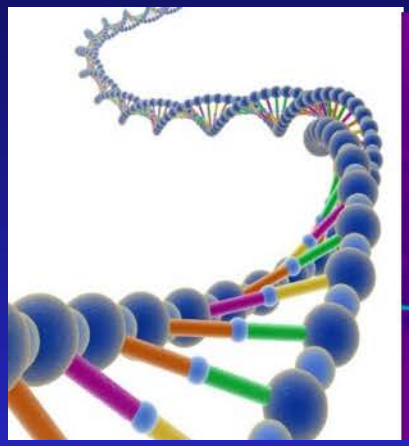
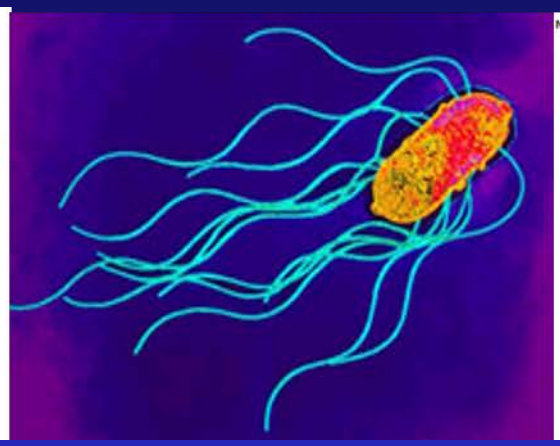
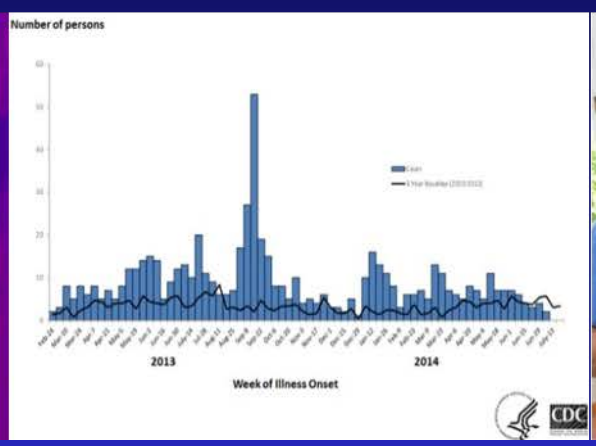
Whole genome sequencing and the transformation of public health surveillance (for enteric infections)

Georgia Emerging Infections Program Annual Conference

March 24, 2017

Atlanta, Georgia

Robert Tauxe, MD, MPH

Director,

Division of Foodborne, Waterborne, and Environmental Diseases

National Center for Emerging and Zoonotic Infectious Diseases

Centers for Disease Control and Prevention

20 years of PulseNet USA 1996 CDC 2016

CDC Centers for Disease Control and Prevention

1946 Atlanta

2012 Bldg 24

What does CDC do?

Protect the health of the public with expert science, by:

- Informing with reliable scientific information for decision makers
- Protecting everyone's health by detecting, solving, stopping and preventing outbreaks
- Innovating to improve scientific methods to public health challenges
- Building capacity in local and state health departments and international partners

Partnership is vital to everything we do

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Looking forward – a few broad themes

- Bringing WGS into daily practice in public health labs for characterizing and subtyping enteric bacteria
- Increasing investigative capacity in state and local health departments
- New strategies for diagnosis – in clinical laboratories and ultimately in the public health labs as well.
- Expanding partnerships
  - Interagency
  - Public-private
  - International

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Improving information for decision making

- Updating the estimates of health burden of enteric illness
  - Starting the new FoodNet population survey in April
  - 2 year cycle, multimodal surveys

- Use to adjust health burden, trends and food source estimates for changes in
  - Diagnostic tests,
  - Physician behavior

- New attribution estimates with expert elicitation for
  - % of infections coming from different pathways:
  - food, water, animal contact, person-to-person contact

Result: New general estimates of burden of foodborne illness

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Making our information more available

- FoodNet Fast
  - Interactive display of graph and tables of FoodNet data
  - Will add more capabilities and mapping
- Web reports on major multistate foodborne outbreaks
  - Will add: more investigative details, relevant to industry
- CDC Foodborne Outbreak Online Database (FOOD) Tool
  - Interactive search and summary of reported foodborne outbreaks
  - Will add: ability to search waterborne, animal contact and person-to-person outbreaks for enteric pathogens
- CDC NARMS NOW
  - Interactive display of resistance by drug and pathogen
  - Will add: ability to display selected groups of resistance

A surveillance network combines strain subtyping and patient interviews

- Detecting a dispersed outbreak among many sporadic cases
  - Means finding the signal in background noise
  - Depends on the surveillance system in use
  - Identifies food safety gaps early in food production

- Starts with the report of a diagnosed illness, and referral of isolate
  - Interview the patient
  - Subtype the strain in a public health laboratory

- Subtyping methods have been improving over time

Improved surveillance with subtyping for foodborne bacterial pathogens

- 1940's - present: Routine clinical laboratory cultures
- 1960's - present: Serotyping (*Salmonella, Shigella*)
- 1980's - 1990's: Plasmid profiles
- 1996 - Present: PulseNet molecular subtype-based surveillance (based on PFGE)
  - *E. coli* O157/STEC
  - *Salmonella*
  - *Listeria*
  - *Shigella*
  - *Campylobacter*

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PulseNet 1996-2016: National network for molecular surveillance of bacterial enteric infections

