



Department
of Health

Wadsworth
Center

New Tools for Tracing Contamination Through the Supply Chain

TN Food Safety Task Force seminar 6/2/2016

William Wolfgang, PhD
Wadsworth Center, NYSDOH
william.wolfgang@health.ny.gov

Existing tool: PulseNet

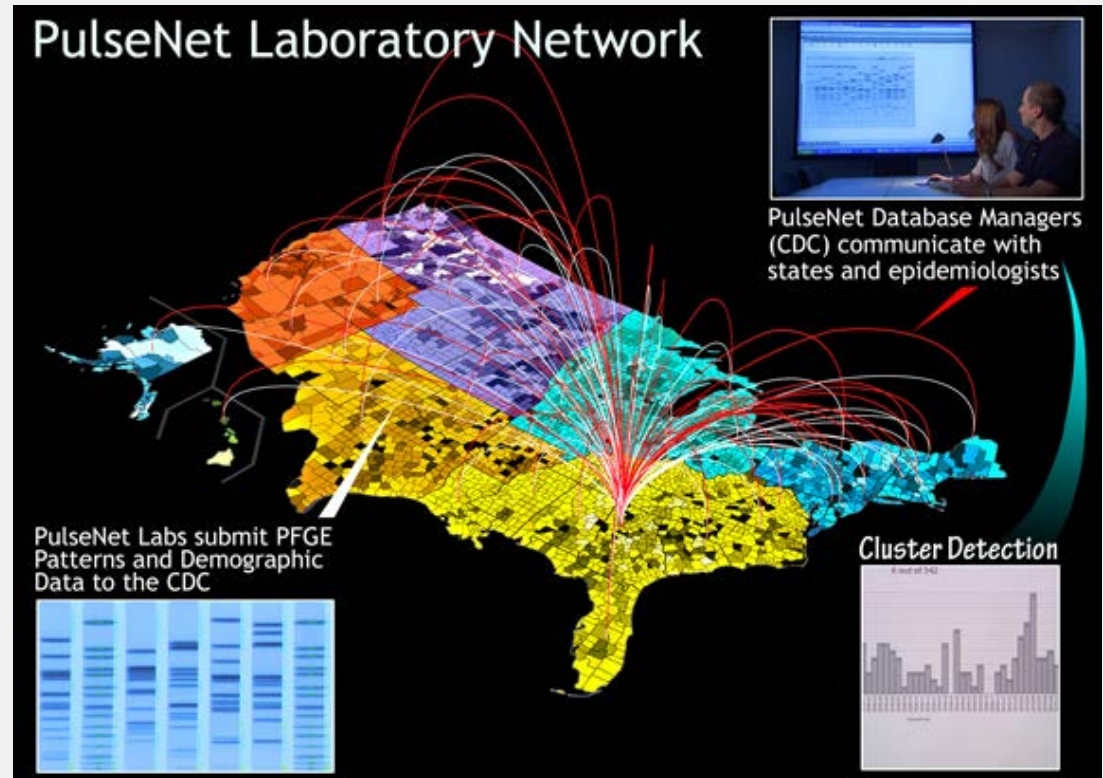
- Illnesses averted annually
 - 266,522 Salmonella,
 - 9,489 Escherichia coli (E. coli), and
 - 56 Listeria monocytogenes
- **“This reduces medical and productivity costs by \$507 million.”**
- And returns **\$70** for every dollar spent.



Scharff et al. 2016 [Am. J. of Preventive Medicine](#)

But challenges exist for PFGE.

- Low resolution
- Instability
- Clusters go undetected
- Clusters may be misleading



We are engaged in 3 Major projects for WGS surveillance of Enteric pathogens

1. FDA GenomeTrakr (fall 2013):- building a genomic database
 - Mostly *Salmonella* from food and environment
2. Wadsworth Center (fall 2013- fall 2015):- real-time *Salmonella* Enteritidis surveillance
 - All *Salmonella* Enteritidis we receive from patients
 - In house bioinformatic analysis
3. CDC-AMD (fall 2014): - real-time surveillance
 - *L. monocytogenes*, *E. coli*, and *Salmonella* from patients.
 - *L. mono.* study just published.

Implementation of Nationwide Real-time Whole-genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation

- Two year collaboration between the CDC, FDA, NCBI, and State PHLs
- All *L. mono.* were sequenced by WGS in real-time.

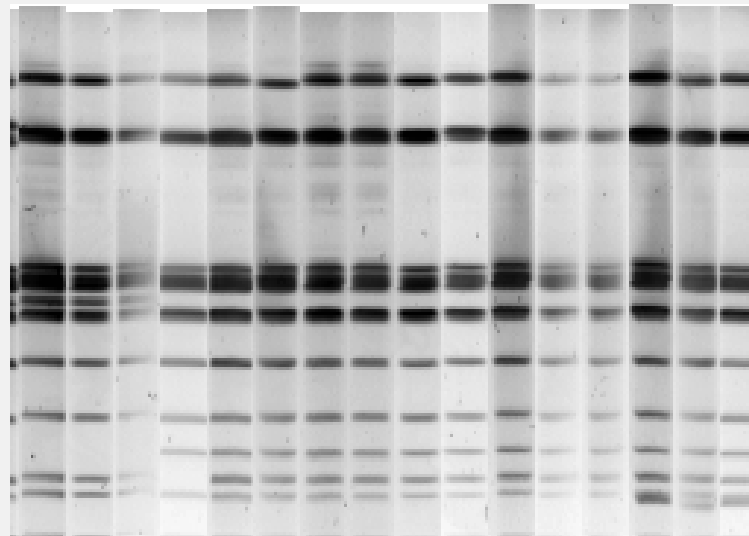
Findings:

- “Increased discriminatory capacity of WGS has helped strengthen links among *L.m.* isolates from food, environment, and patients...”
 - a. Detecting more Listeriosis clusters
 - b. Halt pseudo-cluster investigations
 - c. Focus epidemiologic investigations
 - d. Resulting in solving more outbreaks



At the Wadsworth we receive about 1,600 patient isolates of *Salmonella* in a year

