USDA

United States Department of Agriculture

One Team, One Purpose

Food Safety and Inspection Service

Protecting Public Health and Preventing Foodborne Illness

Food Safety and Inspection Service

FSIS WGS Update

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Success = One-Team-One-Purpose

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FSIS Lab Update: Presentation Outline

- About FSIS and Strategic Commitments
- WGS Activities at FSIS
  - Capacity Building
  - Outbreak Investigations
  - Harborage
  - NARMS and AMR Genes
  - Other Analysis
    - WGS Discriminatory Power Compared to PFGE
    - Matches using NCBI Pathogen Detection Pipeline
    - Pathogenicity Islands and Heat Resistance Genes
- Our Food Safety/Public Health Challenge
- Where do we go from here
- WGS Collaborations - Pivotal for Success
- Concluding Remarks

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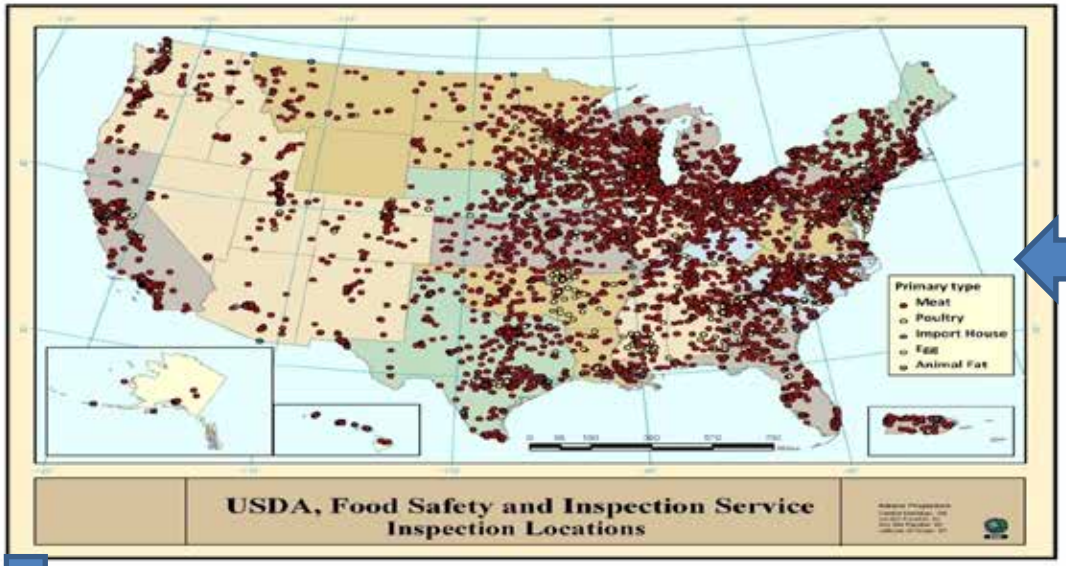
FSIS WGS Update: Our Authority and What We Do!



FSIS is the public health agency in the U.S. Department of Agriculture responsible for ensuring that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged.

Our Authority

- Federal Meat Inspection Act (FMIA), 1906
- Agricultural Marketing Act (AMA), 1946
- Poultry Products Inspection Act (PPIA), 1957
- Humane Methods of Slaughter Act (HMSA), 1958
- Egg Products Inspection Act (EPIA), 1970

Inspection and Sampling

- About 6000+ Establishments
- Over 7000 Inspection Personnel

- > 100 K Microbiological Samples
- > 256,333 Micro Analyses
- About 10,000 bacterial isolates

- 3+ million Scientific Analysis (includes residue samples)

Enrichment
Screening
Isolation
Characterization

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FSIS WGS Update: Mission Critical Investment in 2014 - 2019

Project Description	Goals	Expected Outcomes
q WGS is a multi-year and multi-agency initiative that allows FSIS to: q Characterize bacterial genomes with greater precision and detail q Detect the presence of undesirable genes and genetic elements - Antimicrobial Resistance, Virulence, Plasmids etc. q Utilizes modern sequencing instruments and bioinformatics q Collaboration with the CDC, FDA, NIH, and state agencies q A formal collaboration formed (Gen-FS) and a Charter is being finalized	q To conduct real-time genome sequencing on all FSIS isolates q Collaboratively develop WGS technology as the multi-agency network q Standard for bacterial genome characterization and comparison q Determine Virulence and adaptability factors, Antimicrobial Resistance, Plasmids, etc.	q Enhance FSIS's ability to connect pathogenic bacteria from patients and food & environmental samples q Identify, prevent foodborne illness with a higher level of accuracy and contain outbreaks faster. q Monitor antimicrobial resistance and identify development or introduction of novel/undesirable genes q When fully functional, WGS is expected to consolidate and replace several current laboratory analyses including pulsed-field gel electrophoresis (PFGE) and improve overall speed and efficiency in analysis and reporting across WGS collaboration