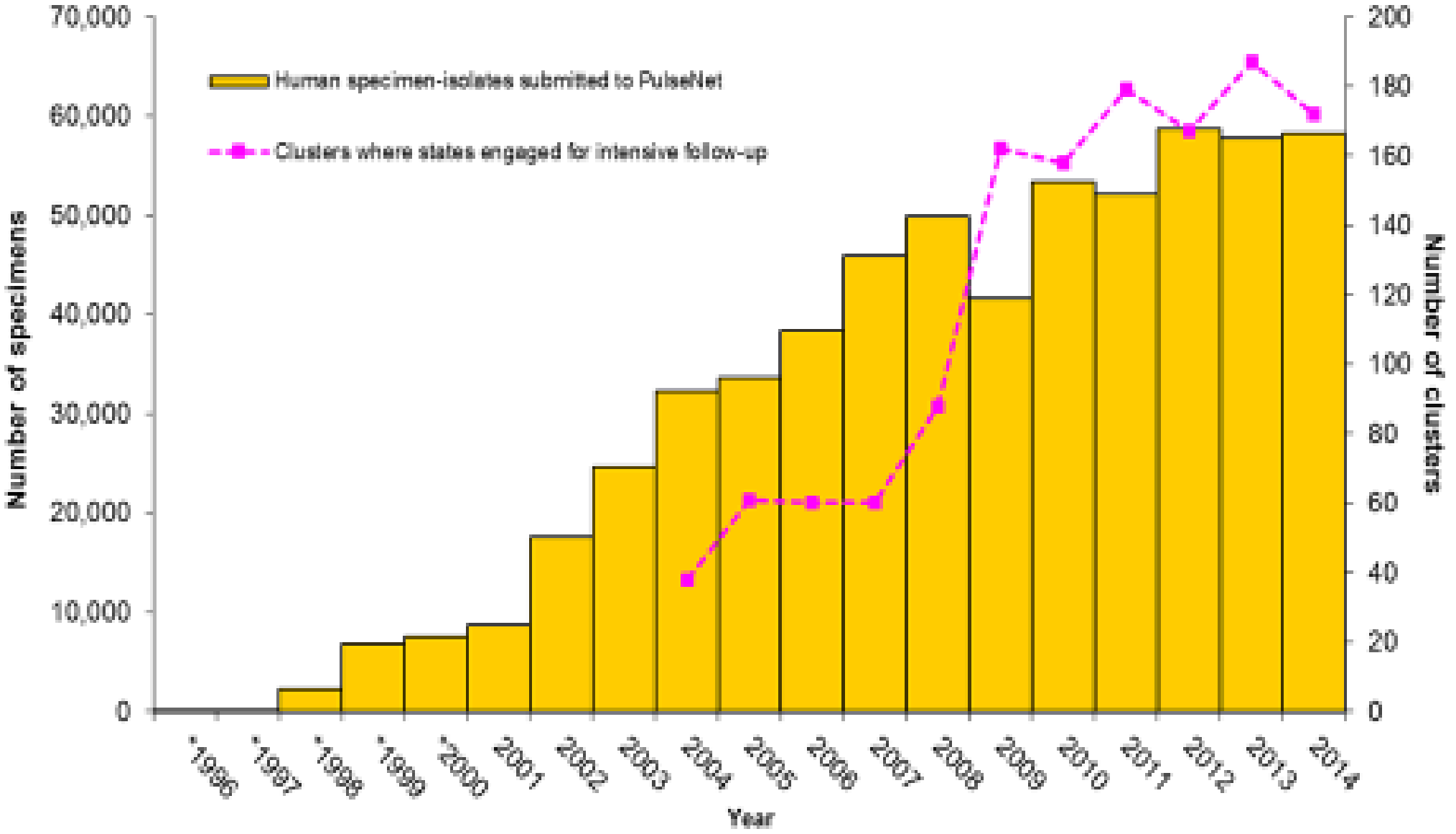
Standard PFGE method
Results in CDC database
All participants can use

87 labs participate:
- All state heath departments
- City health departments
- FDA laboratories
- FSIS laboratories

50,000 bacteria/year from
- ill people
- foods
- animals

Links with:
- PulseNet Canada
- VoluntaryNet (food industry)

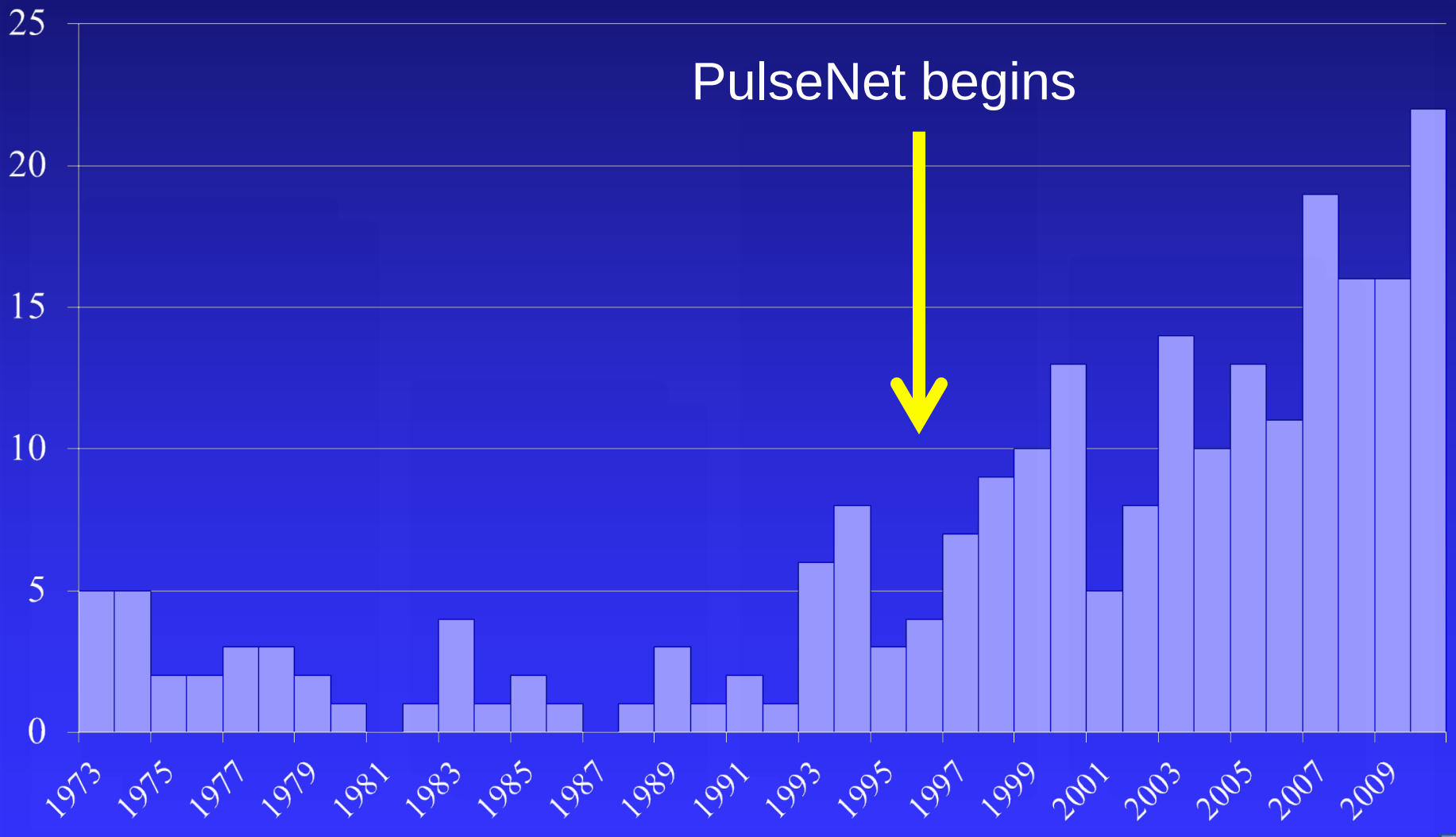
Human Specimen Isolates Uploaded to PulseNet USA and Identified Clusters, 1996-2014†