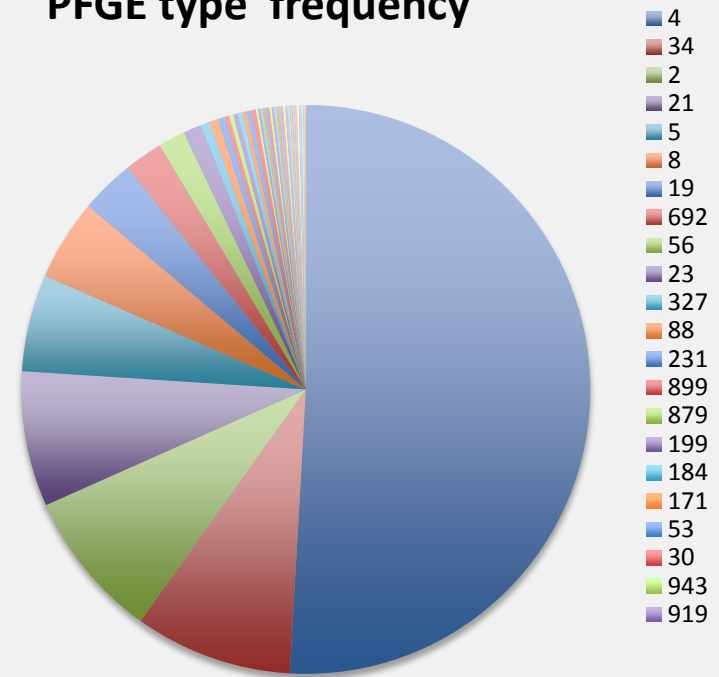
- Each specimen:
 - Serotyped.
 - **DNA is fingerprinted by PFGE.**
- All data is sent to the CDC.
- 300 to 400 are **S. Enteritidis**



Salmonella Enteritidis clusters are poorly resolved by PFGE

- Our most commonly received strain of *Salmonella*
 - a. 1/2 have the same PFGE DNA fingerprint.
 - b. And 2/3 have a very common PFGE DNA fingerprint.
 - c. This DNA fingerprints are so common it is of limited use to our epidemiologists.

PFGE type frequency



52 PFGE types

Outbreaks are rarely resolved for the most common PFGE patterns

2010 Outbreaks

- Montevideo - salami.
- Typhimurium - residential facility for the developmentally disabled.
- Javiana - tomatoes.
- Saintpaul - restaurant.
- **Enteritidis** - long term care facility (4).

2011 Outbreaks

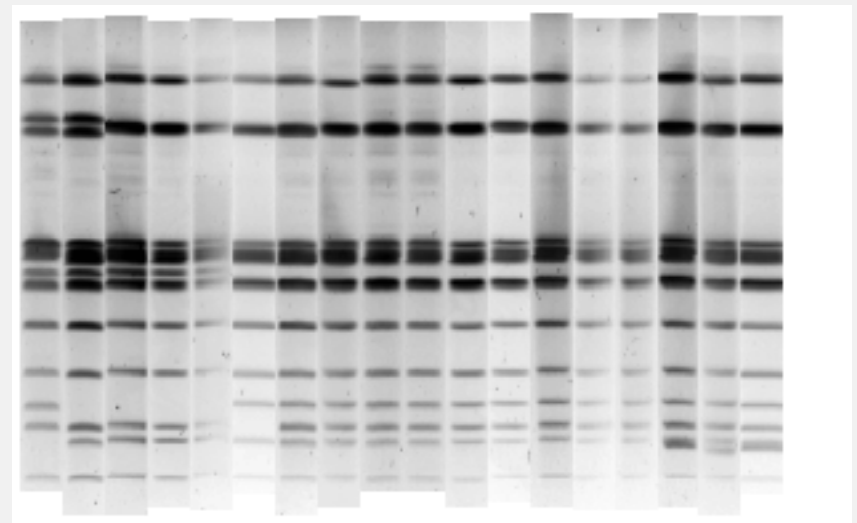
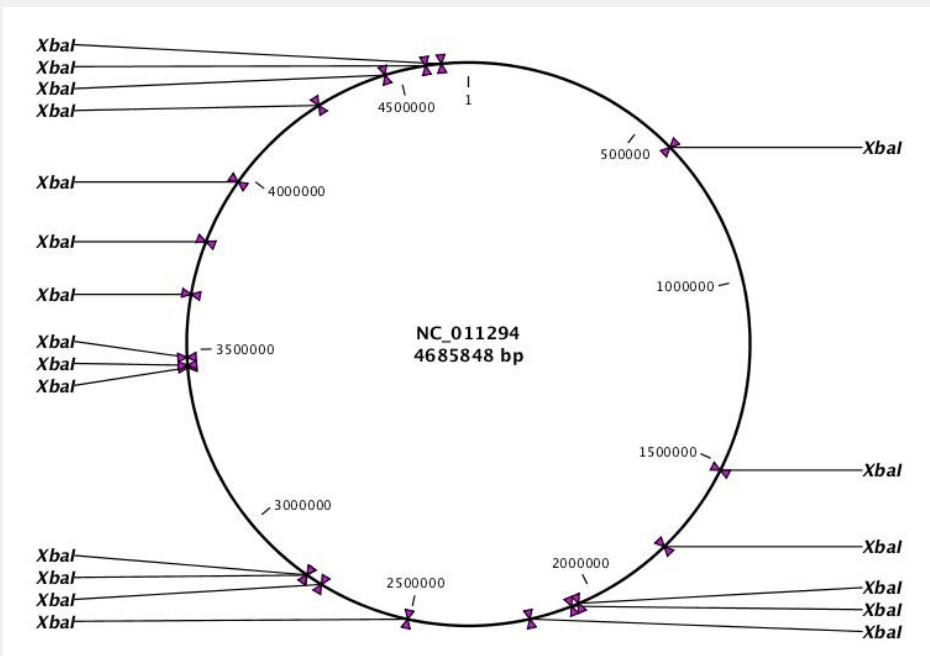
- Typhimurium - aquatic frogs.
- Heidelberg - ground turkey.
- **Enteritidis** - food worker at a deli (692).
- Typhimurium - ground beef.
- **Enteritidis** - Turkish pine nuts (8).

2012 Outbreaks

- Bareilly - sushi.
- Nchanga – sushi.
- Hartford - sub shop worker.
- Newport - chick and duck exposure.
- SanDiego & Poona - small turtles.
- Javiana - Mothers Day fruit baskets.
- **Enteritidis** - ground beef (9).

WGS offers a better way to fingerprint *Salmonella* Enteritidis

- PFGE detects changes at only about 12 sites on the genome.
- Yet there are 4.5 million bases in a *Salmonella* genome.
- Now it is (becoming?) routine to look for changes at all these sites.



