USDA

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Food Safety and Inspection Service

Protecting Public Health and Preventing Foodborne Illness

Food Safety and Inspection Service

Analysis of *Campylobacter* Genomes from Routine Surveillance of Poultry Slaughter and Processing Operations

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Presentation Overview

- Product types tested for *Campylobacter*
- Sequencing of *Campylobacter* in FSIS
- Diversity of *Campylobacter* sequenced
- Sequence type by geography
- How FSIS isolates compare to clinical isolates on NCBI
  - Generalist vs specialist
- Antimicrobial resistance genes in sequenced *Campylobacter*
- Virulence genes in sequenced *Campylobacter*
- Concluding Remarks

Poultry samples collected by FSIS and tests for *Campylobacter*

- Whole carcass chicken rinses
- Chicken parts (legs, breast, wings, halves, quarters)
- Other chicken parts (liver, heart gizzards)
- Ground or comminuted chicken
- Ground or comminuted turkey
- Mechanically separated chicken
- Mechanically separated turkey
- Turkey carcass sponges

In fiscal year 2017 (Oct 1, 2016 –Sept 30, 2017) 22,657 poultry samples were tested for *Campylobacter* ~3.3% positive rate

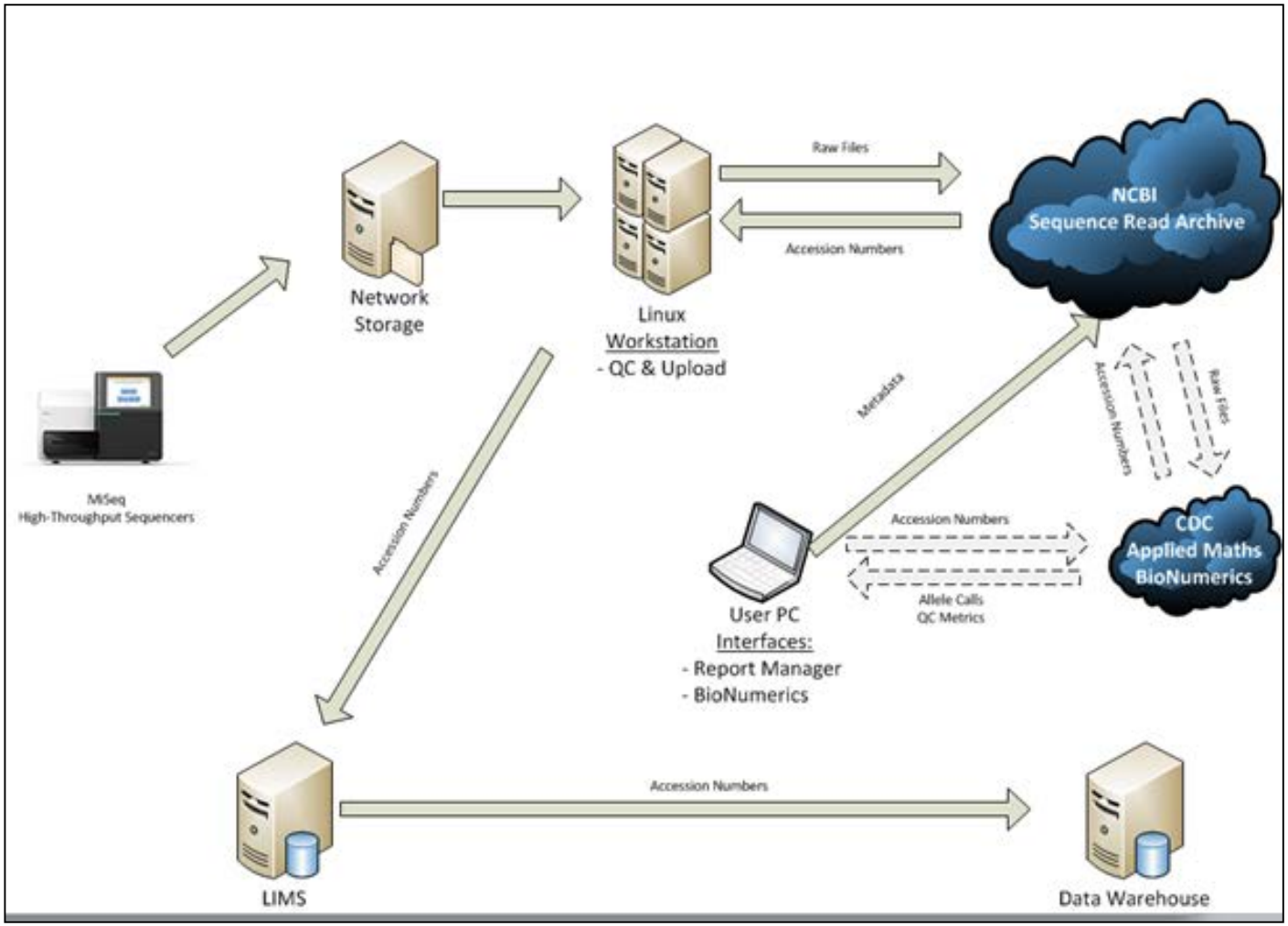
Additional Sample types collected by FSIS as part of the National Antimicrobial Resistance Monitoring System (NARMS)

- FSIS collects cecal samples as part of NARMS, an interagency surveillance program for tracking antimicrobial resistance in foodborne bacteria
- Partnering agencies include: USDA-Food Safety and Inspection Service (FSIS), USDA-Agricultural Research Service (ARS), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC)
- Surveillance data comes from three sources; cecal samples at slaughter, in addition to the previously described product samples (FSIS), Retail meat (FDA) and Humans (CDC)
- FSIS collects cecal samples from Beef cattle, Dairy Cows, Steer, Heifers, Chicken, Turkey, Market Swine and Sows
- Target bacteria: *Salmonella,* ***Campylobacter***, *E. coli*, and *Enterococcus* spp.

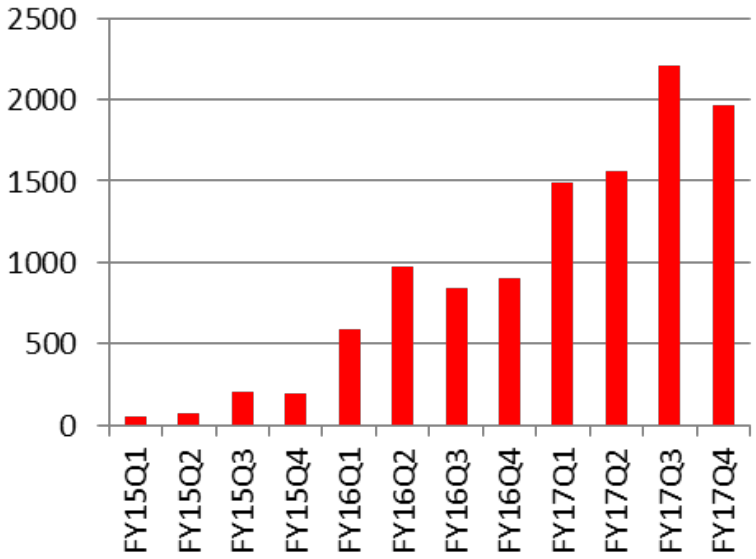
How is FSIS Whole Genome Sequencing (WGS) data compared to other WGS Data?