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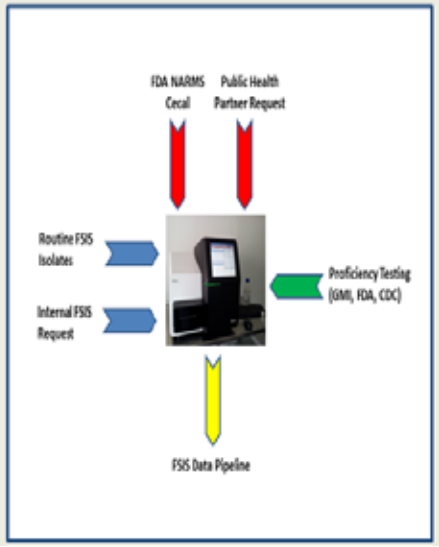
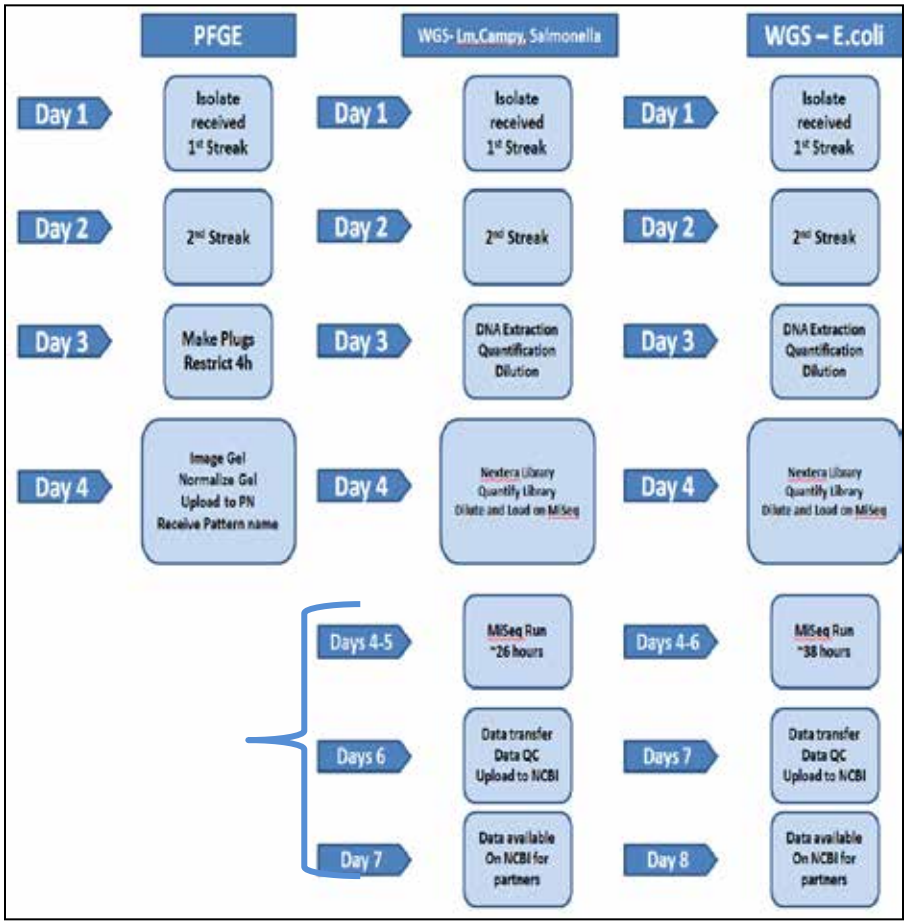
FSIS WGS Update: Isolate Sources and Workflow



Methodology

- Isolates are processed by FSIS laboratory and prepped for WGS
- After sequencing on the MiSeq, data is processed and analyzed
- Sequence data meeting acceptance criteria is uploaded with minimal metadata to NCBI BioSample and Sequence Read Archive (SRA)
- Downstream analyses conducted by USDA-FSIS using bioinformatics tools
- All WGS results (NCBI SAMN Accession number, SRA Accession numbers, antibiotic resistance genes) and associated QC information captured in Laboratory Information System (LIMS)



Note: Currently WGS needs additional time compared to PFGEs, however, informatics can derive variety of information once an isolate is sequenced

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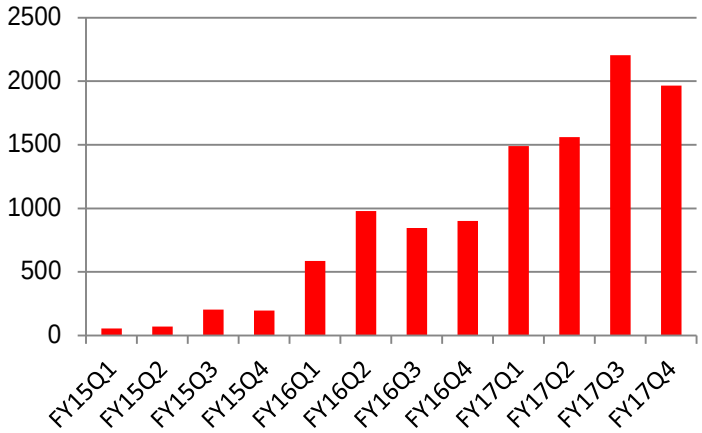
FSIS WGS Update: WGS Milestones and Uploads in to the National Center for Biotechnology Information (NCBI) Genomic Database

Isolates Sequenced



Milestone Dates

- July 2014: *Salmonella* and *Listeria monocytogenes*
- December 2014: STECs
- February 2015: *Campylobacter*
- May 2015: Capability to directly upload WGS files to NCBI

As of 10/01/2017: 10,935 FSIS isolates have been sequenced and uploaded to NCBI

	Listeria monocytogenes	STEC	*Salmonella*	*Campylobacter*	Total
Routine/Special Projects	**435**	**482**	**3825**	**1007**	**5749**
NARMS Cecal Sampling			**2396**	**2790**	**5186**
Total	**435**	**482**	**6221**	**3797**	**10935**
Total for FY 2017	**7219** (Note: In FY 2016 we completed 3313 WGS and in 2015 it was 524. About 121 WGS from NARMS 'E. coli + Enterococcus' are not included in the 10935 total)				

FSIS WGS Updates: Capacity Building and Sequence Uploads to NCBI

- FSIS WGS system comprises of 12 Sequencers in 3 FSIS Labs
- In a short time FSIS has developed capacity to sequence over 7000 FSIS isolates including NARMS *Salmonella* and *Campylobacter,* in FY 2017
- In FY 2018, FSIS intends to sequence all FSIS regulatory isolates, NARMS *Salmonella* and *Campylobacter* and at least 50% of the indicators (*E. coli* and *Enterococcus*)
- All the Sequences and associated metadata are submitted to NCBI in a real-time basis, and the metadata fields include:
  - Product/Source type (Ready to eat product, raw meat/poultry, environmental swab, etc.)
  - Year sample was collected
  - State where sample was collected
  - Subtyping information when available
  - *Salmonella* – serovar
  - Adulterant STECs – O-group
  - *Campylobacter* – species

FSIS WGS Updates: WGS Analysis in FY 2017 and FY 2018

- In FY 2017 FSIS initiated WGS analysis beyond its use in outbreak investigations to include:
  - Geographic distribution of Genotypes
    - Distribution of ST in Beef and pork
  - Antimicrobial Resistance (AMR) and distribution of AMR Genes
  - Presence of *Salmonella* pathogenicity Islands
  - Presence of Heat Resistance Genes
  - Note: A follow-up FSIS presentation will cover some of these analysis and FSIS Pipelines
- In FY 2018 FSIS will focus on understanding further - the occurrence, trend and patterns of genotypes of public health concern in FSIS regulated products
  - This will include exploring harmful genes/traits that can provide further insight into FSIS pathogens of concern
  - Considerations/measures to prevent, control and reduce pathogens of concern in FSIS regulated products