† Data are preliminary and subject to change

* data type information may not be complete for these years

Multistate foodborne outbreaks reported to CDC 1973-2010



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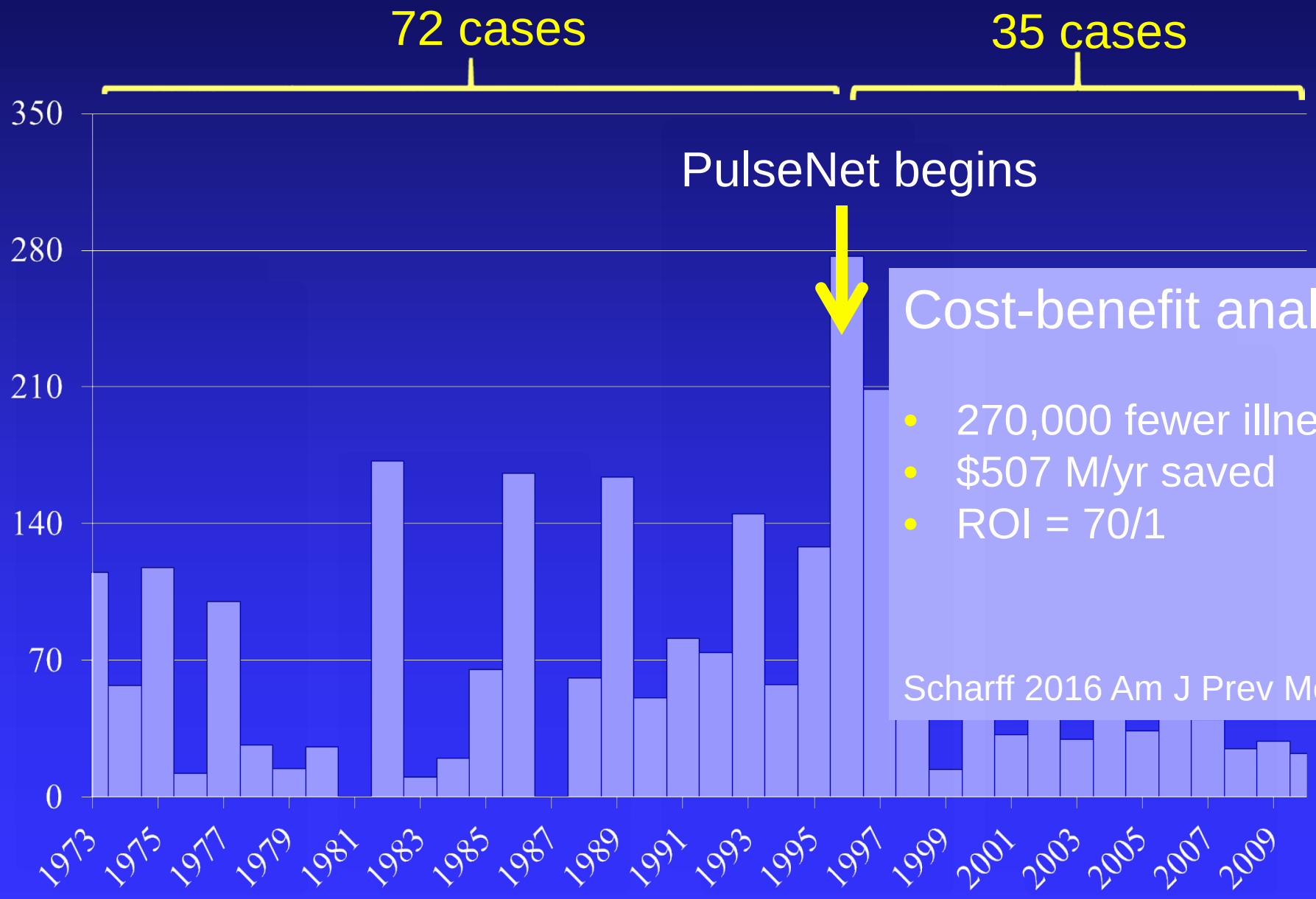
Median size of multistate foodborne outbreaks reported to CDC 1973-2010



Cost-benefit analysis

- 270,000 fewer illnesses/yr
- $507 M/yr saved
- ROI = 70/1

Scharff 2016 Am J Prev Med

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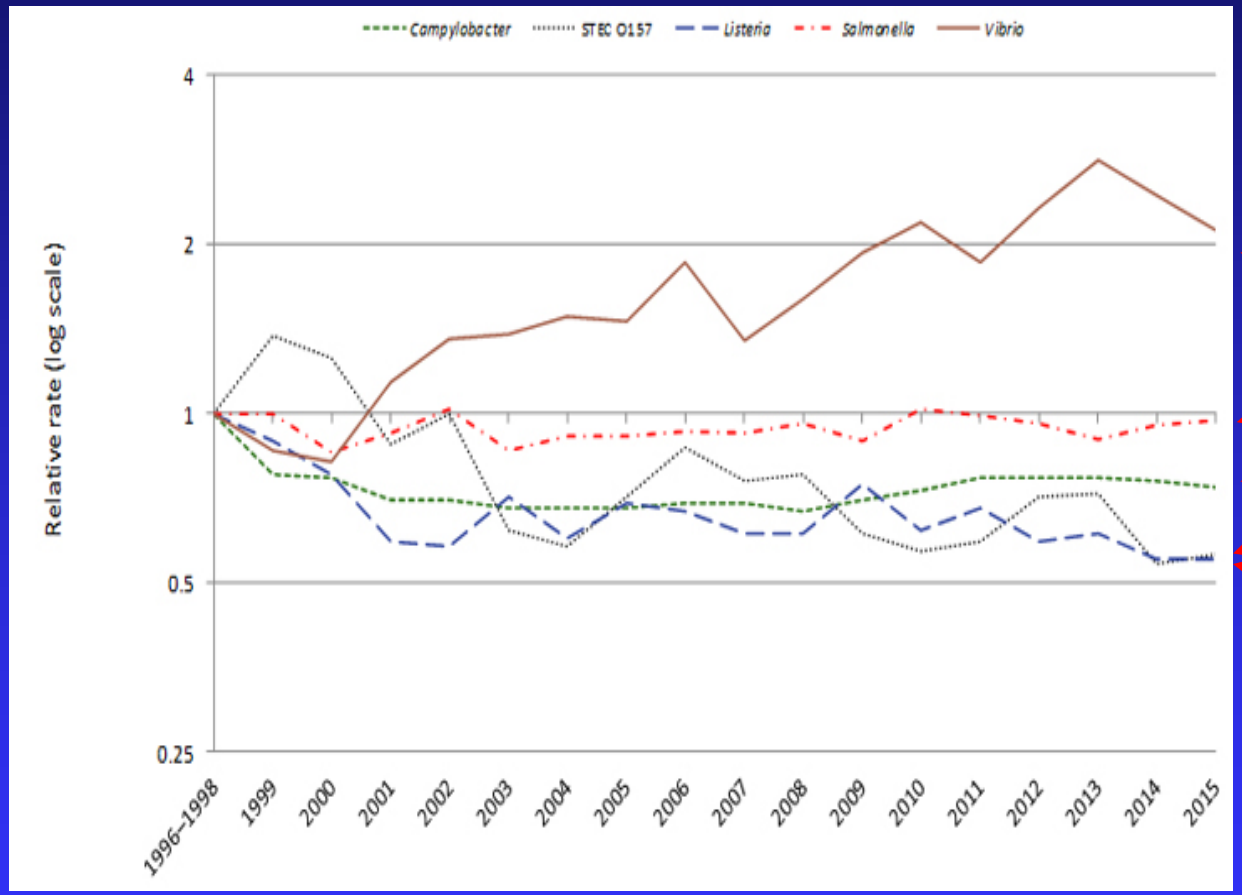
Novel foods implicated in outbreaks since 2006 in the United States