- All FSIS isolate sequences are uploaded to NCBI
- SNP differences between FSIS isolates and other uploaded sequences are calculated via NCBI's Pathogen Detection Pipeline
- In the future, when Bionumerics 7.6 is live for *Campylobacter* FSIS will submit short read sequences via SRA to CDC's calculation engine, where their whole genome MLST will be analyzed.
- Results will also be entered into the CDC PulseNet National Database where they can be compared with those of other PulseNet members

WGS Data Flow

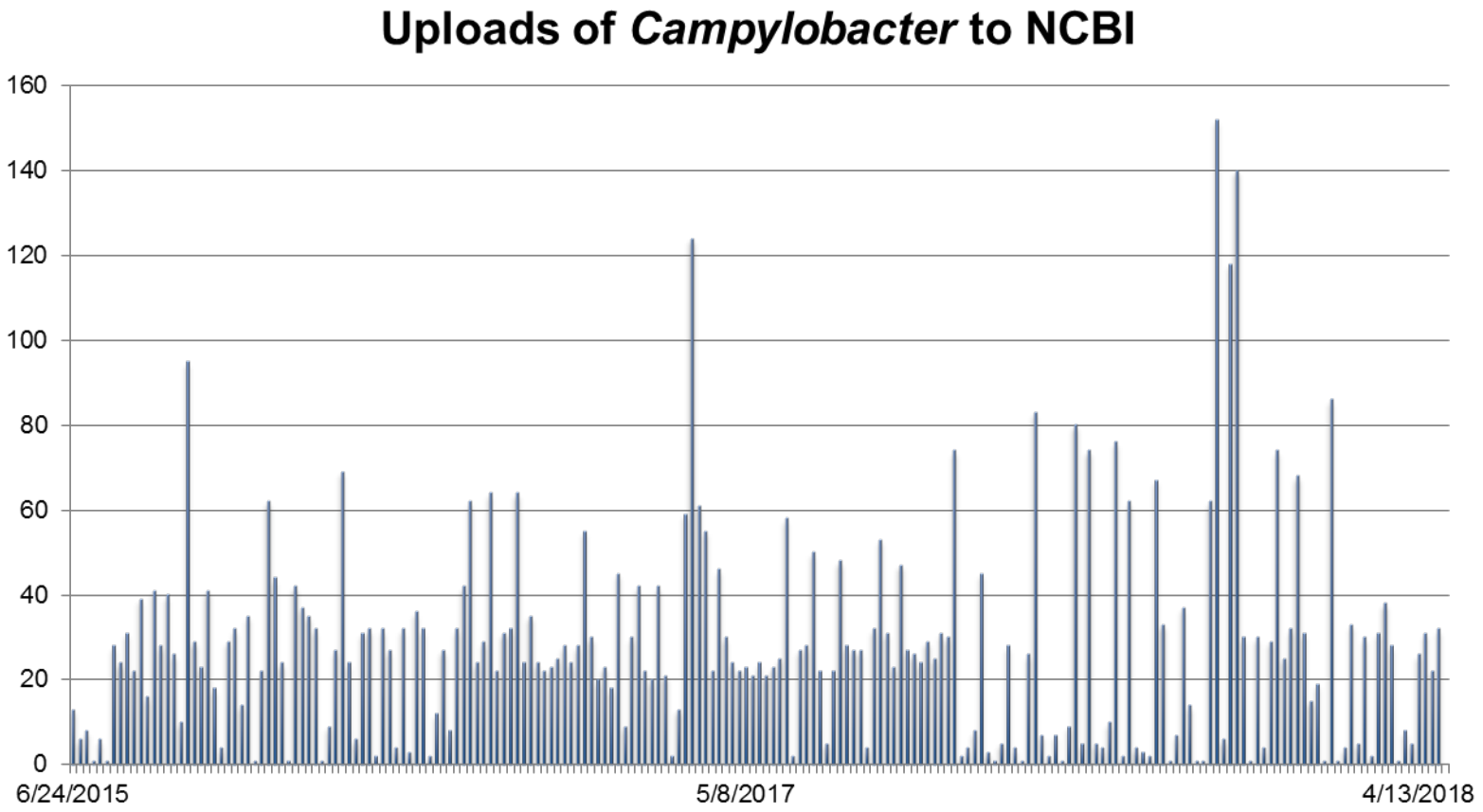


Isolates Sequenced



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*



NCBI accessed 04-26-18

Data set used in this presentation

- **This presentation will focus on 867 isolates from FY2017**

Sample Type	*C. jejuni*	*C. coli*	*C. lari*
Chicken carcass rinse (N = 286)	67.8%	32.2%	
Chicken Parts (N = 267)	64.1%	34.8%	
Ground Chicken (N = 98)	56.1%	42.9%	1.0%
Ground Turkey (N =23)	4.3%	95.7%	
Mechanically Separated Chicken (N = 55)	72.7%	27.3%	
Mechanically Separated Turkey (N = 23)	34.8%	65.2%	
Turkey Carcass Sponge (N = 2)	50%	50%	
Chicken organ samples (N = 113)	78.8%	20.4%	0.8%

WGS Subtyping using 7 gene Multi-Locus Sequence Typing(MLST)

- BLAST database was constructed using allele sequences from pubmlst.org (Jolley and Maiden 2010).
- Typing scheme was first described by Dingle et al (2005).
- Each unique allele combination is assigned a sequence type (ST)
- Sequence types sharing 4 or more MLST alleles with a predefined genotype are members of the same clonal complex(CC)
- Genomes were assembled using CLC genome workbench and used as a query against the above mentioned database

7 gene MLST typing results

- **133 Sequence types were identified**
- **These sequence types belonged to 25 clonal complexes**

Top 5 Sequence Types in FSIS *Campylobacter*
ST-353 (N=67)
ST-829 (N=43)
ST-50 (N=41)
ST-464 (N=28)
ST-3262 (N=28)

Top 5 clonal Complexes in FSIS *Campylobacter*
ST-828 complex (N=245)
ST-353 complex (N=178)
ST-21 complex (N=84)
ST-464 complex (N=32)
ST-48 complex (N=25)
ST-45 complex (N=25)