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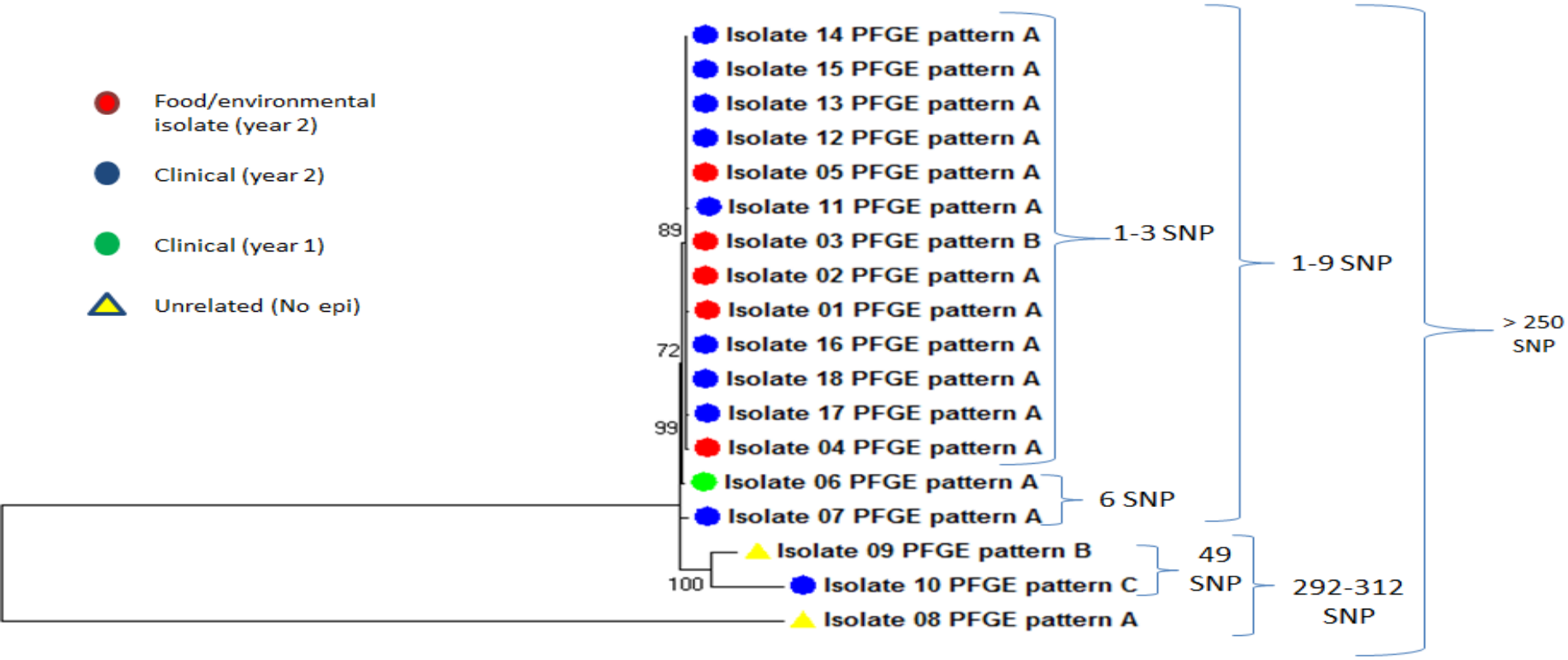
FSIS WGS Update: Use in Outbreak Investigation

Comparison of Clinical isolates to food/environmental isolates from an establishment



Settings: The analysis was generated with Lyve-SET version1.1.4f. Reads were cleaned with CG Pipeline, SNPs were called with Varscan, and Lyve-SET was run with the following options: minimum coverage- 10, min alternative fraction- 0.90, and allowed flanking- 5 bp. Maximum Likelihood Tree built in Mega Version 7 with 500 bootstraps

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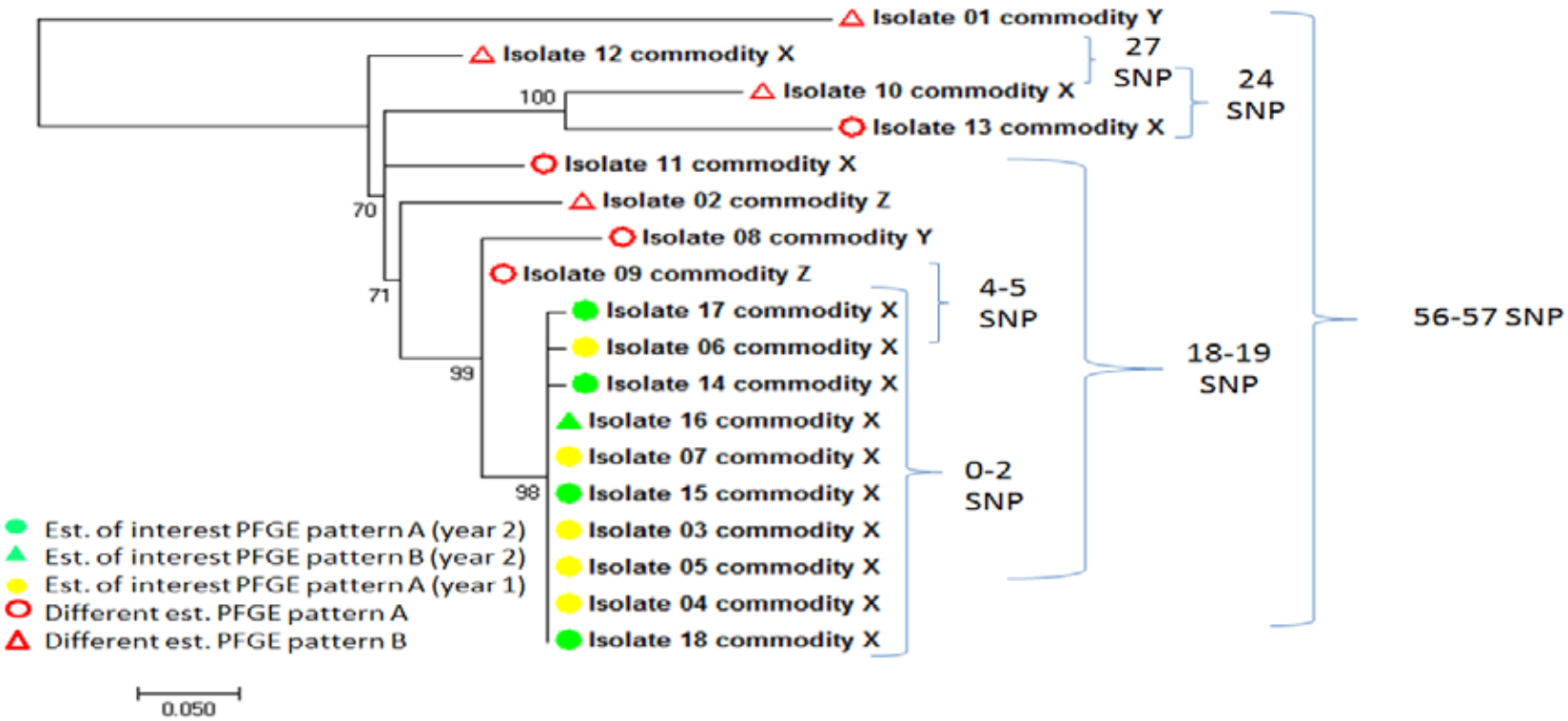
FSIS WGS Update: WGS Use in Outbreak Investigation