- Bagged spinach
- Carrot juice
- Peanut butter
- Broccoli powder on a snack food
- Dry dog food
- Frozen pot pies
- Canned chili sauce
- Hot peppers
- White and black pepper
- Raw cookie dough (flour?)
- Hazelnuts
- Fenugreek sprouts
- Papayas
- Pine nuts
- Raw scraped tuna
- Pomegranate anils
- Torshi
- Cashew nut cheese
- Cucumbers
- Chia sprout powder
- Cheeses cut on the same cutting board as other cheeses

All found
as a result of multi-state investigations

Trends in foodborne illness, United States, FoodNet, 1996-2015



Since 1996, accurate data on diagnosed infections from 10 sites, 15 % of population

***Vibrio* – increased**

***Salmonella* - no change**

Campylobacter,

***E. coli O157,* and**

***Listeria* decreased significantly**

Since 2006-2008, only *E. coli* O157 has decreased significantly

www.cdc.gov/foodnet/reports/data/incidence-trends.html#figure2

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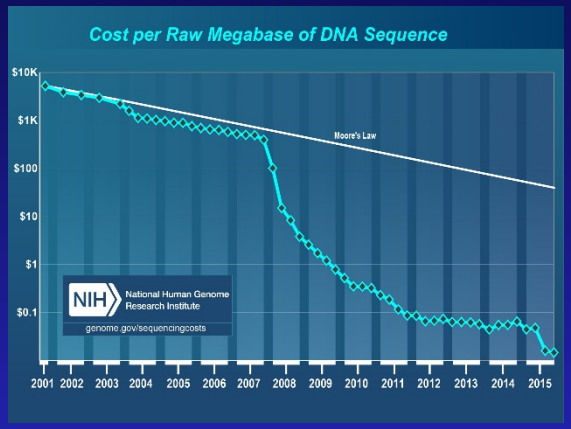
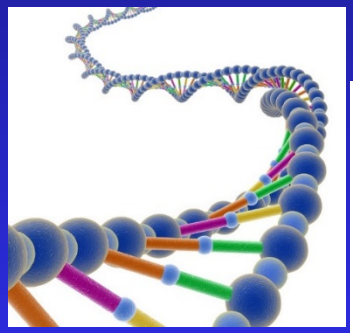
Reading and comparing bacterial DNA sequences
Big data meets microbiology





- In 15 years, cost and speed of sequencing DNA from a bacterium has dropped from $100,000s and a year to $100 and hours
- Reading and interpreting the whole genome sequence is faster and more automated
- 3M base pairs = 1800 pages of text
- Comparing alleles and sequences provides much greater precision than PFGE to say:
  - Strains are closely related (and thus may have same source)
  - Strains are not closely related, (and can be excluded from investigation)
  - Strains from ill people are closely related to strains from suspect foods or environment (a clue to the source)

What happens if we use this new technology in PulseNet?

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2013 Pilot project: Does WGS technology improve listeriosis surveillance?

- Since 1998, State public health laboratories have used PFGE in the PulseNet network for *L monocytogenes*
- In 2013, collaborative multiagency effort began sequencing isolates of *Listeria monocytogenes* as part of routine surveillance
  - Clinical isolates at CDC (~800/year),
  - Food isolates at FDA, USDA
  - WGS data stored at NIH

- State health departments interviewed all listeriosis cases
- Coordination with Canada, UK, France, Denmark, Australia

http://www.hhs.gov/idealab/projects-item/whole-genome-sequencing-future-of-food-safety/

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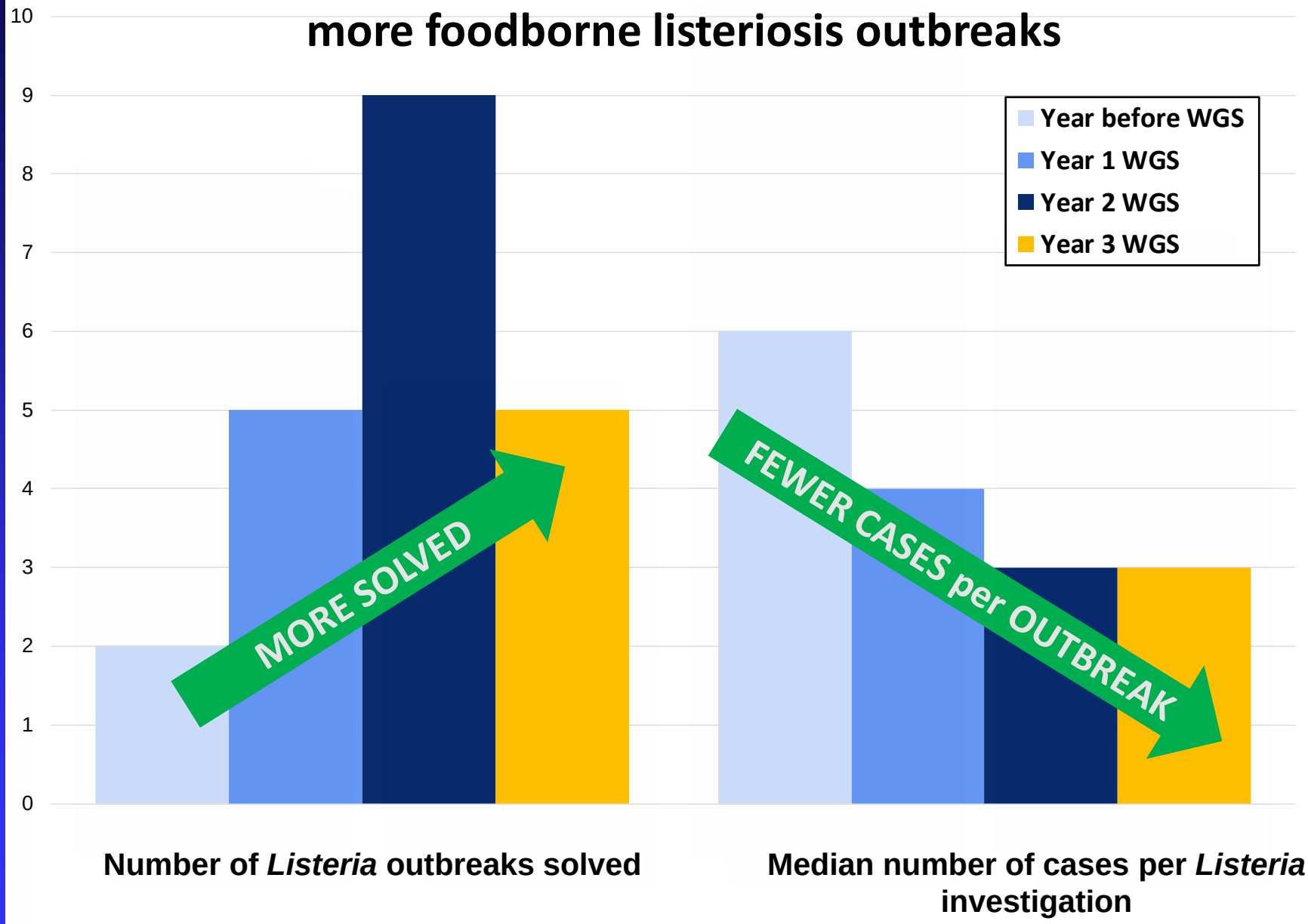
Surveillance based on DNA Sequencing: Solving more foodborne listeriosis outbreaks