7 gene MLST typing results

- 133 Sequence types were identified
- These sequence types belonged to 25 clonal complexes

This ST and CC were frequently found but not in top 5 for chicken isolates submitted to pubMLST. Possibly due to sample types not represented on pubMLST

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ST-353 (N=67)
ST-829 (N=43)
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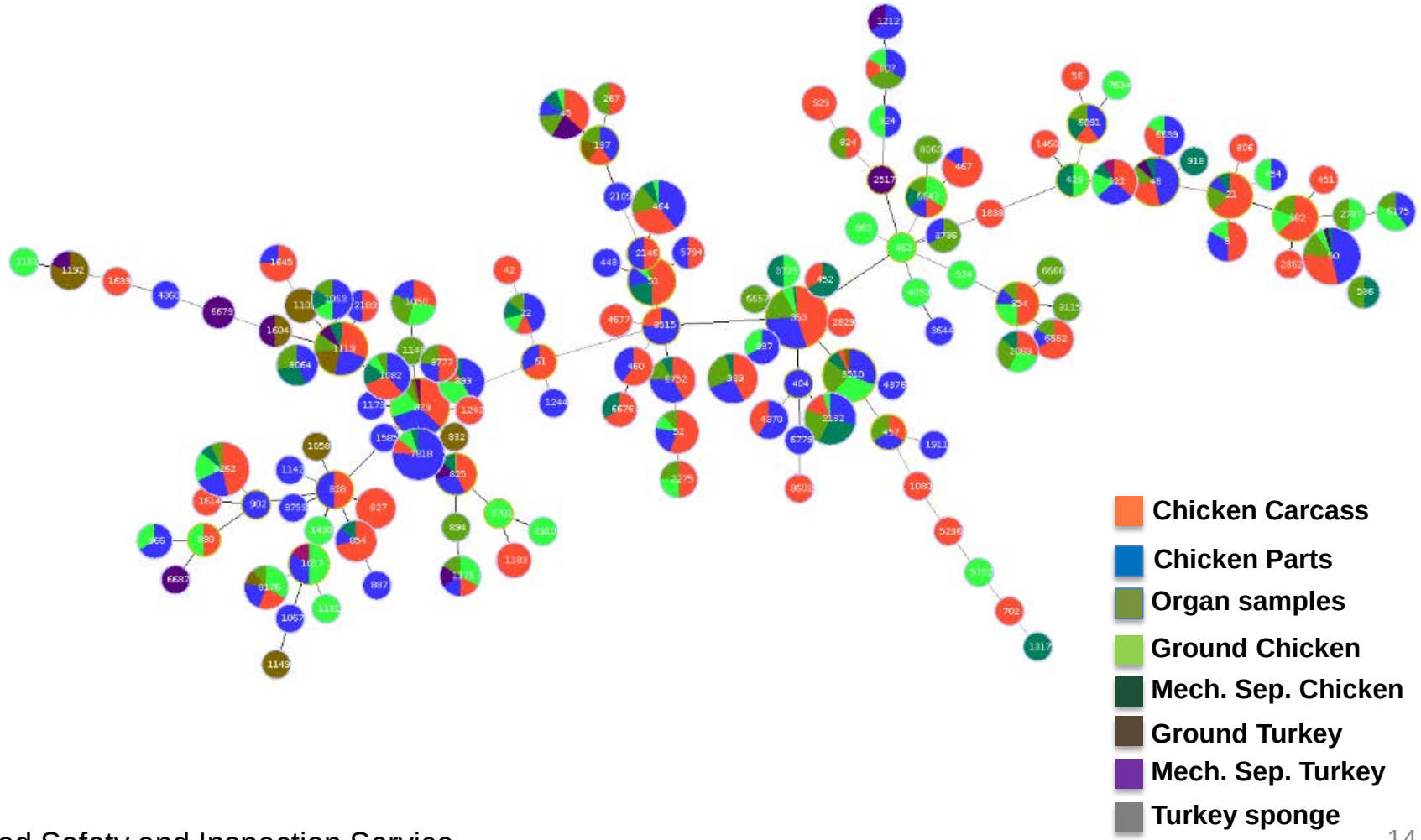
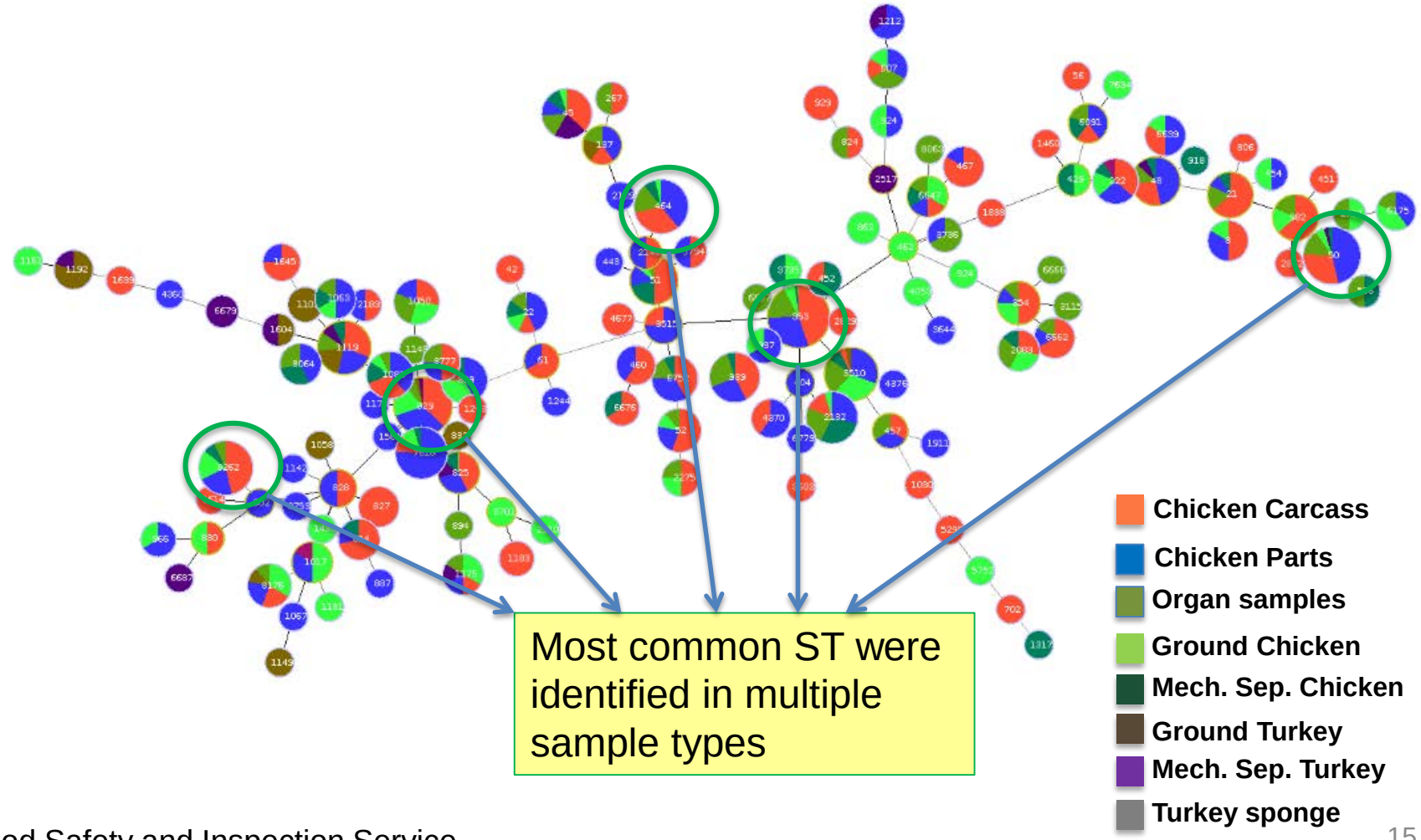
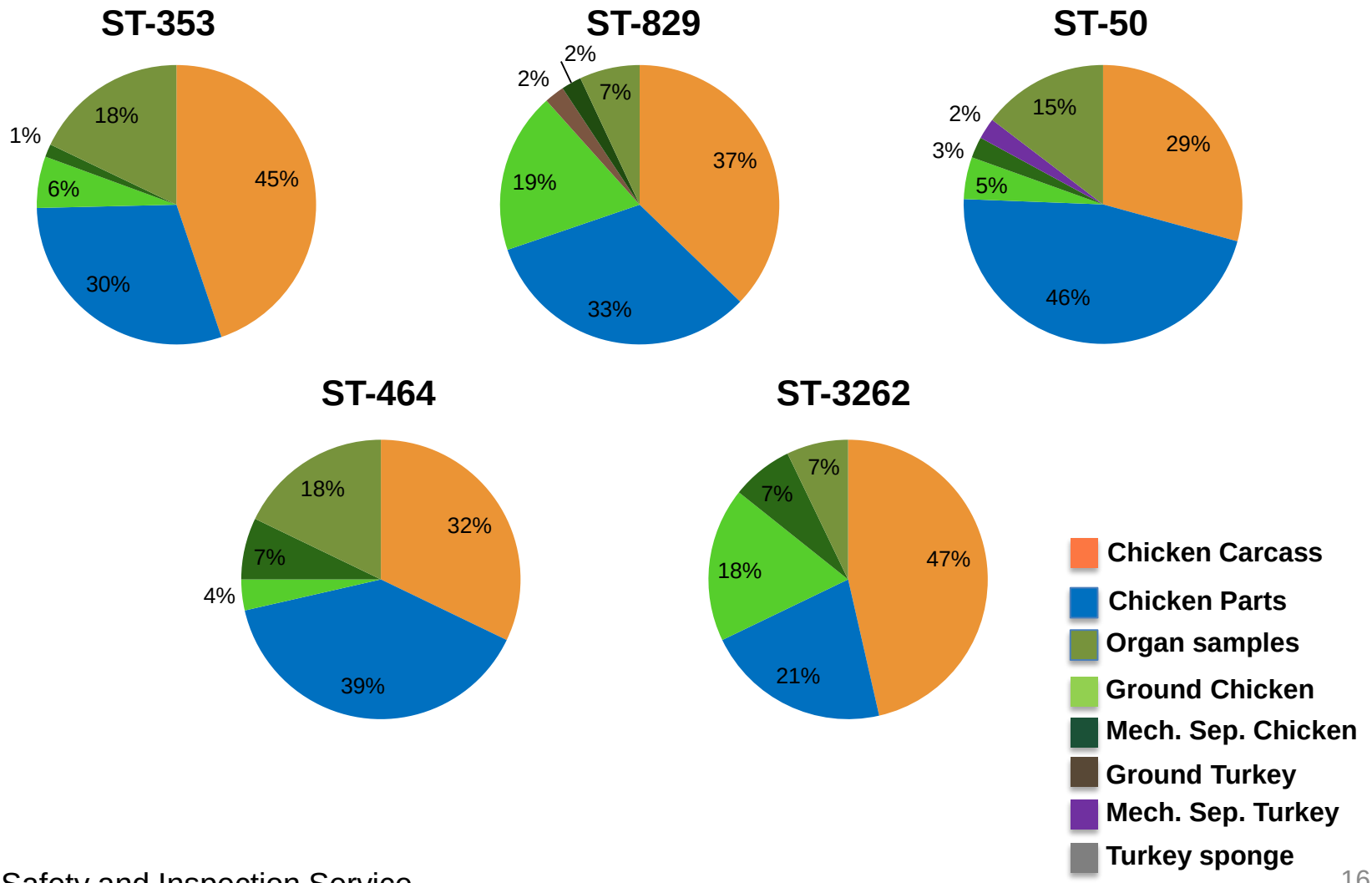
Minimum spanning tree of FY17 *Campylobacter* isolates based on 7 gene MLST