With the arrival of bench top sequencers WGS has become a tool in Clinical Microbiology

- Relatively inexpensive machines can now rapidly sequence the whole genome of a bacterium.



Bioinformatic software tools can analyze large amounts of sequence data

Computer programs can compare these whole genomes to detect Single Nucleotide Polymorphisms (SNP).

Shows phylogenetic relationships

GTCATAGCATTATTATTATTATTTCAGGACTA

CAGTATCGTAATAATAATAATAAAGTCC**T**GAT

1 bp

15



30

GTCATAGCATTATTATTATTATTTCAGG**C**CTA

CAGTATCGTAATAATAATAATAAAGTCC**G**GAT



WGS based cluster analysis revealed a cluster where PFGE failed

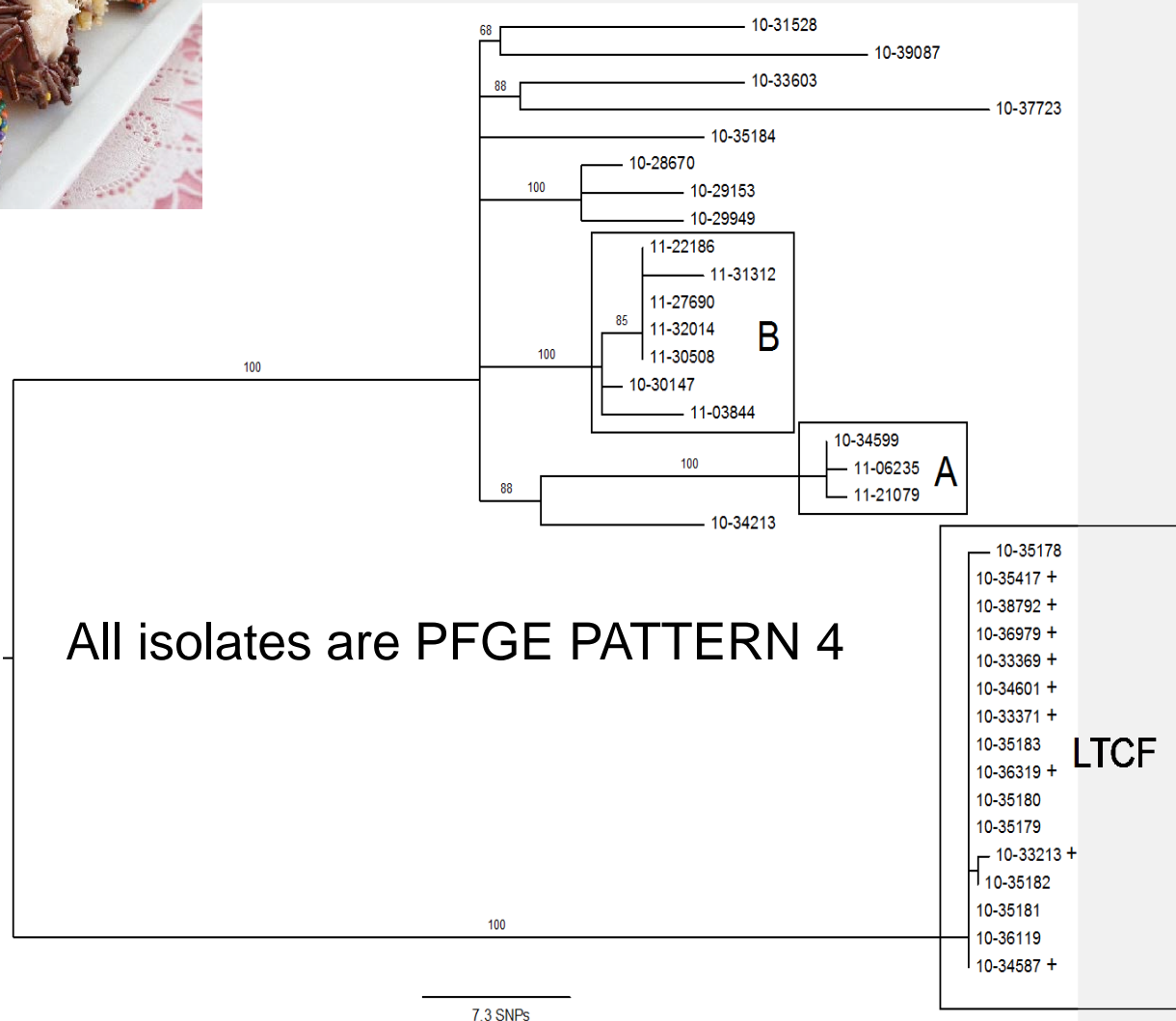
- Sept. 2010 Connecticut Dept. of Health identifies a *Salmonella* outbreak in a long term care facility (LTCF).
- Outbreak was linked to cannoli from a Westchester bakery.
- Both NY and CT cases consumed cannoli's.
- Isolates had the most common PFGE pattern.



Cannoli cohort

Key	County	Date	PFGE
IDR1000029153	Cattaraugus	8/10/10	JEGX01.0004
IDR1000031528	Rockland	8/26/10	JEGX01.0004
IDR1000033213	Putnam	9/10/10	JEGX01.0004
IDR1000033369	Putnam	9/10/10	JEGX01.0004
IDR1000033371	Putnam	9/11/10	JEGX01.0004
IDR1000034601	Washington	9/13/10	JEGX01.0004
IDR1000034587	Westchester	9/20/10	JEGX01.0004
IDR1000035417	Putnam	9/22/10	JEGX01.0004
IDR1000035178	Westchester	9/13/10	JEGX01.0004
IDR1000035179	Greenwich CT	9/12/10	JEGX01.0004
IDR1000035180	Westchester	9/12/10	JEGX01.0004
IDR1000035181	Westchester	9/13/10	JEGX01.0004
IDR1000035182	Westchester	9/12/10	JEGX01.0004
IDR1000035183	Greenwich CT	9/16/10	JEGX01.0004
IDR1000036119		9/17/10	JEGX01.0004
IDR1100035184	Westchester	9/16/10	JEGX01.0004
IDR1000036319	Putnam	9/28/10	JEGX01.0004
IDR1000036979	Putnam	10/8/10	JEGX01.0004
IDR1000038792	Nassau	10/29/10	JEGX01.0004
IDR1000034599	Orange	9/15/10	JEGX01.0004
IDR1100006235	Westchester	2/21/11	JEGX01.0004
IDR1100021079	Rockland	7/13/11	JEGX01.0004
IDR1000030147	Out-Of-State	8/22/10	JEGX01.0004
IDR1100003844	Onondaga	2/1/11	JEGX01.0004
IDR1100022186	Yates	7/22/11	JEGX01.0004
IDR1100027690	Erie	9/6/11	JEGX01.0004
IDR1100030508	Madison	10/9/11	JEGX01.0004
IDR1100031312	Suffolk	10/5/11	JEGX01.0004
IDR1100032014	Onondaga	10/22/11	JEGX01.0004
IDR1000028670	Nassau	8/8/10	JEGX01.0004
IDR1000029949	Suffolk	8/16/10	JEGX01.0004
IDR1000033603	Erie	9/14/10	JEGX01.0004
IDR1000034213	Erie	9/13/10	JEGX01.0004
IDR1000037723	Westchester	10/4/10	JEGX01.0004
IDR1000039087	Westchester	10/27/10	JEGX01.0004