Jackson 2016. Clin Infect Dis 63:380-6

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Foods implicated in listeriosis outbreaks since 2013 in the United States (in the WGS era)

➢ Expected foods:
- Raw milk
- Soft cheeses
- Mung bean sprouts
- (Not processed meats)

➢ Novel foods:
- Caramel-dipped apples
- Ice cream
- Packaged leafy green salads
- Stone fruits (nectarines)
- Frozen raw vegetables

Found as a result of multi-state investigations

Contamination often occurred at the packing shed or processing facility

Most investigations started with ill people. A few started with finding *Listeria* in a food, that matched strains from patients who ate that food

New efforts in food industry now to reduce contamination with listeriosis

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Listeria and raw milk, 2014-2016

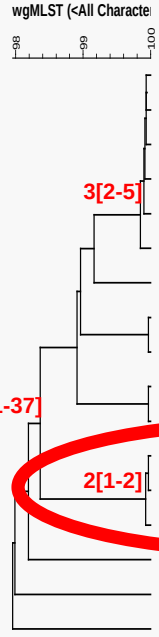
- November 2015: FDA collected raw milk sample at a raw milk conference in CA
- January 2016: *Listeria* isolated from raw milk, WGS matched 2 infections from 2014
- Patients in FL and CA
- Mean age 77: both hospitalized, 1 died

- PFGE: Two different patterns
- WGS: Extremely close: Within 2 SNPs

By PAULA COHEN / CBS NEWS / March 18, 2016, 4:12 PM

Deadly listeria outbreak linked to raw milk

A 2014 listeria outbreak has been traced to raw milk. / OKSANA BRATANOVA



WGS_id	PFGE-AscI-pattern	PFGE-ApaI-pattern	Outbreak	SourceState	SourceSite	IsolatDate
2011L-2609	GX6A16.0029	GX6A12.0069	1109COGX6-1	TX	Blood	2011-08-20
2011L-2944	GX6A16.0029	GX6A12.0069	1109COGX6-1	CO	Unknown	2011-10-23
2011L-2793	GX6A16.0029	GX6A12.0069	1109COGX6-1	CO	Blood	2011-09-21
PNUSAL000005	GX6A16.0029	GX6A12.0069	1109COGX6-1	CO	Blood	2011-08-29
2011L-2804	GX6A16.0029	GX6A12.0069	1109COGX6-1	MO	Cantaloupe	
2011L-2659	GX6A16.0029	GX6A12.0069	1109COGX6-1	MO	Blood	2011-09-07
PNUSAL000447	GX6A16.0029	GX6A12.0069		SC	BLOOD	2013-10-20
PNUSAL002119	GX6A16.0029	GX6A12.0069	1604MLGX6-1		Urine	2016-03-17
PNUSAL002126	GX6A16.0029	GX6A12.0069	1604MLGX6-1	GA	Urine	2016-03-17
FDA00009849	GX6A16.0029	GX6A12.0069		NJ	Environmental Swab	2016-01-14
[illegible]	GX6A16.0029	GX6A12.0069		NJ	Environmental Swab	2016-01-14
PNUSAL000811	GX6A16.0029	[illegible]12.0069	1601MLGX6-1WGS	CA	Blood	2014-06-21
PNUSAL001231	GX6A16.0029	GX6A12.0069	1601MLGX6-1WGS	FL	blood	
FDA00009686	GX6A16.0087	[illegible]12.0069	1601MLGX6-1WGS	CA	Raw, Chocolate Milk	2015-11-20
[illegible]	GX6A16.0029	GX6A12.0069		MI	liver	2014-11-03
NYAG_13B07958D_1	GX6A16.0087	GX6A12.0069		NY	Milk	2013-08-19
CFSAN032476	GX6A16.0029	GX6A12.0069		TX	Butternut Squash	2015-04-01

- Both drank raw milk
- One reported to get milk online from same PA farm that FDA had sampled
- Source of other unclear

- PA farm sold milk interstate over the web
- Private membership organization
- March 18: CDC warned public

www.cdc.gov/listeria/outbreaks/

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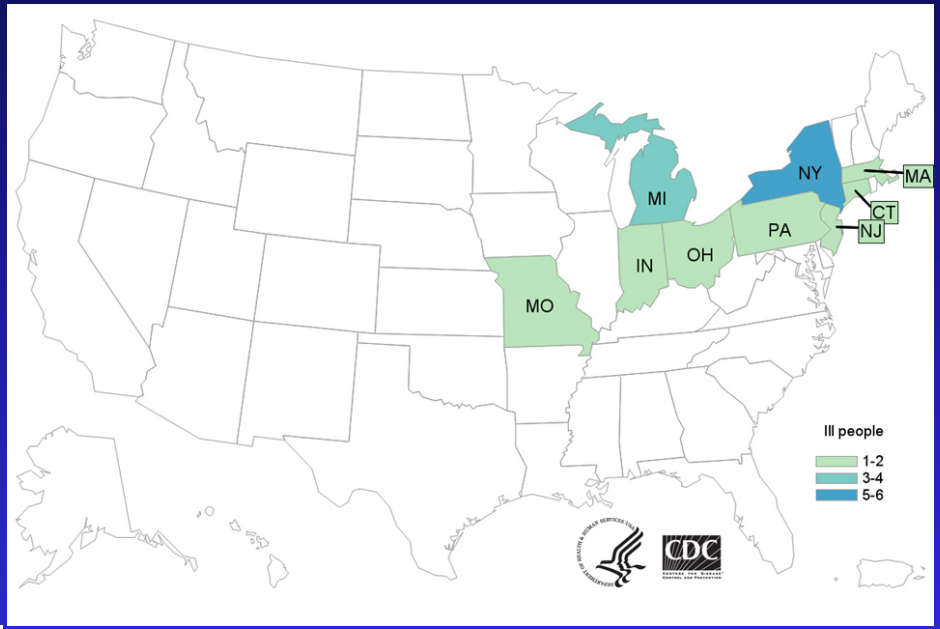
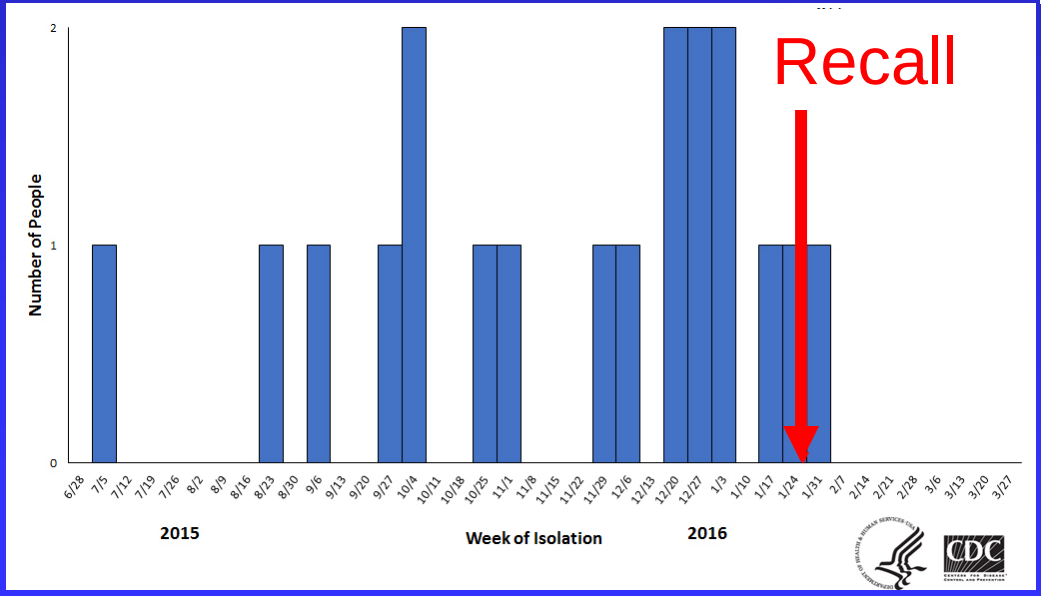
Listeria and bagged salads, 2015-2016