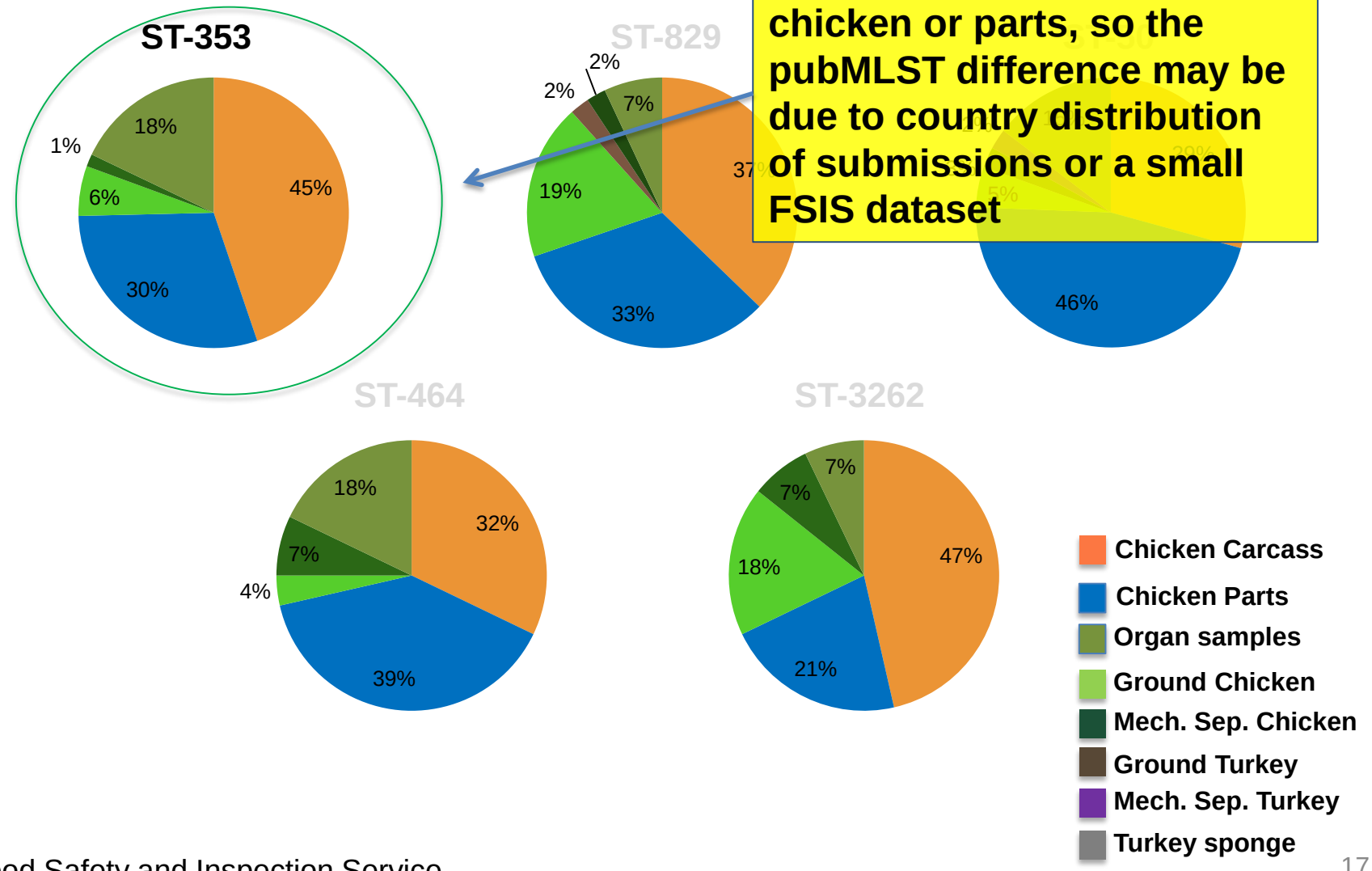
Minimum spanning tree of FY17 *Campylobacter* isolates based on 7 gene MLST



