Culture-Independent Diagnostic Testing
The Epidemiologist's Perspective

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Overview

- Public health surveillance in Massachusetts and the United States
- Benefits of CIDT in the clinical setting and in public health
- Implications of CIDT in public health
- National trends
- Case studies

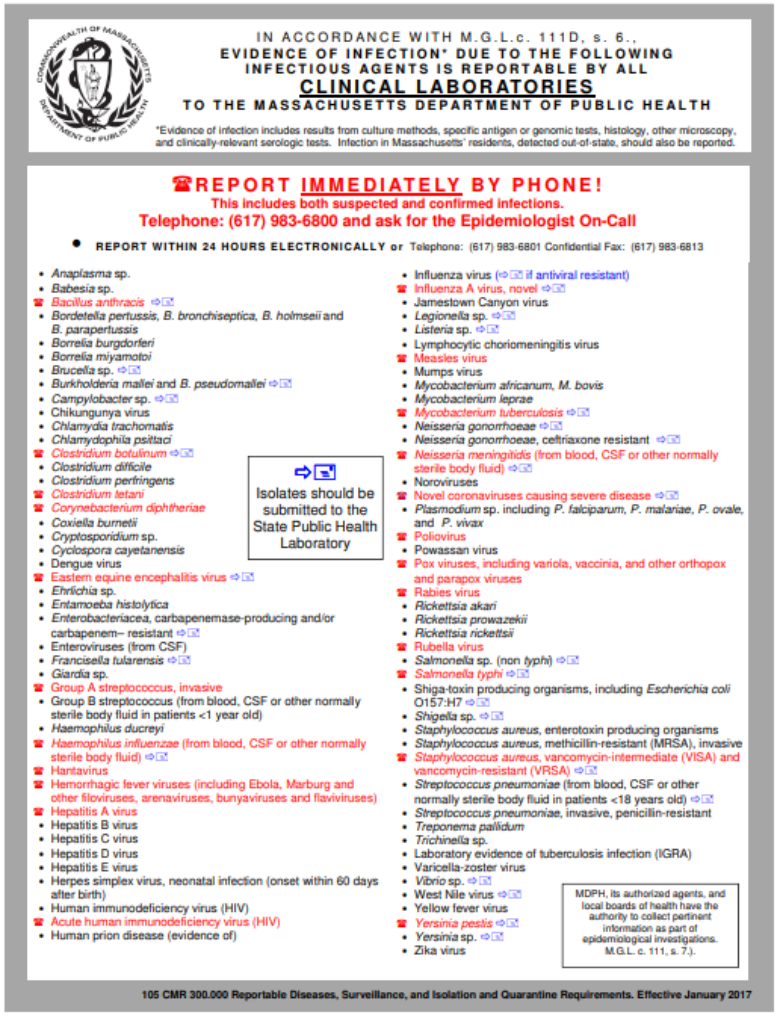
PUBLIC HEALTH SURVEILLANCE

Objectives of surveillance

1) Case investigation for disease control
2) Assessment of disease burden and trends to prioritize and evaluate population-based control measures
3) Outbreak detection
4) Microbiologic characterization of reported infections

Reportable diseases in Massachusetts

- List of over 70 reportable infections
- Positive lab results are reported via Electronic Lab Reporting into the state's case management system



IN ACCORDANCE WITH M.G.L.c. 111D, s. 6.,
EVIDENCE OF INFECTION* DUE TO THE FOLLOWING INFECTIOUS AGENTS IS REPORTABLE BY ALL
CLINICAL LABORATORIES
TO THE MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH

*Evidence of infection includes results from culture methods, specific antigen or genomic tests, histology, other microscopy, and clinically-relevant serologic tests. Infection in Massachusetts' residents, detected out-of-state, should also be reported.

☎REPORT IMMEDIATELY BY PHONE!
This includes both suspected and confirmed infections.
Telephone: (617) 983-6800 and ask for the Epidemiologist On-Call

- REPORT WITHIN 24 HOURS ELECTRONICALLY or Telephone: (617) 983-6801 Confidential Fax: (617) 983-6813