Comparison of isolates from an establishment over a 2 year period



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FSIS WGS Update: WGS Application – Potential Use in Harborage

- FSIS is also currently exploring use of WGS as a tool to understand potential harborage or reoccurrence of contamination in establishments
- FSIS works collaboratively with FDA in dual-jurisdiction establishments that produce both FDA and FSIS-regulated products
  - When one agency identifies potential harborage through bacterial characterization of Lm isolates (PFGE and/or WGS), information is shared to inform a collaborative regulatory response within the establishment

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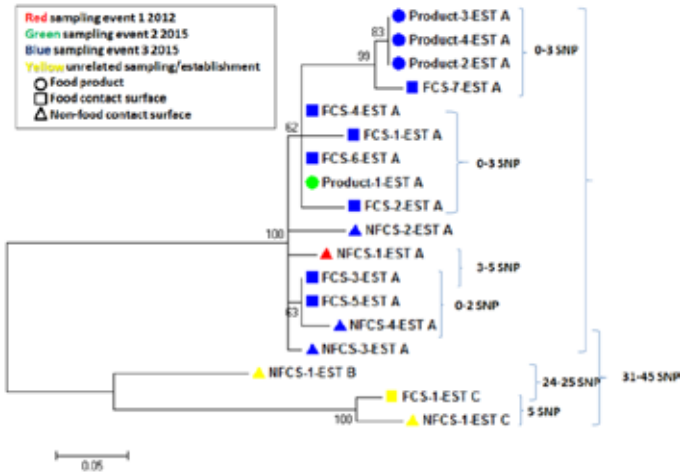
FSIS WGS Update: Harborage 'WGS and PFGE' – *Lm* Experience

- **WGS and PFGE results are generally in agreement**
- **WGS can sometimes exclude isolates from a group with the same PFGE pattern or include isolates in a group with different PFGE patterns**
- **In the examples below inclusion or exclusion considered all information including sample metadata (establishment, isolation date, etc.)**



Lm harborage: WGS and PFGE comparison		
	High quality SNPs	wgMLST
Same PFGE pattern: WGS agreement	0-2 SNPs	0-7 allele differences
Same PFGE pattern: WGS exclusion	34-45 SNPs	29-33 allele differences
Different PFGE: WGS inclusion	0-3 SNPs	0-5 allele differences

Note the trend between the SNP and wgMLST approaches

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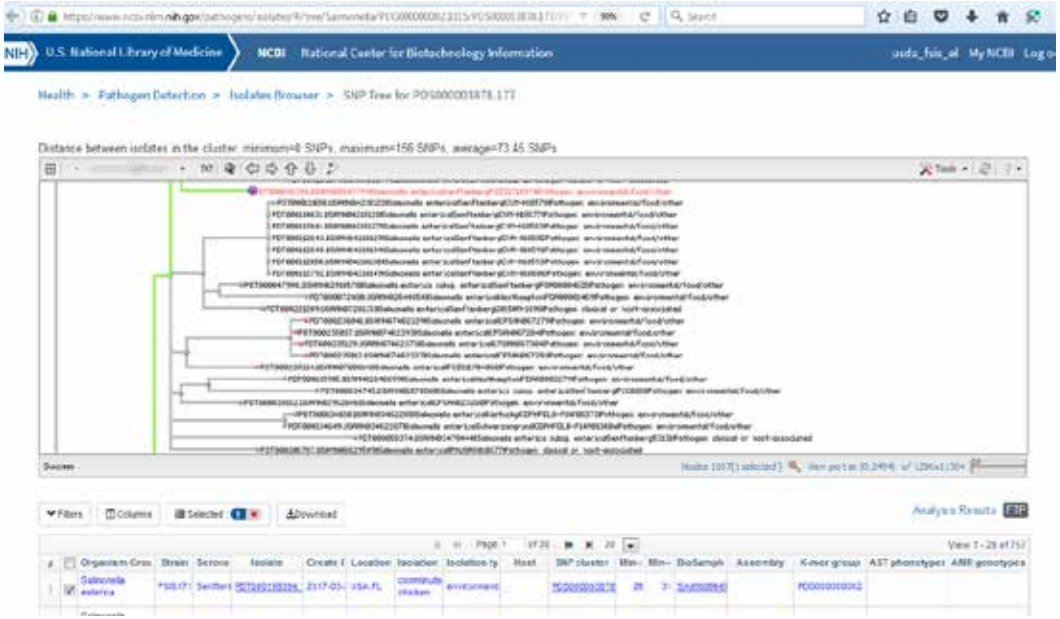
FSIS Update - WGS: FY17 *Salmonella* Isolates from Chicken Commodities and NCBI Pathogen Detection Pipeline

Product	Serotype	Total #	% in SNP cluster (#)	% w/in 20 SNPs of clinical	% w/in 10 SNPs of clinical
Total Serotypes	Enteritidis	447	99.8% (446)	98.9% (442)	90.6% (405)
	Kentucky	607	99.8% (606)	0% (0)	0% (0)
	Heidelberg	74	98.6% (73)	98.6% (73)	86.4% (64)
	Schwarzengrund	122	100% (122)	47.5% (58)	7.3% (9)
	Infantis	218	94.5% (206)	92.2% (201)	50.9% (111)
	Typhimurium	155	96.8% (150)	30.3% (47)	10.3% (16)

- Using NCBIs pathogen detection pipeline food commodity isolates can be placed in a clinical context: So what does this really mean and how can this information be useful?