Top 5 Sequence type by sample type

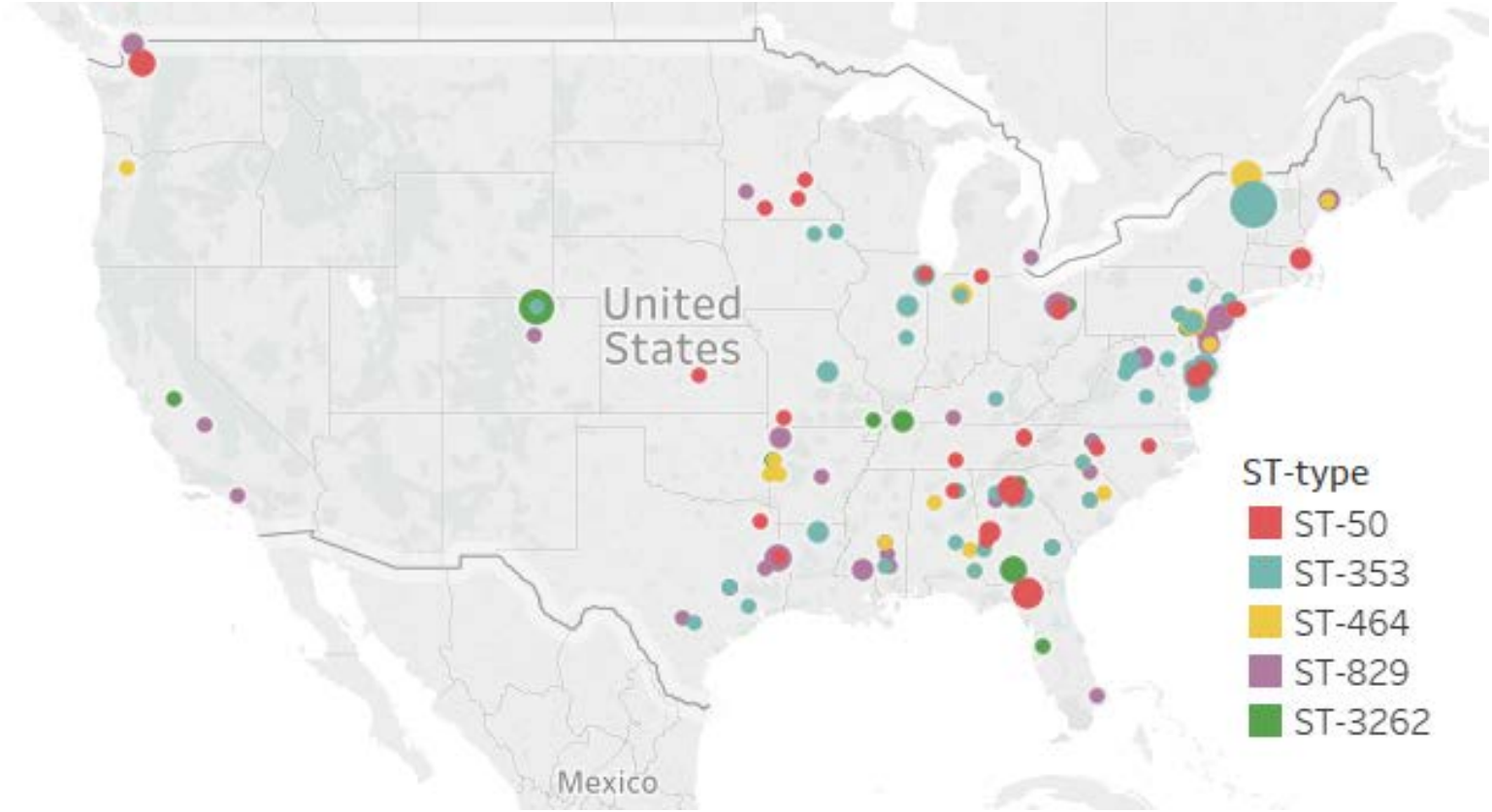


Top 5 Sequence type by sample type



ST-353 is mostly whole chicken or parts, so the pubMLST difference may be due to country distribution of submissions or a small FSIS dataset

Geographic Distribution of Top 5 Sequence Types in FSIS non-cecal poultry Samples