- *Anaplasma* sp.
- *Babesia* sp.
- ☎ *Bacillus anthracis*
- *Bordetella pertussis, B. bronchiseptica, B. holmseii* and *B. parapertussis*
- *Borrelia burgdorferi*
- *Borrelia miyamotoi*
- *Brucella* sp.
- *Burkholderia mallei* and *B. pseudomallei*
- *Campylobacter* sp.
- Chikungunya virus
- *Chlamydia trachomatis*
- *Chlamydophila psittaci*
- ☎ *Clostridium botulinum*
- *Clostridium difficile*
- *Clostridium perfringens*
- ☎ *Clostridium tetani*
- ☎ *Corynebacterium diphtheriae*
- *Coxiella burnetii*
- *Cryptosporidium* sp.
- *Cyclospora cayetanensis*
- Dengue virus
- ☎ Eastern equine encephalitis virus
- *Ehrlichia* sp.
- *Entamoeba histolytica*
- *Enterobacteriacea*, carbapenemase-producing and/or carbapenem– resistant
- Enteroviruses (from CSF)
- *Francisella tularensis*
- *Giardia* sp.
- ☎ Group A streptococcus, invasive
- Group B streptococcus (from blood, CSF or other normally sterile body fluid in patients <1 year old)
- *Haemophilus ducreyi*
- ☎ *Haemophilus influenzae* (from blood, CSF or other normally sterile body fluid)
- ☎ Hantavirus
- ☎ Hemorrhagic fever viruses (including Ebola, Marburg and other filoviruses, arenaviruses, bunyaviruses and flaviviruses)
- ☎ Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Hepatitis D virus
- Hepatitis E virus
- Herpes simplex virus, neonatal infection (onset within 60 days after birth)
- Human immunodeficiency virus (HIV)
- ☎ Acute human immunodeficiency virus (HIV)
- Human prion disease (evidence of)

⇨☐ Isolates should be submitted to the State Public Health Laboratory

- Influenza virus (⇨☐ if antiviral resistant)
- ☎ Influenza A virus, novel
- Jamestown Canyon virus
- *Legionella* sp.
- *Listeria* sp.
- Lymphocytic choriomeningitis virus
- ☎ Measles virus
- Mumps virus
- *Mycobacterium africanum, M. bovis*
- *Mycobacterium leprae*
- ☎ *Mycobacterium tuberculosis*
- *Neisseria gonorrhoeae*
- *Neisseria gonorrhoeae*, ceftriaxone resistant
- ☎ *Neisseria meningitidis* (from blood, CSF or other normally sterile body fluid)
- Noroviruses
- ☎ Novel coronaviruses causing severe disease
- *Plasmodium* sp. including *P. falciparum, P. malariae, P. ovale,* and *P. vivax*
- ☎ Poliovirus
- Powassan virus
- ☎ Pox viruses, including variola, vaccinia, and other orthopox and parapox viruses
- ☎ Rabies virus
- *Rickettsia akari*
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- ☎ Rubella virus
- *Salmonella* sp. (non *typhi*)
- ☎ *Salmonella typhi*
- Shiga-toxin producing organisms, including *Escherichia coli* O157:H7
- *Shigella* sp.
- *Staphylococcus aureus*, enterotoxin producing organisms
- *Staphylococcus aureus*, methicillin-resistant (MRSA), invasive
- ☎ *Staphylococcus aureus*, vancomycin-intermediate (VISA) and vancomycin-resistant (VRSA)
- *Streptococcus pneumoniae* (from blood, CSF or other normally sterile body fluid in patients <18 years old)
- *Streptococcus pneumoniae*, invasive, penicillin-resistant
- *Treponema pallidum*
- *Trichinella* sp.
- Laboratory evidence of tuberculosis infection (IGRA)
- Varicella-zoster virus
- *Vibrio* sp.
- West Nile virus
- Yellow fever virus
- ☎ *Yersinia pestis*
- *Yersinia* sp.
- Zika virus

MDPH, its authorized agents, and local boards of health have the authority to collect pertinent information as part of epidemiological investigations. M.G.L. c. 111, s. 7.).

105 CMR 300.000 Reportable Diseases, Surveillance, and Isolation and Quarantine Requirements. Effective January 2017

Disease investigation

- Individual cases of illness investigated by local board of health nurses in conjunction with epidemiologists at DPH
- In general, case investigation includes:
  - Collection of clinical information
  - Evaluation of risk and exposures during incubation period
  - Education to individual on infection and prevention
  - Control of spread of illness through recommendations to close contacts and possible restriction from work
    - 105 CMR 300.000 outlines restrictions on foodhandlers diagnosed with a reportable infection on criteria that needs to be met to return to work

Classifying cases

- Surveillance case definitions are used to uniformly define diseases for public health surveillance
  - Allow for public health officials to classify and count cases consistently across jurisdictions
  - Not intended to be used by healthcare providers for making a clinical diagnosis or treatment decisions
- Established nationally by the Council of State and Territorial Epidemiologists (CSTE)

Case definition example: Vibriosis

Clinical Description

- An infection of variable severity characterized by watery diarrhea, primary septicemia, or wound infection. Asymptomatic infections may occur, and the organism may cause extra-intestinal infection.