- Of the 1734 Salmonella isolates analyzed, 773 isolates (44.58%) were pan-susceptible and had no resistance genes
- The most common combination of genes detected (strA, strB, tet(B)) was found in 290 isolates (16.72%)

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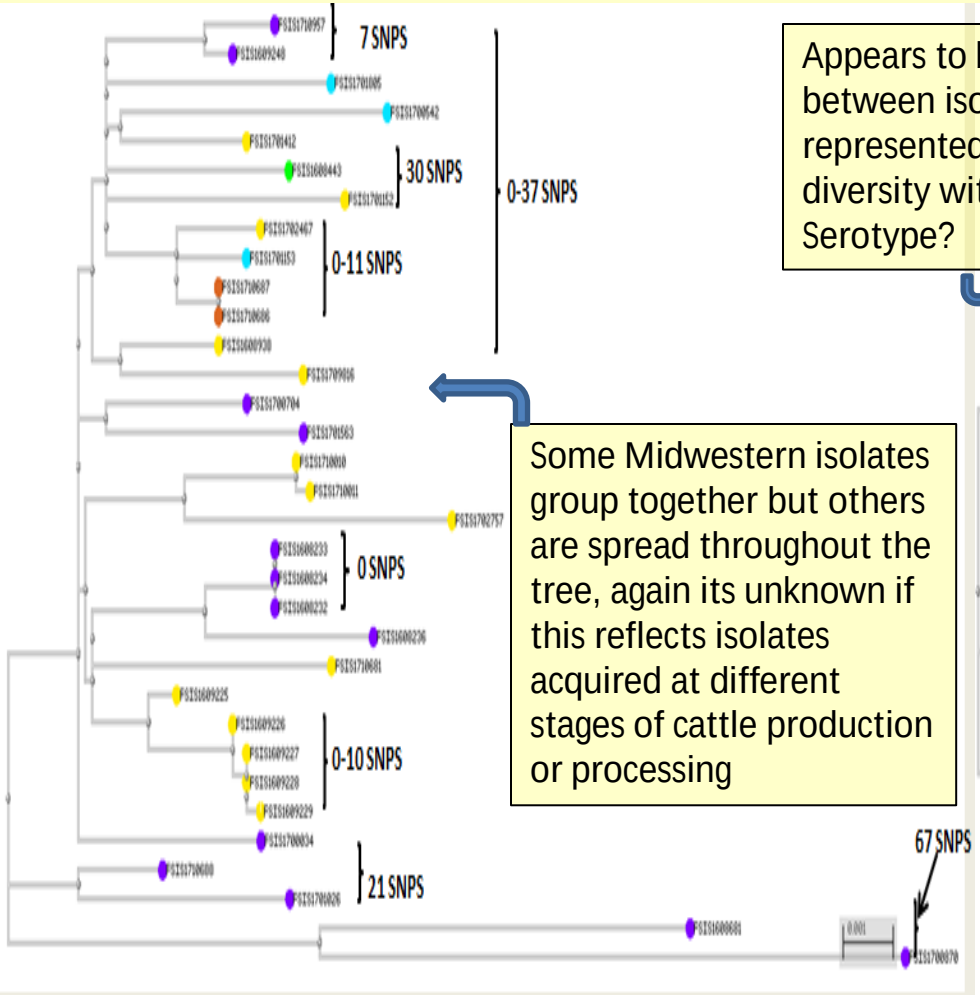
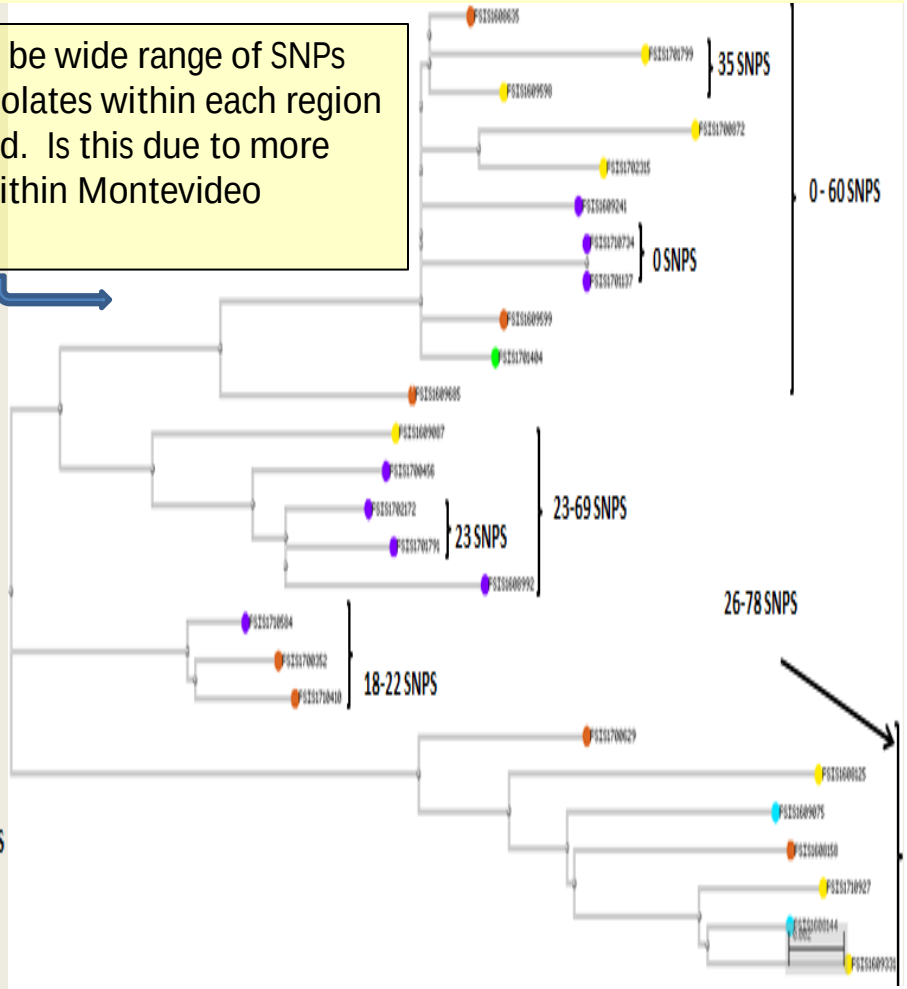
FSIS WGS Update: *Salmonella* Geographic Distribution

Dublin



Montevideo



Appears to be wide range of SNPs between isolates within each region represented. Is this due to more diversity within Montevideo Serotype?