WGS can identify an outbreak cluster not detected by PFGE



WGS can identify an outbreak cluster not detected by PFGE



WGS based cluster analysis:

- 1) Improved resolution
 - i. detected clusters within a common PFGE type.
- 2) Identified isolates in the cluster that were missed.
- 3) Isolates associated with a single source were 0 to 4 SNPs apart.
 - This range of SNP diversity has been seen in many studies.

Initiated a 2 year prospective study.

In a 2 year study from Oct. 2013 to Oct. 2015

- **684 isolates** sequenced and analyzed in real time.
- **41 PFGE types**
 - 607 (89%) isolates had endemic PFGE patterns
- **Identified 118 Genomic Clusters (4 SNPs or less)**
 - 287 isolates (42%) reside in clusters
 - **Only 7** genomic clusters contained non-endemic PFGE types.
 - Most clusters contain 4 or fewer isolates; mean 3.5
 - i. Largest cluster contained over 17
 - Can persist for almost 2 years
 - 31 (26%) genomic clusters contained multiple PFGE types.
- Clusters were reported to our epidemiologists.

WGS detects many clusters

PFGE JEGX01.0004
contains 62 genomic
clusters

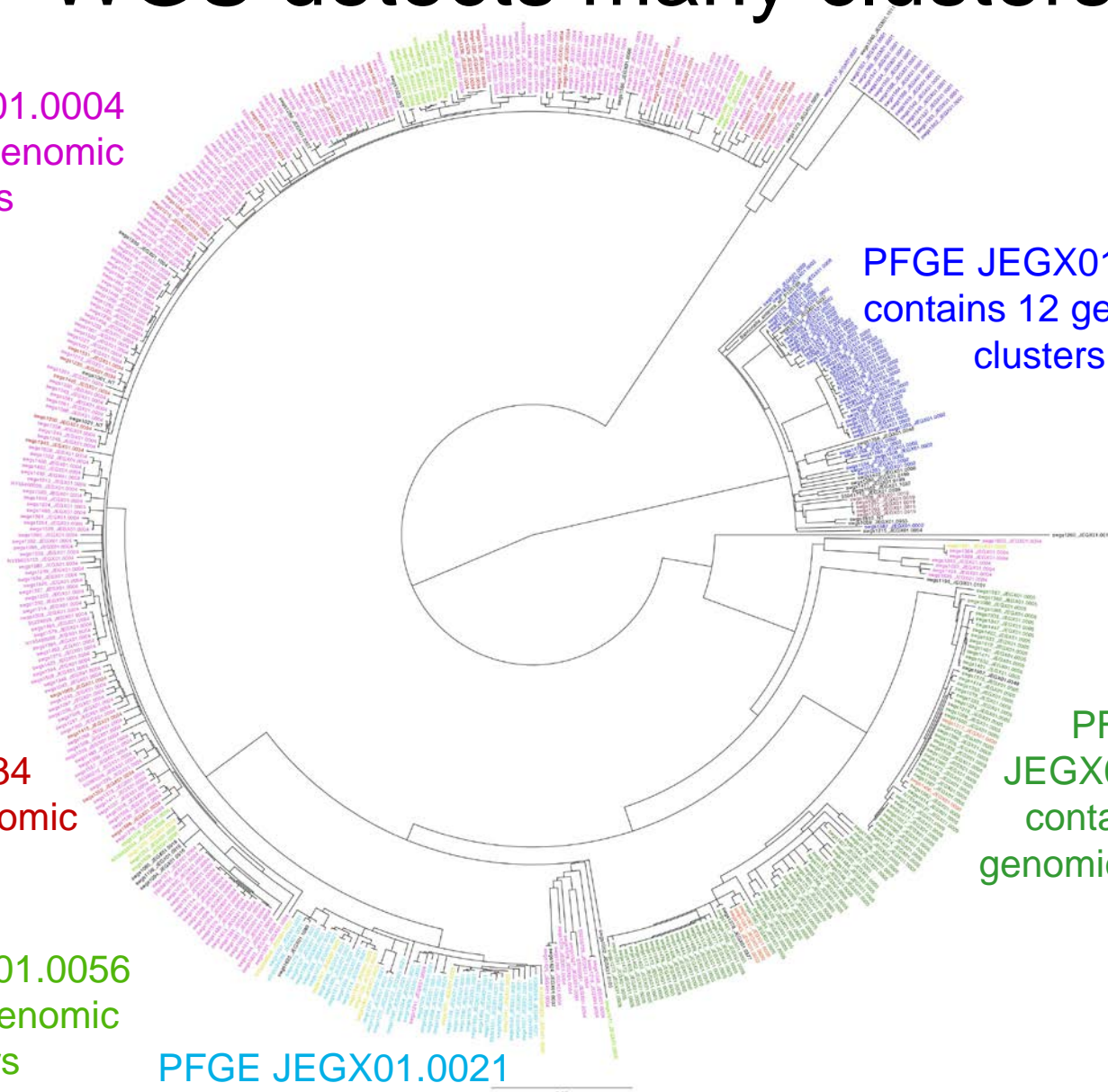
PFGE JEGX01.0002
contains 12 genomic
clusters

