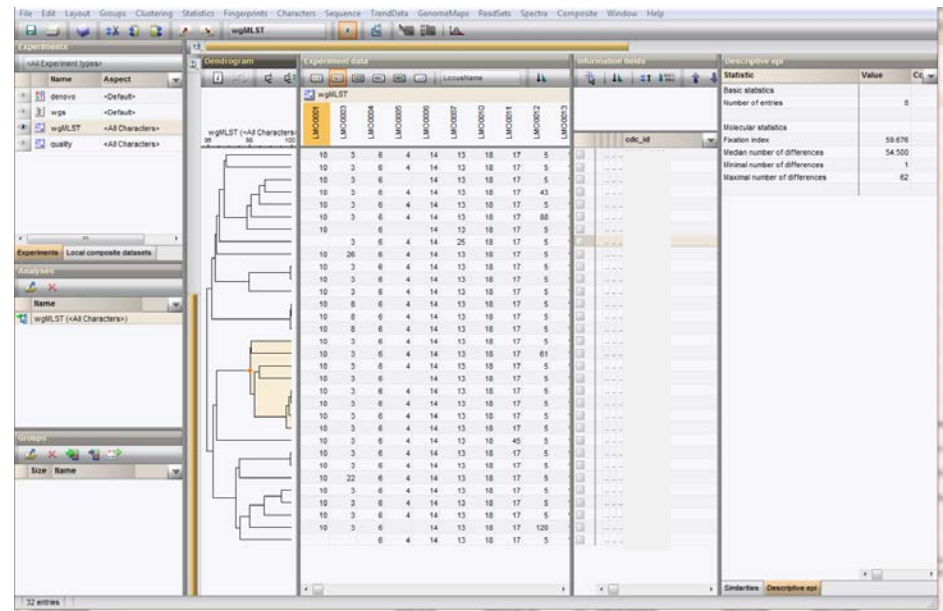
View 1 - 5 of 18

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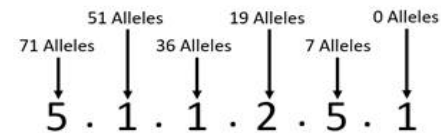
USA, PNUSAE008453, PDT000227537.1
 17-09-01, USA, PNUSAE009239, PDT000238418.1
 clinical, 2017-11-03, USA, PNUSAE010709, PDT000260756.1
 clinical, 2018-03-21, USA, PNUSAE012670, PDT000296209.1
 clinical, 2016-09-23, USA, PNUSAE004527, PDT000150131.1
 clinical, 2016-05-03, USA, Stool, PNUSAE002953, PDT000128459.1
 clinical, 2016-11-28, USA, PNUSAE005234, PDT000163241.2
 clinical, 2017-09-22, USA, PNUSAE009715, PDT000242791.1
 clinical, 2017-07-03, USA, PNUSAE007139, PDT000222572.1
 clinical, 2016-05-25, USA, Stool, PNUSAE003127, PDT000132453.1
environmental/other, 2015-07-24, USA:NY, Animal-Calf-Formula-fed Veal, FSIS1501068, PDT000072730.1
 clinical, 2017-09-22, USA, PNUSAE009713, PDT000242789.1

Food Safety and Inspection Service: Whole genome Multi-locus Sequence Typing (wgMLST)

- 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



Listeria Nomenclature



**These allele definitions are approximate! The nomenclature is based off of the core genome (cgMLST) and wider allele differences are to be expected when comparing wgMLST results.

Food Safety and Inspection Service: WGS at FSIS: Where Do We Go From Here

*WGS:
Future*

Illness Prevention
Focus and
Collaborations

- ❑ **WGS in Risk and Attribution**
 - ❑ **Phenotype to Genotype focus**
 - ❑ **Virulence, Pathogenicity, Adaption, 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



Food Safety and Inspection Service:

Questions?



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