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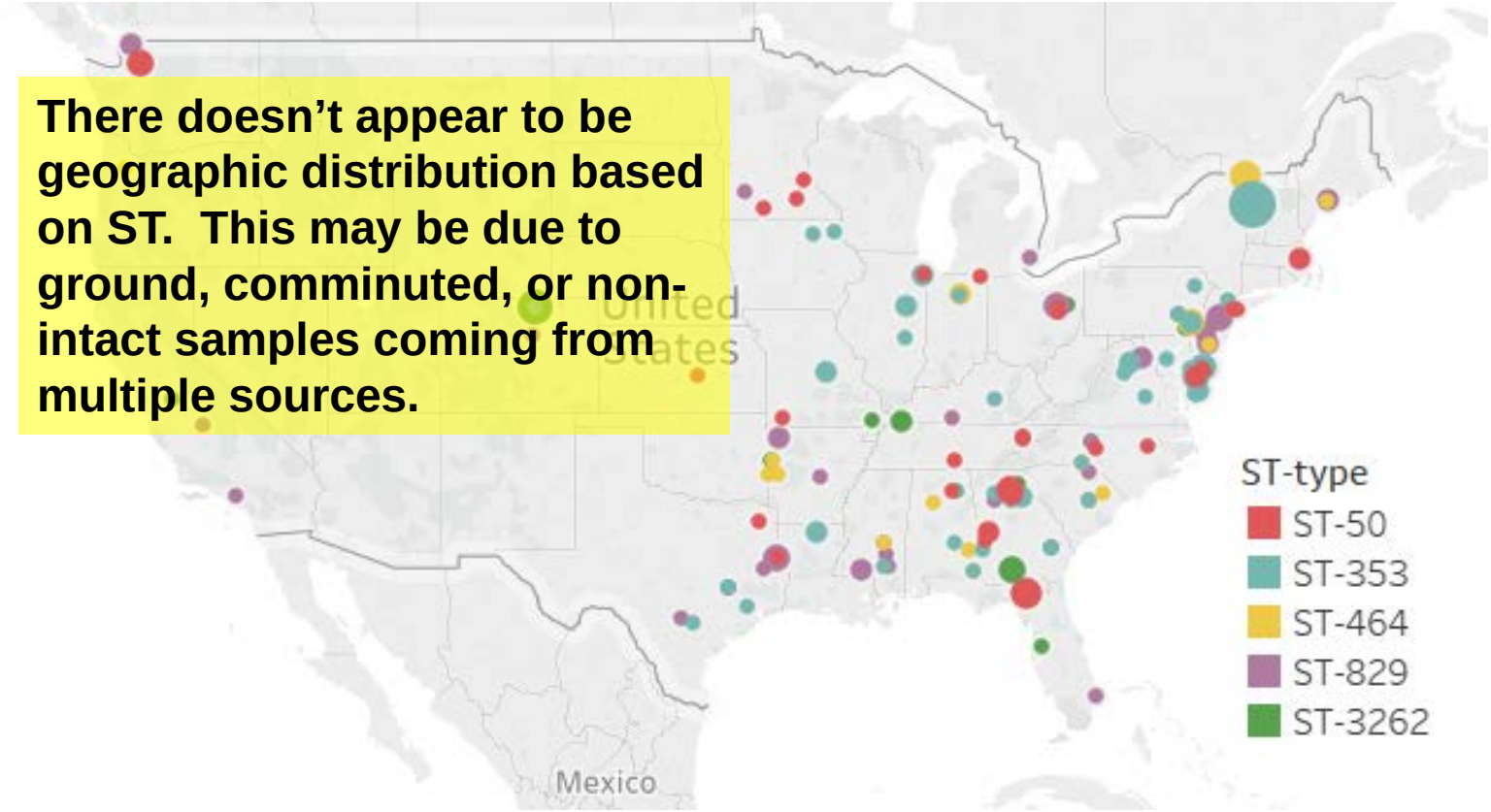
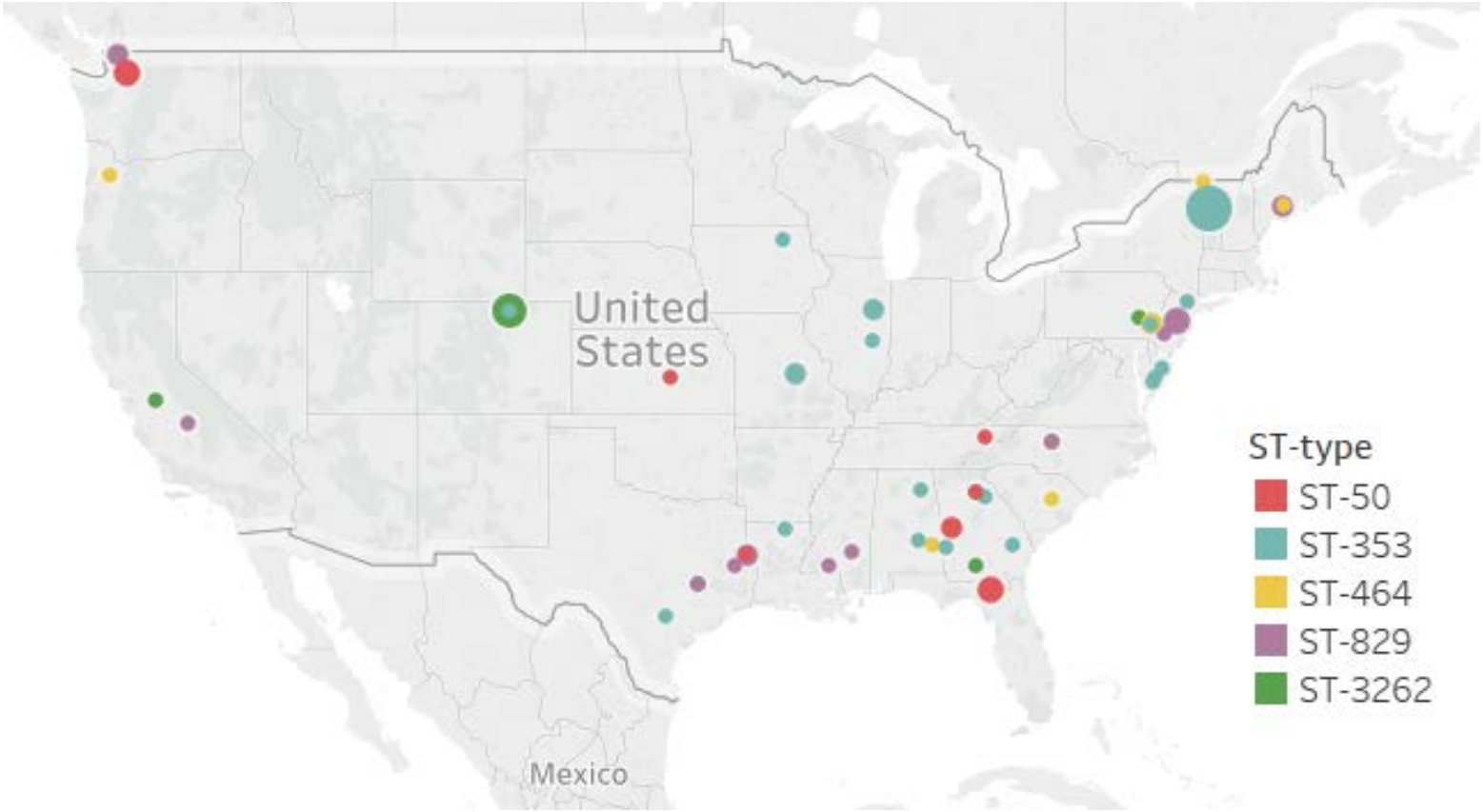
Geographic Distribution of Top 5 Sequence Types in FSIS non-cecal poultry Samples