PFGE
JEGX01.0034
contains 5 genomic
clusters

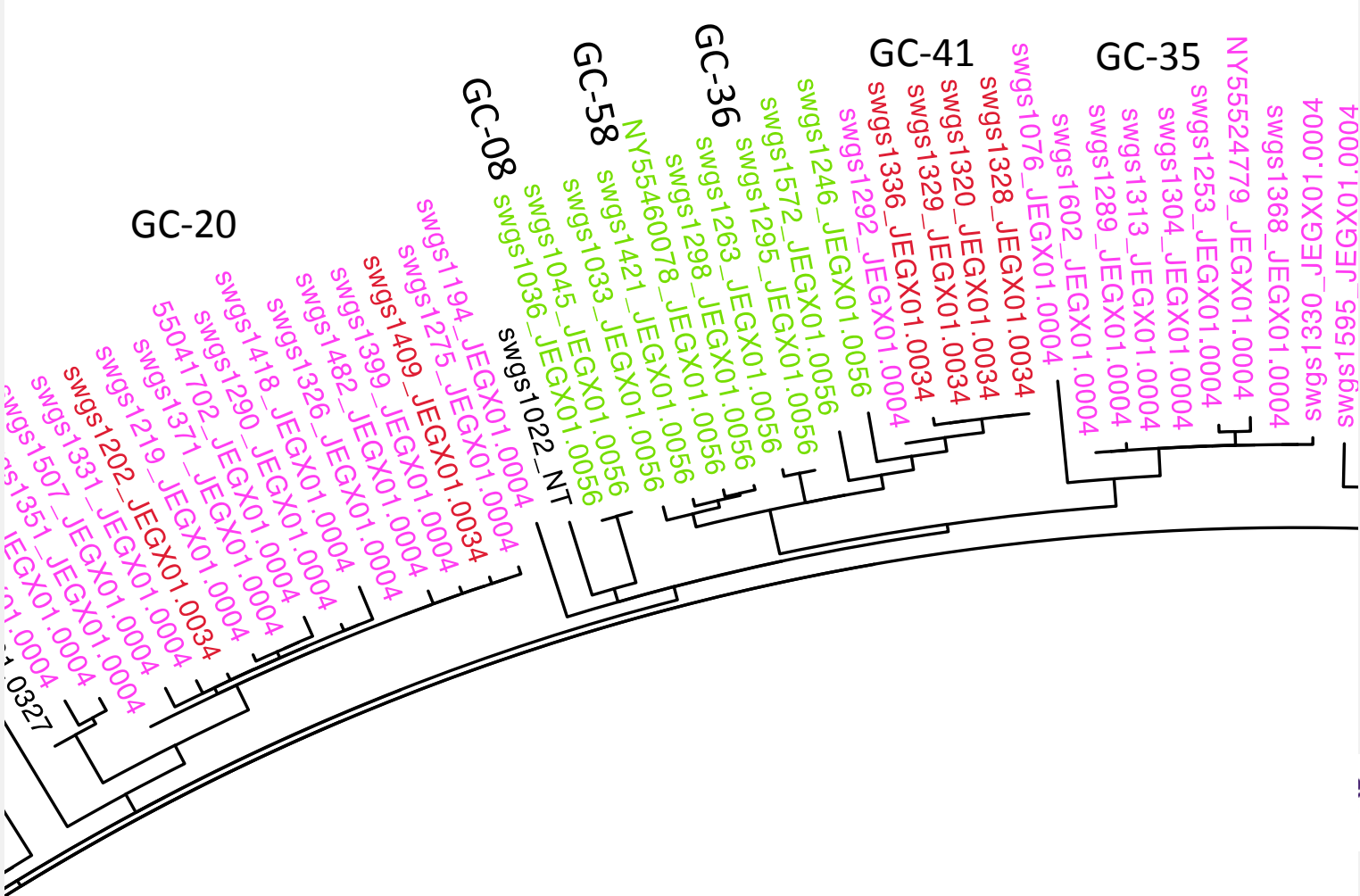
PFGE
JEGX01.0005
contains 18
genomic clusters

PFGE JEGX01.0056
contains 4 genomic
clusters

PFGE JEGX01.0021
contains 12 genomic
clusters

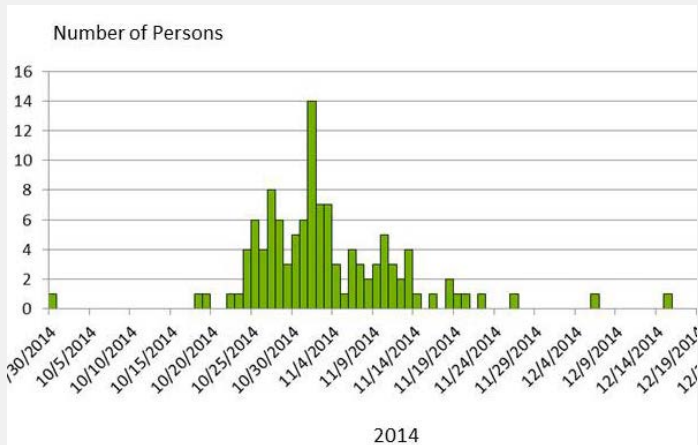
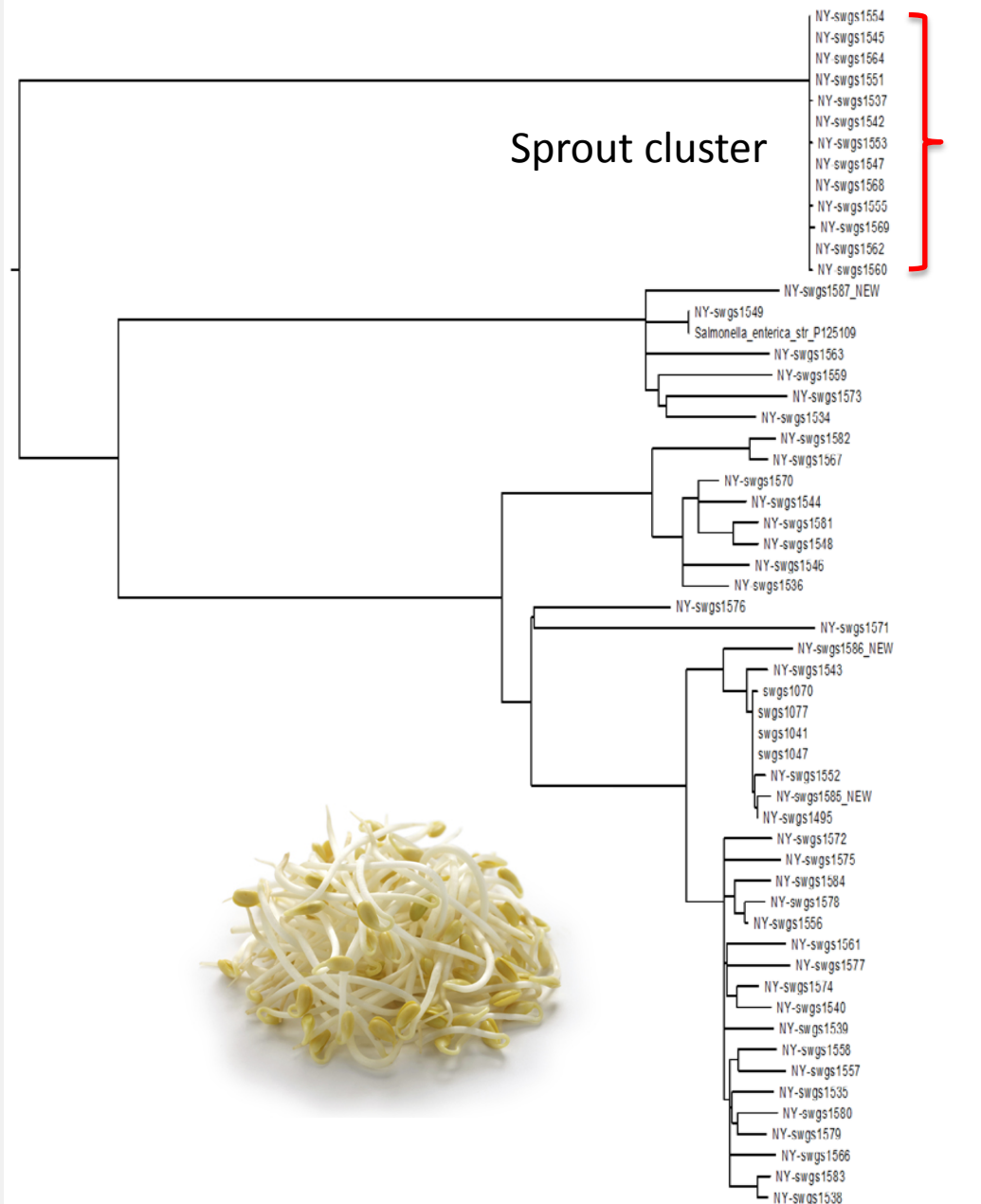