- July 2015 - Jan 2016
- 19 cases in US, 14 in Canada (33 total)
- Closely related by WGS
- 9 states, 5 provinces
- All hospitalized, 4 died
- Median age 64 years, 74% female
- 13/14 ate bagged salad, 9 named 1 brand

- WGS match to *Listeria* from same brand
  - regulatory salad sample in Ohio
  - bagged salad tested in Canada





- Concern focused on multiple products made at one packaging plant in Ohio
- CDC and FDA shared information with company
- Halted production, and recalled product
- Plant closed for 4 months
- Intensive assessment, sanitation
- New program of in-plant monitoring

www.cdc.gov/listeria/outbreaks/

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Salmonella Enteritidis (SE) and frozen stuffed breaded raw chicken products – Minnesota, 2015

- Minnesota DH began sequencing SE
- Found 2 clusters in summer of 2015
- Cluster #1: 5 illnesses
- Ate one brand of frozen stuffed breaded raw chicken entrée
- Same strain found in product
- Product distributed to many states
- 2.4 M pounds recalled

www.fsis.usda.gov

- Cluster #2: 15 illnesses (including 7 in other states)
- Ate a different brand of frozen stuffed breaded chicken products
- Same strain found in frozen product
- Product distributed to many states
- 1.7 M pounds recalled

- Most patients knew the product was raw, and followed cooking instructions
- Some even checked the internal temperature
- UDSA now considering further standards for products like this

www.cdc.gov/salmonella/outbreaks/ and thanks to Carlota Medus

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Value added by using whole genome sequencing

- Close genetic similarity means greater confidence that a group of infections may share a common origin, so more focused investigations
  - Split up common PFGE patterns into more related subgroups
  - Join strains with different PFGE types that are closely related
  - Compare strains from foods, animals, and production environments
  - Better determination of which cases to interview, which to exclude
- Whole genome sequencing as part of public health surveillance can
  - Replace serotyping, toxin typing, resistance testing
  - Provide fine-grained subtyping that is phylogenetically relevant
  - Like serotyping in the 1960's, PFGE in the 1990's, detecting clusters
- Empowers epidemiologists, rather than replacing them
  - Still need to find out what patients ate, that others did not
  - Still need to trace foods to their sources

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Improving our ability to detect, solve and stop outbreaks

- Routine whole genome sequencing is swiftly becoming part of routine public health surveillance
- 3 year collaborative pilot using WGS with *Listeria* 2013 – 2016
- Now placing WGS capacity in state and city health departments
  - Starting with *Listeria*
  - Expanding to STEC, *Salmonella*, *Campylobacter*, *Shigella…*
  - Likely to start finding more clusters
  - Expanding investigative capacity in states as well

Anticipate using WGS as main subtyping method in 2019

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Accelerating deployment of WGS is part of the plan to Combat Antibiotic Resistant Bacteria

- 2016 Federal Budget included support for CDC to address the threat of antimicrobial resistance in many pathogens, including some foodborne ones (The Combatting Antibiotic Resistant Bacteria plan, or CARB)
- With WGS, resistance is known as soon as an outbreak of *Salmonella* infections is detected
- Outbreaks caused by multi-drug resistant (MDR) strains can be prioritized for investigation and control
- Goal: Reduce MDR *Salmonella* infections by 25% by 2020