There doesn't appear to be geographic distribution based on ST. This may be due to ground, comminuted, or non-intact samples coming from multiple sources.



Map image generated in Tableau

Geographic Distribution of Top 5 Sequence Types in FSIS whole carcass rinses



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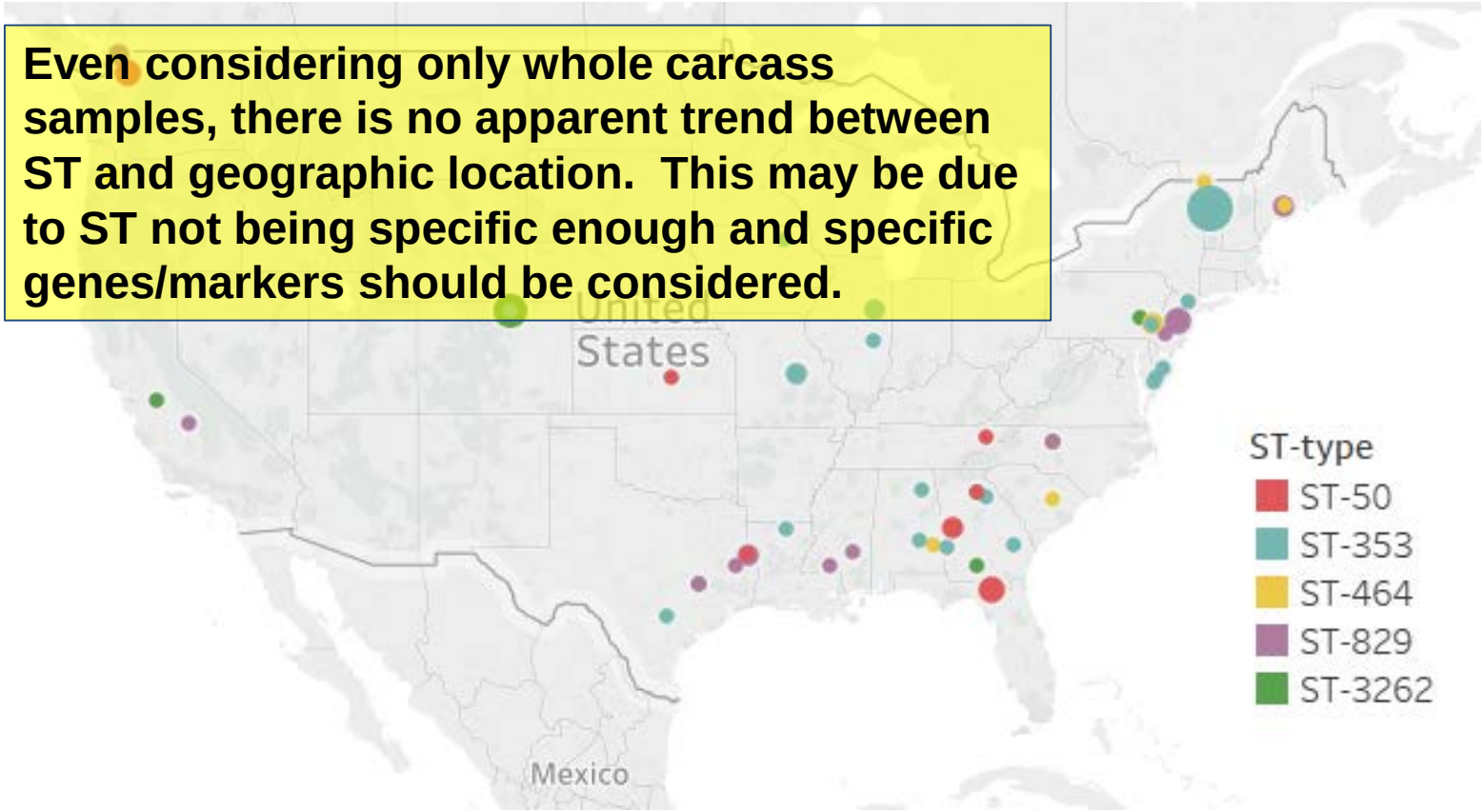
Geographic Distribution of Top 5 Sequence Types in FSIS whole carcass rinses

Even considering only whole carcass samples, there is no apparent trend between ST and geographic location. This may be due to ST not being specific enough and specific genes/markers should be considered.



Map image generated in Tableau

Antimicrobial resistance genotypes of FSIS *Campylobacter*

- BLAST databases were built from the Resfinder database hosted at http://www.genomicepidemiology.org/
- Genomes were assembled using CLC genome workbench and used as a query against the above mentioned database
- Genes were considered present if the BLAST hit was >= 60% coverage and >=80% identity
- Additionally gyrA genes were analyzed for mutations for potential quinolone resistance

Antimicrobial resistance genotypes of FSIS *Campylobacter*

23.99% of isolates had no acquired antibiotic resistance genes detected

Acquired Resistance Genotype	Antimicrobial Class(es)	Percentage of Isolates
blaOXA-61	Beta-lactam	22.49%
blaOXA-61, tet(O)	Beta-lactam, Tetracycline	14.64%
tet(O)	Tetracycline	8.76%
aph(3')-III, blaOXA-61, tet(O)	Aminoglycoside, Beta-lactam, Tetracycline	6.68%
blaOXA-184	Beta-lactam	3.92%

Decreased Susceptibility to Ciprofloxacin (DSC) for FSIS *Campylobacter*

MIC (µg/ml)	Number of isolates	Mutational genotype
1	1	no mutations
2	4	no mutations
4	13	61.5% gyrA T86I (8/13), 30.8% gyrA T86V (4/13)
8	38	94.7% gyrA T86I (36/38)
16	36	97.22% gyrA T86I (35/36)
32	17	100% gyrA T86I (17/17)
64	3	100% gyrA T86I (3/3)