PFGE types are not monophyletic and Genomic clusters can contain multiple PFGE types



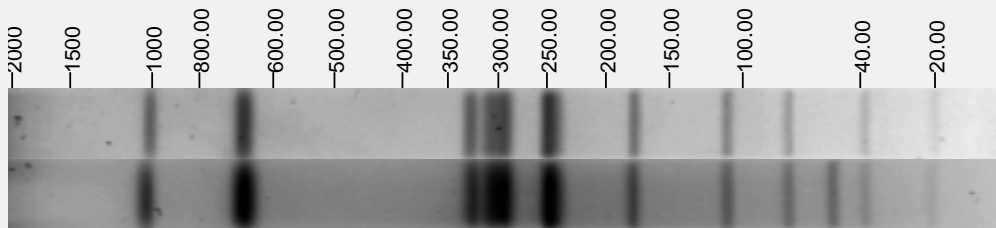
Sprout associated outbreak from 2014

- CDC identified an outbreak associated with Sprouts
- 115 people from 12 states
- NYS collected 13 patient isolates collected in Oct. and Nov.
- 0 to 2 SNPS apart



NYC restaurant outbreak

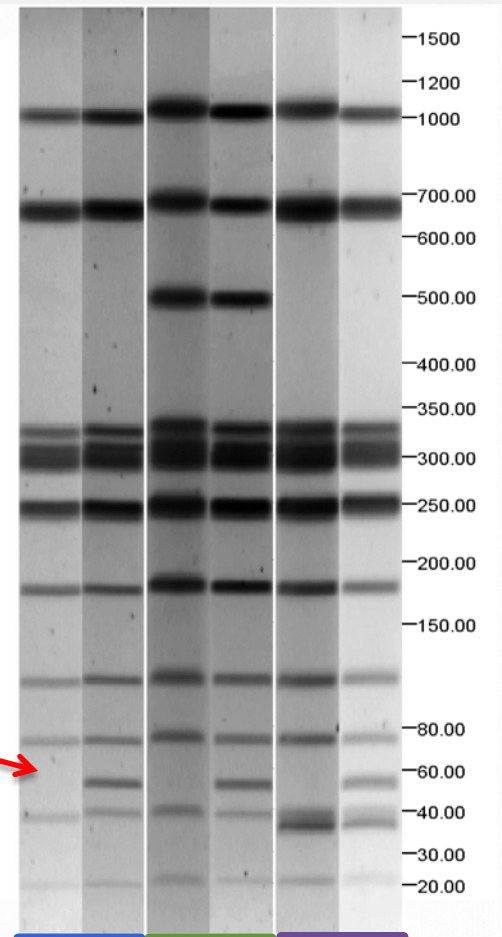
- NYCDOHMH requested WGS.
- 6 isolates were JEGX01.0034
- 1 isolates was JEGX01.0004
- Q: Was the outlier related?
- A: Yes; There was 0 SNPs difference.



- Enteritidis JEGX01.0034
- Enteritidis JEGX01.0004

26% of Genomic clusters harbor two PFGE types

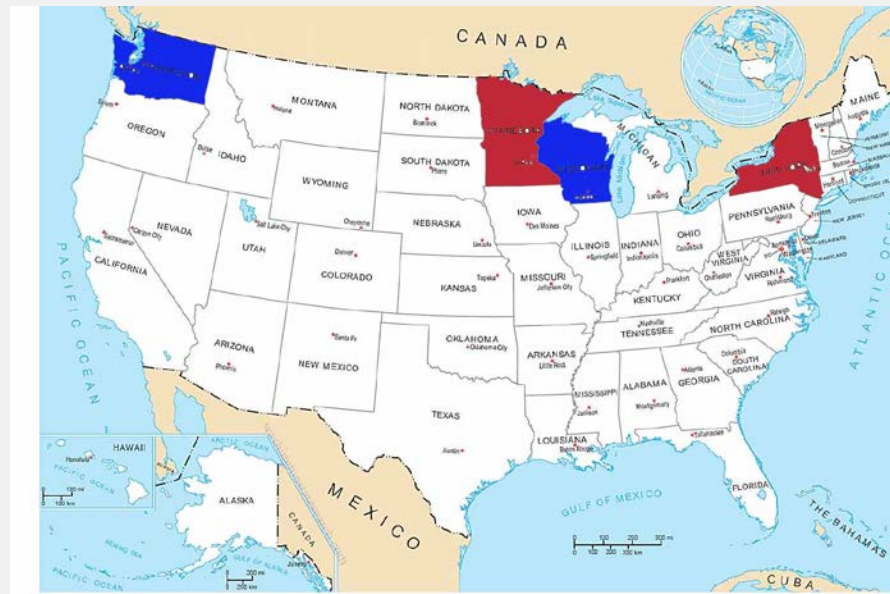
- Commons pairs have all lost the same plasmid
 - SLA5
- Genomic types are potentially more stable than PFGE types.