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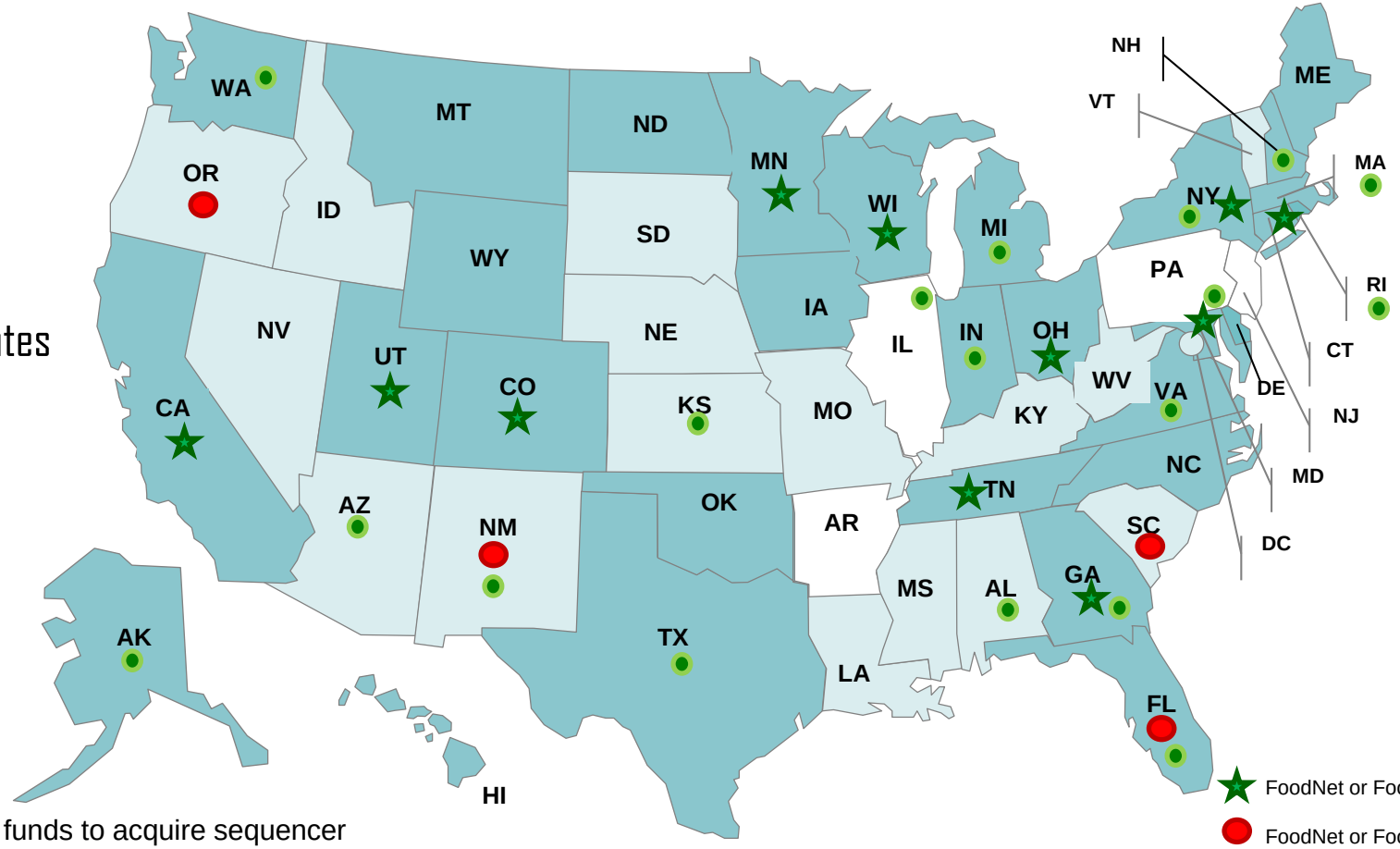
PulseNet Sequencing Capacity – February 2017

33 labs in 30 states certified;

4 states without sequencers



Awarded ELC funds to acquire sequencer
Has sequencer but not certified
PulseNet certified

FoodNet or FoodCore with WGS capacity
FoodNet or FoodCore without WGS capacity
Outbreak Net Enhanced

Innovation – After Whole Genome Sequencing, what do we do for an encore?

- WGS depends on
  - Isolating of a pure culture
  - Shipping it to the public health laboratory
  - Sequencing and interpreting
  - *Can take a week*

- To make surveillance faster, public health needs more advanced molecular diagnostic tools for use on the clinical specimen that
  - Provide species identification
  - Predict serotype, virulence, antimicrobial resistance, subtype
  - Report results in hours, rather than days

- We and others have begun exploratory work in this area
  - Amplifying around key targets, like Shiga toxin-coding phage genes
  - Metagenomic approaches with long-read sequencing
  - Single cell sequencing

Will be of great use where cost and complexity prohibit culture

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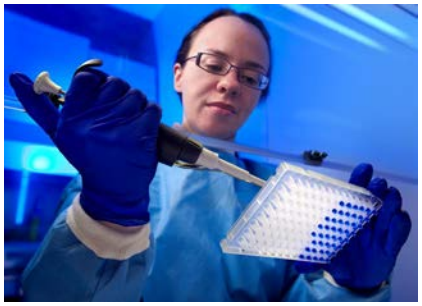
Diagnostic Methods Through Time

1860s:
Culture-based tests
Invented by Louis Pasteur, Robert Koch, et al

1980s-90s:
Antigen-based tests
Detects antigens specific to pathogen type



2000s:
Polymerase Chain Reaction (PCR) tests
Detects short genetic sequences specific to pathogen type

2010s:
Multiplex PCR panels
Uses PCR to detect multiple pathogens simultaneously, often designed for disease syndromes

Culture-independent Diagnostic Tests

Innovation – The culture-independent diagnostic tests (CIDTs) are starting to change clinical practice

- The most popular CIDT platform tests for 22 pathogens in an hour
- Physicians can now base immediate treatment decisions on lab diagnosis
- Test routinely for pathogens rarely considered before
  - *Cryptosporidium*
  - Enterotoxigenic *E. coli*
  - Norovirus
  - *Vibrio parahaemolyticus*
  - *Yersinia enterocolitica*
- Laboratory workflow is simplified
- Rapidly increasing in use

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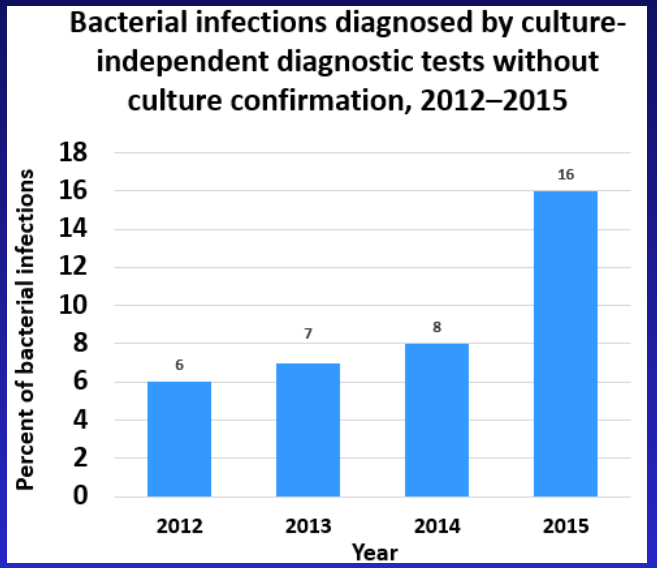
Innovation – CIDTs will likely lead to increases in reported case numbers

Use of CIDTs in clinical practice may increase

- Likelihood of testing (which drives reported case numbers up)
- Sensitivity of detection, perhaps because the test identifies damaged or dead organisms in the specimen (which drives reported case numbers up)
- Reports of infections that were previously rarely diagnosed (which drives those case numbers up even more)

We are developing models to account for these effects, so that we can continue to track progress in prevention

Innovation – Minding the gap, as culture-independent diagnostic tests (CIDTs) become more common



- FoodNet tracks the uptake of CIDTs
- Between 2012 and 2015, bacterial infections identified only by CIDT, without culture confirmation, went from 6% to 15%
- As CIDTs become more common, we see a gap as fewer cultures are available to public health
- To preserve access to isolates, states are changing their disease reporting requirements to encourage “reflex culture”
- Culture CIDT-positive specimens at clinical lab, or ship specimen to public health laboratory
- Public health surveillance will depend on those isolates for at least the next 5 years

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Culture-independent diagnostic tests: Challenges to public health programs