Some Midwestern isolates group together but others are spread throughout the tree, again its unknown if this reflects isolates acquired at different stages of cattle production or processing

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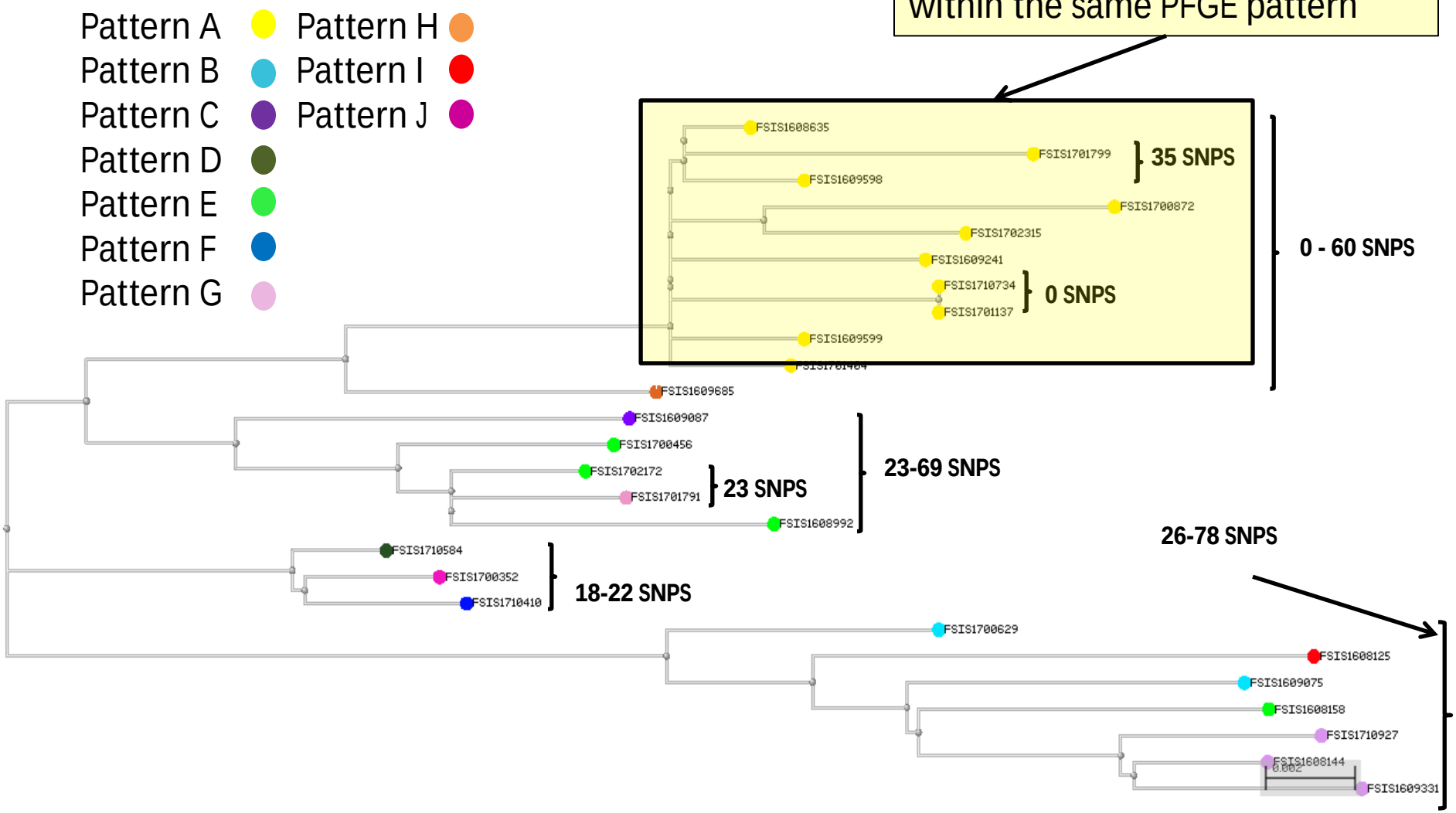
FSIS WGS Update: *Salmonella* Montevideo – WGS and PFGE

We see wide range of SNPs within the same PFGE pattern



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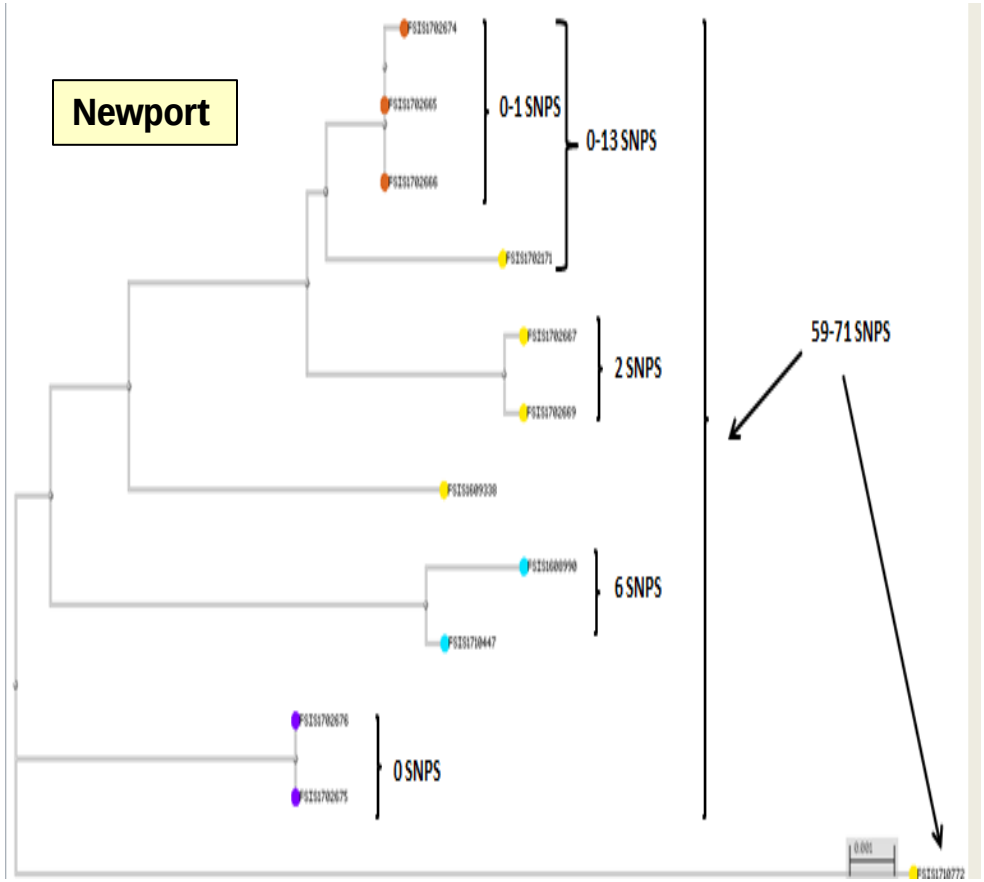
FSIS WGS Update: *Salmonella* Regional Distribution

Some grouping by geographic region, but data skewed by multiple isolates from single establishment and Midwestern region still has large SNP range