Laboratory Criteria for Diagnosis

- Isolation of a species of the family *Vibrionaceae* (other than toxigenic *Vibrio cholerae* O1 or O139, which are reportable as cholera) from a clinical specimen.

Case Classification

Probable

- A clinically compatible case that is epidemiologically linked to a confirmed case.

Confirmed

- A case that meets the laboratory criteria for diagnosis.

Estimating true burden of infection

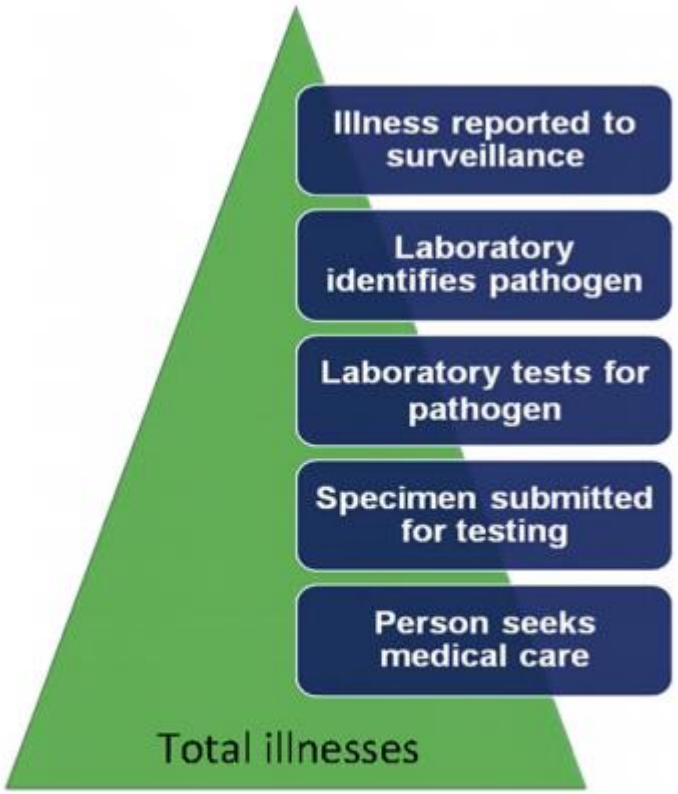


Figure 1. Surveillance steps that must occur for a laboratory-diagnosed case to be reported as part of notifiable disease surveillance.

Pathogen	Laboratory-confirmed	Multipliers	
		Under-reporting†	Under-diagnosis‡
Bacteria			
Bacillus cereus, foodborne¶	85§	25.5	29.3
Brucella spp.	120**	1.1	15.2
Campylobacter spp.	43,696††	1.0	30.3
Clostridium botulinum, foodborne¶	25**	1.1	2.0
Clostridium perfringens, foodborne¶	1,295§	25.5	29.3
STEC O157	3,704††	1.0	26.1
STEC non–O157	1,579††	1.0	106.8
ETEC, foodborne¶	53§	25.5	29.3
Diarrheagenic E. coli other than STEC and ETEC	53	25.5	29.3
Listeria monocytogenes	808††	1.0	2.1
Mycobacterium bovis	195††	1.0	1.1
Salmonella spp., nontyphoidal‡‡	41,930††	1.0	29.3
S. enterica serotype Typhi	433††	1.0	13.3
Shigella spp.	14,864††	1.0	33.3

National laboratory surveillance systems
PulseNet

- Is a national laboratory network that connects foodborne illness cases to detect outbreaks
- PulseNet labs perform PFGE and/or WGS to identify a DNA fingerprint for bacterial isolates which are then uploaded into a database and reviewed for more-than-expected numbers of matching DNA fingerprints suggestive of a cluster.
- Developed as a result of a large *E. coli* outbreak in 1993
- Plays a vital role in surveillance and the investigation of foodborne outbreaks
- Is instrumental in the identification and resolution of outbreaks where the cases are geographically dispersed
- Allows for outbreaks to be identified in hours rather than days or even weeks