	T34	T4	T30	T5	T23	T21
pSLA5		+		+		+
6kb Island	+	+	+	+	+	+
Phage RE2010	+	+	+	+	+	+
T21 Plasmid*					+	+
T5 Island			+	+		

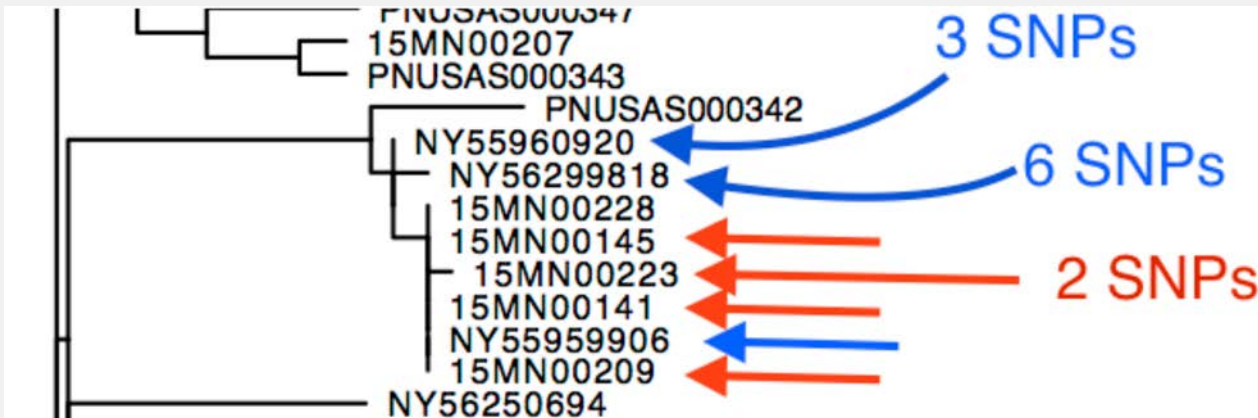
Four State SE surveillance Network

- Collaborating with MN, WA, and WI.
- Analyze their WGS data.
- Combine it with ours.



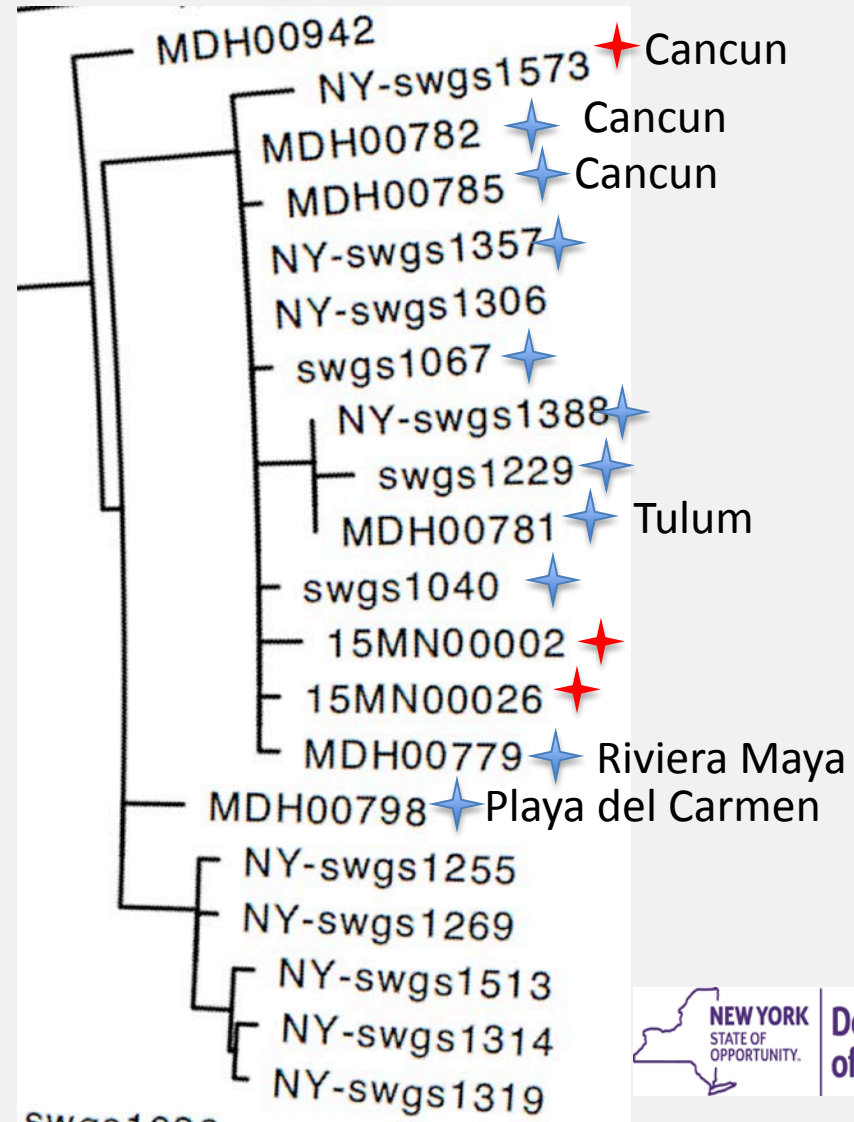
MN Frozen Chicken product outbreak

- 1506MNJEG-2 posted 6/25
- Collected 4/13 to 6/12
- 4 isolates have 0 SNPs.
 - 1 isolate had 2 SNPs difference
- pattern JEGX01.0004
- NY isolates in the cluster.
 - 4/28 to 5/20
 - 1 isolate 0 SNPs difference



WGS reveals the same *Salmonella* imported from Mexico to NY and MN

- Starred have a travel history to Mexico.
- 0 to 8 snps
- Red star travelled 2014.
- Blue stars 2015.