- WGS and *Salmonella* Regional Distribution – what can we conclude?
  - Cattle production, movement and region of slaughter impact WGS observations
  - Data skewed based on the number of samples from the establishments
  - Need to determine if range of SNPs differs by serotypes
  - May need to identify key genes as geographic markers as opposed to looking at entire genome

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FSIS WGS Update: WGS - AST Determination and Concordance

FSIS WGS Update: AST Genotype and Phenotype Concordance - NARMS Cecal *Salmonella* CY 2016		
	Number of Isolates	Percent concordance
Overall Concordance	1130	95.0%
Pan-susceptible phenotype	769	99.1%
Beta-lactam resistance phenotype	110	99.1%
Aminoglycoside resistance phenotype	167	98.8%
Phenicol resistance phenotype	42	97.6%
Tetracycline resistance phenotype	330	99.7%

FSIS Update: NARMS - Application of WGS and Detection of Novel Genes

Genotypic screening for antimicrobial resistance using whole genome sequencing (WGS)

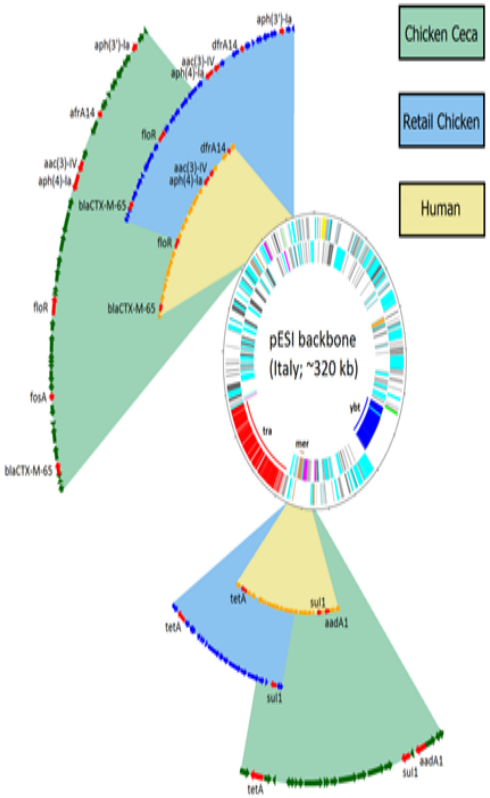
2015
Salmonella and *Campylobacter* from **cecal** with R ≥ 1 antimicrobial

2016
All *Salmonella* and *Campylobacter* from **cecal**

2017
All *Salmonella* and *Campylobacter* from both **cecal** and **HACCP**
AMR *E. coli* and *Enterococcus*

- Ability to rapidly identify new genes of concern
  - Work with NARMS and other partners in a real-time to identify the presence, magnitude and impact of undesirable gene(s)
  - Proactively work with stakeholders to start taking the necessary actions
- Examples of WGS application to novel gene detection and actions
  - ESBL $bla_{CTX\text{-}M\text{-}65}$
  - Colistin Resistance
  - Quinolone Resistance
  - Linezolid Resistance
  - Daptomycin Resistance

- The $bla_{CTX_m_65}$ gene is located in a multiresistance region and confers resistance to 5 other antimicrobial classes:
  - Florfenicol/Chloramphenicol
  - Sulfisoxazole, Trimethoprim/Sulfamethoxazole
  - Tetracycline
  - Fosfomycin
  - Aminoglycosides



An FDA-CDC-FSIS Paper: Antimicrob Agents Chemother. 2017 Jun 27;61(7). pii: e00488-17. doi: 10.1128/AAC.00488-17. Print 2017 Jul Press
Title: Comparative Analysis of Extended-Spectrum-β-Lactamase CTX-M-65-Producing Salmonella enterica Serovar Infantis Isolates from Humans, Food Animals, and Retail Chickens in the United States

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FSIS WGS Update: *Salmonella* Pathogenicity Islands and Heat Resistance Genes from FSIS Beef Sources

Salmonella Pathogenicity Islands (SPI)

- SPI are chromosomally-encoded gene regions within *Salmonella* and other pathogenic bacteria where virulence genes can be found in defined locations
- In Salmonella there are 5 SPIs
  - SPI-1: Type III Secretion Systems (T3SS), iron transport, membrane fusion, host cell reorganization, host invasion (*invA*, *orgA*, *sptP*, *sopE*, *sipA*)
  - SPI-2: Type III Secretion Systems (T3SS),Survival and replication within host cells (*sifA*, *spiC*, *sseABC*)
  - SPI-3: Intracellular adaptation within host cells, including magnesium uptake (*mgtB*, *mgtC*, *misL*)
  - SPI-4: Genes involved in Type I secretion system (T1SS) and binding to host cells
  - SPI-5: Genes involved effector protein secretion for T3SS and T1SS (*sopB*, *pipB*)

Top 4 Pathogenicity Island profiles (No. genes present)	Serotypes
SPI-1 (25), SPI-2 (17), SPI-3 (9), SPI-4 (5), SPI-5 (10)	
	Newport (11)
	Meleagridis (5)
	Meunchen (4)
	Uganda (3)
	Agona (2)
	Anatum (2)
	Infantis (2)
	Lexington (1)
	Litchfield (1)
SPI-1 (25), SPI-2 (17), SPI-3 (11), SPI-4 (5), SPI-5 (10)	
	Dublin (25)
	Enteritidis (1)
	London (1)
SPI-1 (23), SPI-2 (17), SPI-3 (9), SPI-4 (5), SPI-5 (10)	
	Montevideo (14)
	Meunster (5)
SPI-1 (25), SPI-2 (17), SPI-3 (12), SPI-4 (5), SPI-5 (10)	
	Typhimurium (6)
	I 4,[5],12:i:- (5)
Note: Total gene/orf targets tested: SPI-1 (42), SPI-2 (44), SPI-3 (12), SPI-4 (10), SPI-5 (10)	

The top 4 most prevalent pathogenicity profiles shared a majority of the same genes with a maximum of 4 genes being different

Heat resistance genes in Salmonella isolates from FSIS collected beef samples

- Mercer et al. (2015) reported high heat resistance in a food isolated E. coli was attributed to a 14-kb genomic island termed locus of heat resistance (LHR)
- LHR is present in a diverse group of enterobacteriaceae, including *Cronobacter sakazakii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *E. coli*, and *Salmonella enterica*
- To detect LHR gene in FSIS isolates, a BLAST database including genes contained in the LHR, was built based on the published sequence of E. coli AW1.7.
- Salmonella isolates from beef products collected in FY17 were sequenced, assembled and queried against the LHR BLAST database.