- Current CIDT platforms do not provide isolates

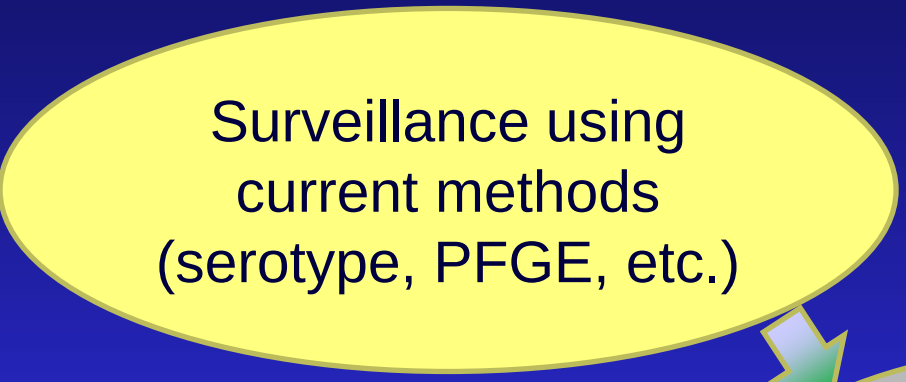
- Public health currently depends on the isolate to:
  - Detect and investigate outbreaks
  - Track cases and trends accurately
  - Estimate the overall burden from specific sources
  - Track changes in antimicrobial resistance
  - Drive public health prevention

Meeting the challenge to public health of culture-independent diagnostic tests

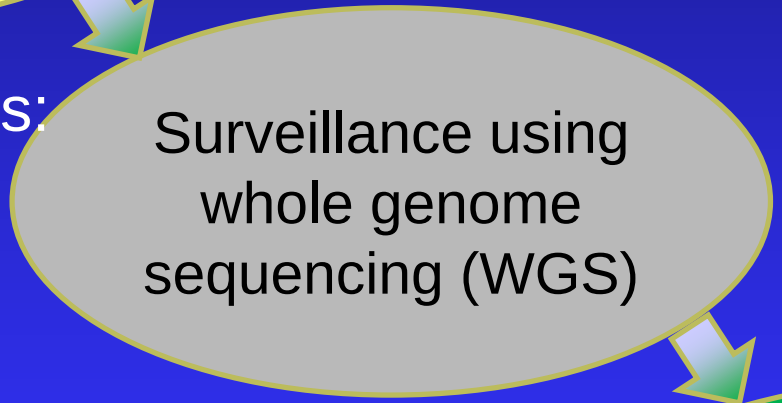
- Shorter term: Preserve access to isolates:
  - Work with medical community, state public health labs, FDA and diagnostic testing industry, clinicians
  - Request reflex culture of positive specimens to confirm them, and provide isolates
  - In clinical labs or public health labs

- Longer term: Diagnostic assays that get critical information directly from stool specimen, that are culture-independent
  - Selective amplification at diagnostic regions of genome?
  - Metagenomic shotgun sequencing?
  - Single cell sequencing?

Evolutionary path of public health surveillance
Preparing for a culture-independent environment

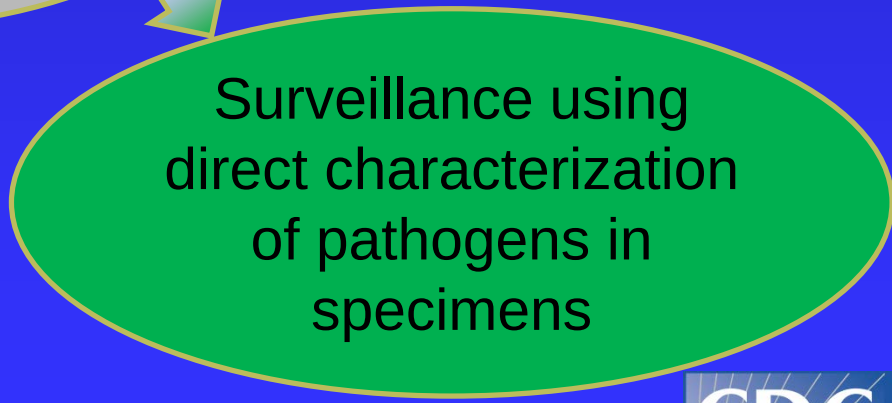


1) Need cultured isolates:
Reflex culture of
specimens positive in
multiplex PCR panels



3) New culture-
independent methods
that provide public
health data

2) Need reflex cultures:
Develop large genome
databases



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CONTROL AND PREVENTION

Long track record of success in controlling and preventing foodborne diseases – to be continued

- Identified major problems in outbreak investigations, which triggered extensive applied research, and then multipronged prevention
  - *Salmonella* Enteritidis and shell egg laying flocks
  - *E. coli* O157:H7 and ground beef
  - *Listeria monocytogenes* in processed meats

- More recently:
  - *Listeria monocytogenes* in caramel apples (2013-14)
  - *Salmonella* Heidelberg in chicken parts (2013-14)

- With new approaches, we can solve challenges like
  - STEC in raw flour
  - MDR *Salmonella* in veal calves and roaster pigs

Can expect more issues to emerge, in need of new solutions

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Could 2017 be a tipping point for improving foodborne disease prevention?

- USDA/FSIS: Implementing new performance standards for poultry parts, ground poultry, for *Salmonella* and *Campylobacter*

- FDA: New regulations under FSMA for
  - Preventive controls for processed foods, animal feeds
  - Produce safety
  - Foreign supplier verification

- Companies imposing new requirements for suppliers, making food safety part of corporate culture

- Consumers demanding food that is responsibly produced

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Future foodborne outbreaks more likely to be

- Detected by sequence-based surveillance
- Dispersed in space: Multi-state, multi-national
- Dispersed in time: Multi-year
- Detected as contaminated product first

- Associated with
  - fresh produce and minimally processed foods
  - imported foods
  - novel food vehicles
  - novel routes and pathways of contamination

Control of foodborne disease in the 21st century: An evolving public health approach

- Whole genome sequence-based surveillance is a major evolutionary step forward
- Better outbreak detection, investigation and attribution
- Requires patient interviews and traceback of suspect foods
- With WGS we can anticipate that
  - more outbreaks are detected and stopped while smaller,
  - new food safety gaps are identified
- New diagnostic tests will be increasing the number of reported cases and driving efforts to preserve access to isolates (for WGS)
- Collaboration with many partners is vital to
  - investigate contamination events throughout food chain
  - focus and improve prevention measures
  - reduce the number of foodborne infections

Thank you

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention

Our websites

FoodNet:
www.cdc.gov/foodnet

PulseNet:
www.cdc.gov/pulsenet

Foodborne outbreak surveillance:
www.cdc.gov/foodsafety/outbreaks/index.html

General Information About Foodborne Diseases:
www.cdc.gov/foodsafety/
www.cdc.gov/vitalsigns/foodsafety/

FoodNet Fast
wwwn.cdc.gov/foodnetfast/

CDC NARMS NOW
wwwn.cdc.gov/narmsnow/

Foodborne Outbreak Online Database (FOOD) Tool
wwwn.cdc.gov/foodborneoutbreaks/

Foodborne Outbreak Updates
www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/index.html