A national / international surveillance network is being built by federal and state Public Health Labs

FDA GenomeTrakr

State Health labs

- Wadsworth
- Florida
- Arizona
- Washington
- Minnesota
- Virginia
- Maryland
- Alaska
- Hawaii
- New Mexico
- NY Agriculture
- N. Carolina

CDC AMD

State Health Labs

- Wadsworth
- Connecticut
- Michigan
- Washington
- Minnesota
- Virginia
- Maryland
- Ohio
- Tennessee
- Wisconsin
- and 10 additional states

CFSAN Labs

- 9 FDA field labs
- CFSAN - MOD1
- CFSAN - Wiley
- IEH (contracting lab)

International labs

- Mexico
- Ireland
- UK (FERA)

Contributors

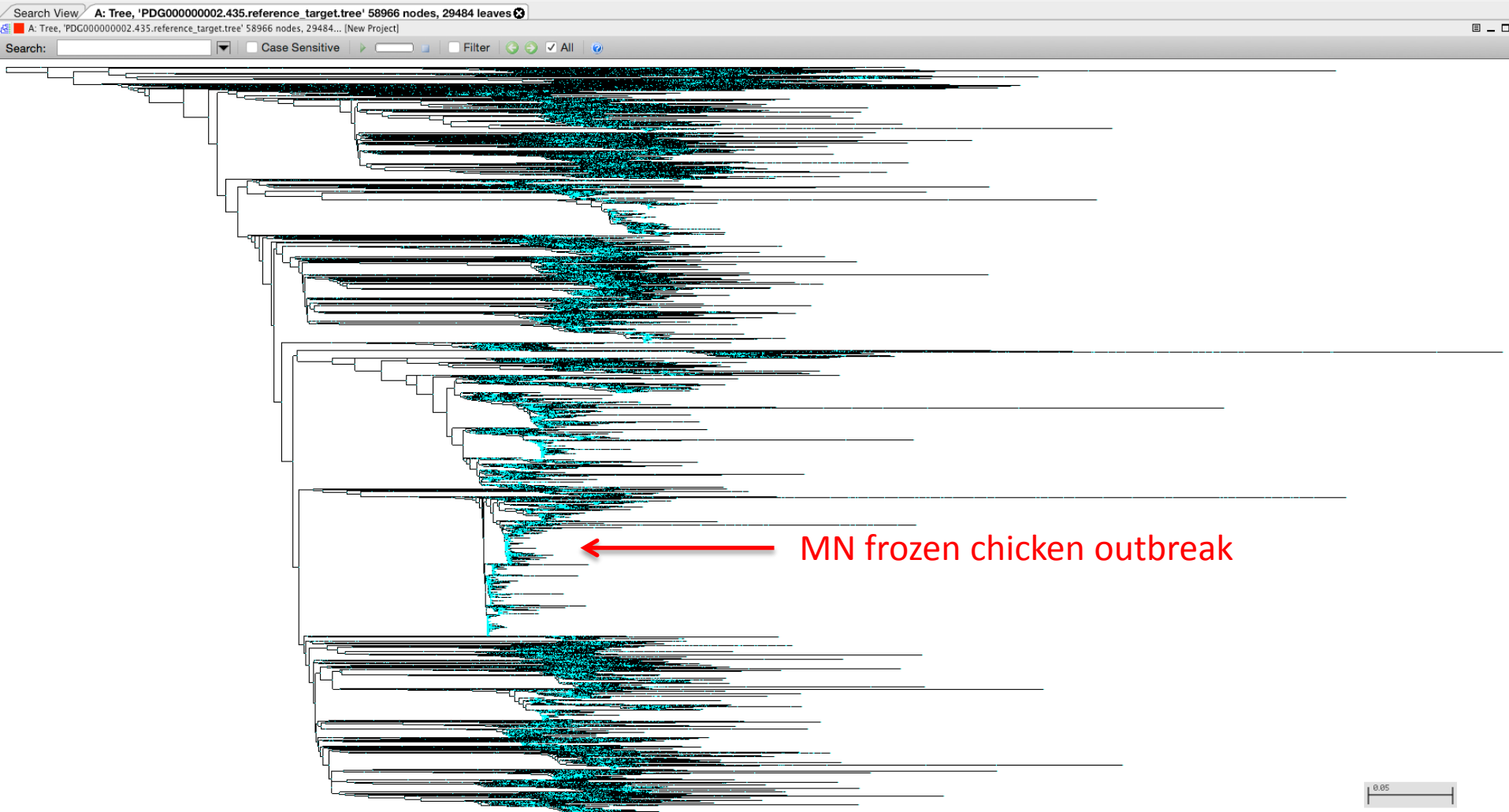
- Turkey
- Brazil
- Italy
- Columbia

National Genomic Surveillance Machine of the future

- NCBI provides a centralized public repository for all sequences and metadata.
 - Historical as well as contemporary isolates
 - Publicly accessible
- CDC and NCBI provide centralized analysis and reporting to states.
- Decentralized analysis by states- using CDC and NCBI tools and databases.



NCBI tree with 29,484 Salmonella genomes



Zoom in



- Red isolates contain outbreak isolates from from MN and NY.
- Still a work in progress

WGS is great!!!

- It improves resolution.
- It is more stable.
- And data can be readily shared.

- It works really great to confirm or refute epi. findings.
 - Subdivide Endemic PFGE types
 - Provides ultimate resolution

- But challenges still exist.
 - Standardization of analysis
 - Communication of results
 - Increased number of clusters detected:
 1. Prioritization
 2. How we define clusters

Acknowledgments

Wadsworth Bacteriology

Kim Musser
Michelle Dickinson
Samantha Wirth
Kara Levinson
Kara Mitchell

Wadsworth Center Genomics Core

Matt Shudt
Zhen Zhang
Melissa Leisner
Mike Palumbo
Pascal LaPierre

Wadsworth PFGE Lab

Dianna Bopp
Deb Baker
Lisa Thompson
Michelle Dickinson

BCDC NYSDOH

Madhu Anand
Alex Newman

FDA

Eric Brown
Peter Evans
Marc Allard
Errol Strain
Ruth Timme

NCBI

Bill Klimke
Martin Shumway

CDC

Eija Trees
Heather Carleton

Cornell

Henk den Bakker
Martin Wiedmann

Minnesota DOH

David Boxrud
Angie Taylor
Victoria Lappi

Washington DOH

Ailyn Perez-Osorio

Wisconsin DOH

Sara Wagner
Tim Monson