- **None of the FSIS Salmonella beef isolates in FY17 contained LHR genes.**

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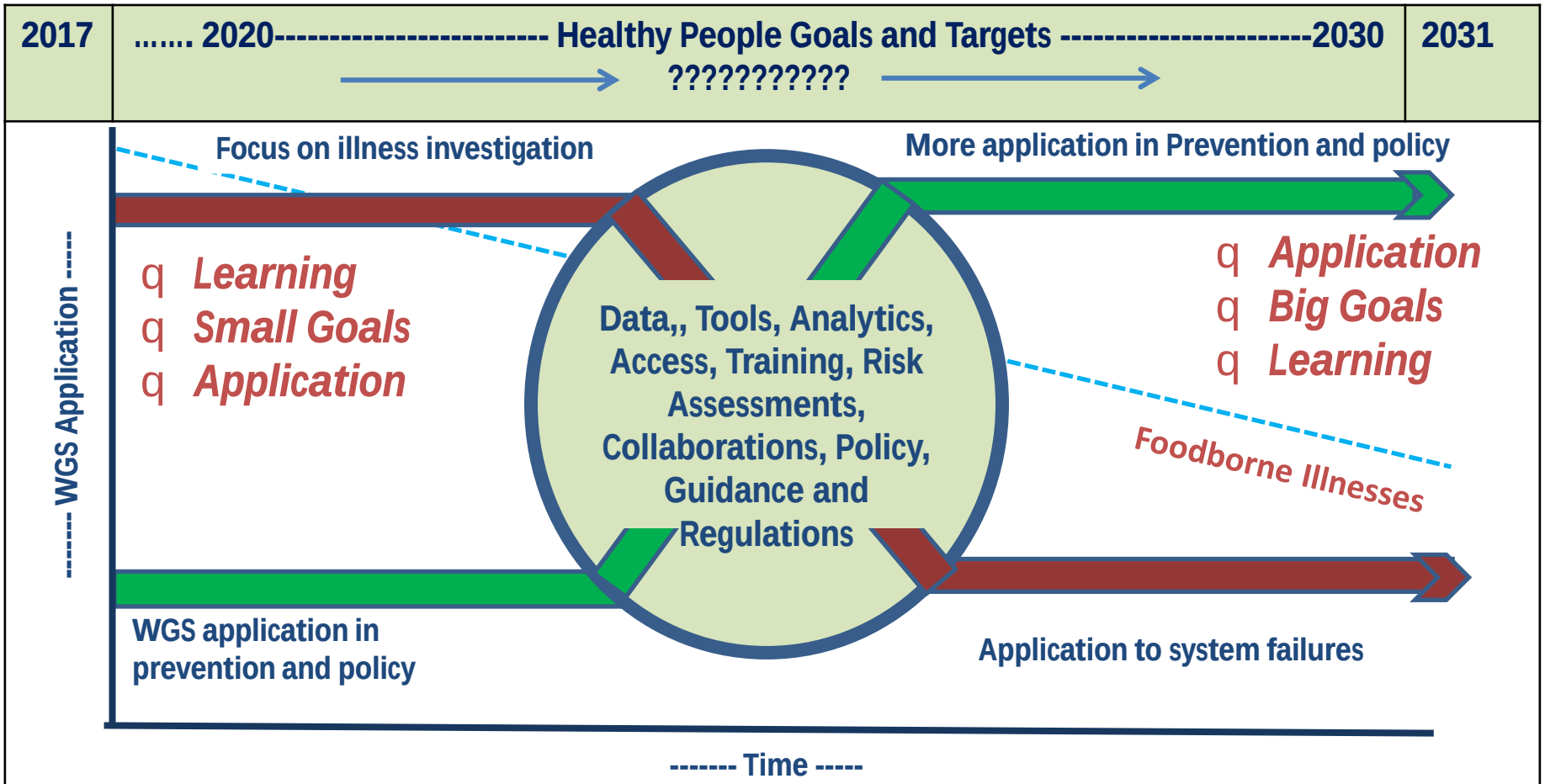
FSIS WGS Update: WGS Application and Expected Impact



Strategic Initiatives and Collaborative Efforts by Gen-FS Partners
Ex: FSIS Strategic Plan 2017-2021, Goal 2: Modernize Inspection Systems , Policies and the Use of Scientific Approaches

FSIS WGS Update: WGS - A Collaborative Undertaking

- FSIS WGS Collaborators

U.S. Department of Health and Human Services

FDA U.S. FOOD & DRUG ADMINISTRATION

Illinois Institute of Technology

IFSH Innovation Through Collaboration INSTITUTE FOR FOOD SAFETY AND HEALTH

CDC Centers for Disease Control and Prevention

CDC 24/7: Saving Lives, Protecting People™

NCBI National Center for Biotechnology Information

USDA United States Department of Agriculture Food Safety and Inspection Service

FSIS is actively engaged in partnerships/collaborations

- ❑ Gen-FS: An Interagency Collaboration on Genomics and Food Safety
- ❑ IFSH: A FDA-Industry-IIT collaboration
- ❑ GMI: Global Microbial Identifier – Mission is to establish a global and connected genomic database/system
- ❑ WHO – WHO/PAHO guidance on WGS for developing countries
- ❑ IRAC – Use of WGS in QMRA

USDA ARS and APHIS

World Health Organization

Global Microbial Identifier

GMI Global Microbial Identifier

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FSIS WGS Update: Summary and Future Directions

- FSIS' new strategic plan is focused on the use of new technology to preventing foodborne illnesses and protect public health protection
  - Based on Healthy People 2020 (soon 2030) our pathogen reduction goals and our challenges are well defined
- With the acquisition of 12 Sequencers, FSIS has build sufficient capacity for conducting WGS on all FSIS isolates and in FY 2017 FSIS sequenced over 7200 isolates with the future targets of ~ 10,000 isolates/year
- In FY 2017 FSIS initiated WGS data analysis beyond its use in outbreak investigations and in FY 2018 FSIS will focus on further understanding the occurrence, trend and patterns of genotypes of public health concern in FSIS regulated products
- In FY 2018 FSIS and partners will seek stakeholder input to understand the scope, applicability and perception, associated with the application of WGS in a regulatory setting
- FSIS continues to engage with National and International partners to stay at the cutting-edge of utilizing WGS technology
  - Work with National Antimicrobial Resistance Monitoring System (NARMS) partners (FDA, CDC) to understand the occurrence or introduction of antimicrobial resistance genes in pathogens and indicators
  - The WGS interagency collaboration Gen-FS will have an operational charter in early FY 2018
- <u>Note: In our investigative decision making we utilize WGS findings/interpretations - as a part of the totality of available evidence</u>