Decreased Susceptibility to Ciprofloxacin (DSC

A lack of mutations to contribute to phenotype, and the same genotype having different phenotypes may be due to other mechanisms, such as efflux pump expression

MIC (µg/ml)	Number of isolates	Mutational genotype
1	1	no mutations
2	4	no mutations
4	13	61.5% gyrA T86I (8/13), 30.8% gyrA T86V (4/13)
8	38	94.7% gyrA T86I (36/38)
16	36	97.22% gyrA T86I (35/36)
32	17	100% gyrA T86I (17/17)
64	3	100% gyrA T86I (3/3)

Comparison of FSIS *Campylobacter* to clinical isolates on NCBI

	C. jejuni (N=559)		*C. coli* (N=303)	
Product Type	**Number of isolates**	**% of isolates <=10 SNPS of Clinical Isolate**	**Number of isolates**	**% of isolates <=10 SNPS of Clinical Isolate**
Carcass rinse	194	23.2 (45/194)	92	8.7 (8/92)
Chicken Parts	171	20.4 (35/171)	93	19.4 (18/93)
Ground chicken	55	30.9 (17/55)	42	9.5 (4/42)
Ground turkey	1	0.0	22	0.0
Mechanically Separated Chicken	40	17.5 (7/40)	15	13.3(2/15)
Mechanically Separated Turkey	8	0.0	15	0.0
Organ Samples	89	19.1 (17/89)	23	0.0
Turkey Carcass Sponge	1	0.0	1	0.0

NCBI accessed 04-26-18

Comparison of FSIS *Campylobacter* to clinical isolates on NCBI

Generalist versus Specialist

- To determine if an isolate belongs to a generalist or specialist sequence type we will use NARMS cecal data
- A generalist ST will have isolates from bovine, porcine, and poultry sources
- A specialist ST will have isolates from only one animal source
- Isolates similarity to human clinical isolates will be based on minimum SNP distance from a clinical determined by NCBI's pathogen detection pipeline

Comparison of FSIS *Campylobacter* to clinical isolates on NCBI

Generalist versus Specialist

Generalist ST	% of Isolates <=20SNPs	% of Isolates <=10SNPs	% of Isolates <= 5SNPs
ST-45	32.72% (18/55)	12.72% (7/55)	7.27% (4/55)
ST-829	28.5% (24/84)	5.95% (5/84)	1.19% (1/84)

Specialist ST	% of Isolates <=20SNPs	% of Isolates <=10SNPs	% of Isolates <= 5SNPs
ST-464 (poultry)	23.33% (7/30)	13.33% (4/30)	3.33% (1/30)
ST-922 (bovine)	50% (24/48)	31.25% (15/48)	16.67% (8/48)
ST-1096 (porcine)	0%	0%	0%

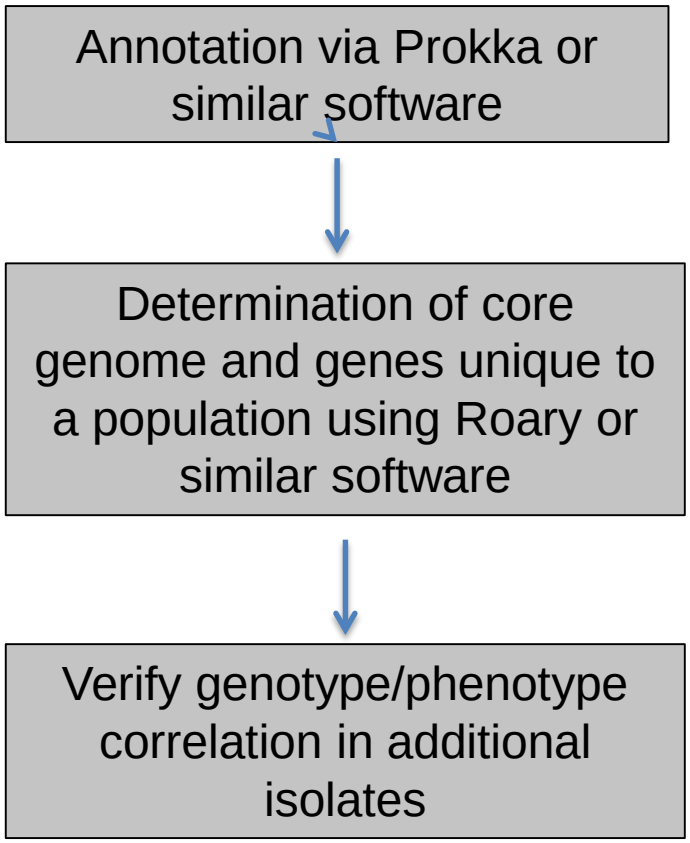
NCBI accessed 04-26-18

Presence of reported* virulence and survival genes

Gene	Function	Percentage of isolates with gene identified
cdtB	Cytolethal distending toxin	99.77% (865/867)[a]
ceuE	Invasion	99.42% (862/867)[b]
ciaB	Invasion	99.42% (862/867)[b]
iamA	Invasion	99.42% (862/867)[b]
racR	Adherence	100% (867/867)
cadF	Adherence	99.42% (862/867)[b]
katA	Oxidative Stress	100% (867/867)
sodB	Oxidative Stress	100% (867/867)

[a]Two *C. coli* ST-6679 from mechanically separated Turkey
[b]Five *C. lari*
** See references*

Moving Forward: Identification of genes associated with a phenotype



Example: Comparison of subtypes found more frequently in meat samples than cecal samples to subtypes found more frequently in cecal samples than meat samples

This can give insights to genes contributing to survival during processing, or the isolation process

This could be used for microbial risk assessments or changes to isolation procedures to be more inclusive

Concluding Remarks

- FSIS is fully committed to utilizing the analytic power and resolution of WGS in its 'pathogen reduction – public health protection' pursuit
- Use of wgMLST will give us more information about discrete genes and possible markers for geography, clinical association etc.
- As NCBI database grows we can feel more confident in hypotheses related to host-specificity/sample type prevalence
- FSIS continues to collaborate with the CDC, FDA and NCBI to further understand the 'scope and applicability' of WGS findings in FSIS's regulatory context
- WGS is only one piece of evidence; phenotypes need verification, differences may be at transcriptional level, and epidemiology is necessary

Acknowledgements

- FSIS Field Services Laboratories
- FDA Center for Veterinary Medicine
- Glenn Tillman Ph.D.
- Jovita Haro
- Jamie Wasilenko Ph.D.
- FSIS – OPHS
- FSIS – OPPD

Questions?

References

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