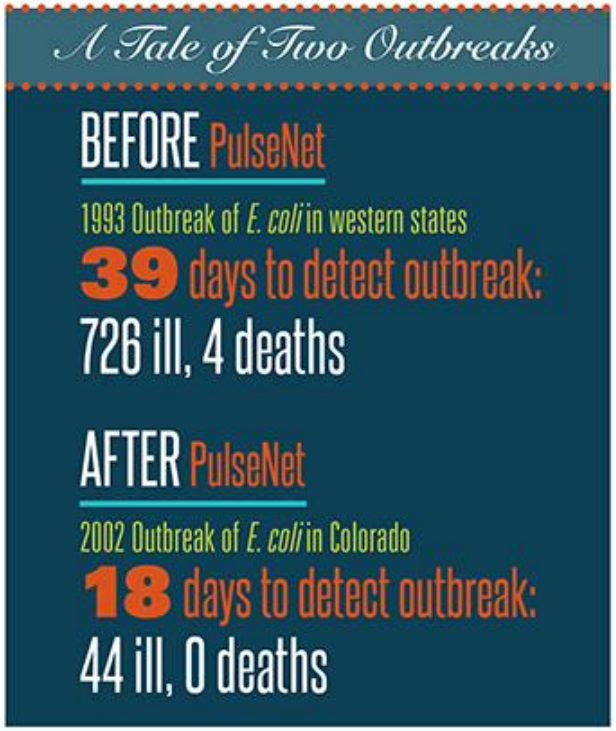
PulseNet USA
The National Molecular Subtyping Network for Foodborne Disease Surveillance
CDC

National laboratory surveillance systems

PulseNet

PulseNet by the numbers:

- **1 billion** pounds of contaminated food recalled since PulseNet was launched
- **$507 million** saved each year (medical costs and lost productivity) with quick outbreak detection
- **1 million** DNA fingerprints of foodborne bacteria in the PulseNet USA database
- **89,000** DNA fingerprints of bacteria submitted to PulseNet in 2015 – a record number
- **1,500** clusters of illness from *Salmonella*, *E. coli*, and *Listeria* infections annually identified by PulseNet member labs
- **280** multistate clusters of illness caused by *Salmonella*, *E. coli*, and *Listeria* infections identified by PulseNet each year
- **83** federal, regional, state, and local laboratories in the PulseNet network
- **40** clusters of human illnesses tracked weekly

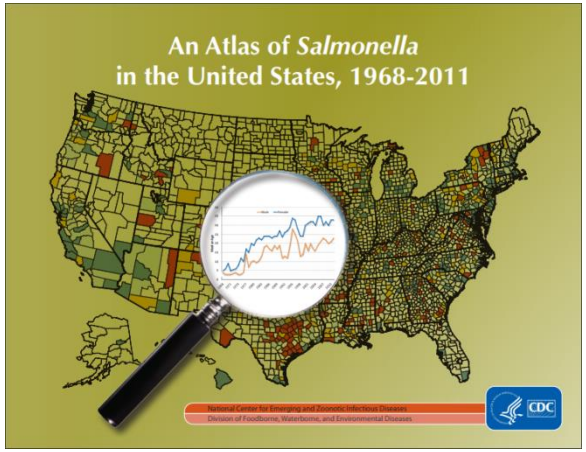


National laboratory surveillance systems
NARMS

- Monitors antimicrobial resistance among enteric bacteria from humans, retail meats, and food animals
  - Detects emerging trends of resistance;
  - Links enteric illnesses (resistant and susceptible) to specific sources and risk factors;
  - Understands the genetic mechanisms that confer resistance and its spread among enteric bacteria;
  - Educates consumers about foodborne antimicrobial resistance threats
  - Provides information and recommendations that promote the judicious use of antimicrobials
  - Guides public health priorities

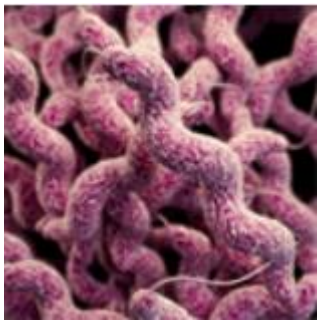
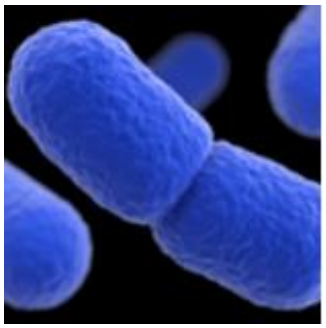
National laboratory surveillance systems

- In 2013, CDC published an atlas summarizing 42 years of lab-confirmed surveillance data on *Salmonella* isolates
  - Includes the top 30 *Salmonella* serotypes
- Clinical isolates are analyzed by age, sex, geography, and season
- Includes animal breakdown of isolates from animals and related sources (e.g., environment and feeds)



National laboratory surveillance systems

Bottom line:
Isolate driven

THE BENEFITS OF CIDT

PROs: CIDT in the clinical setting

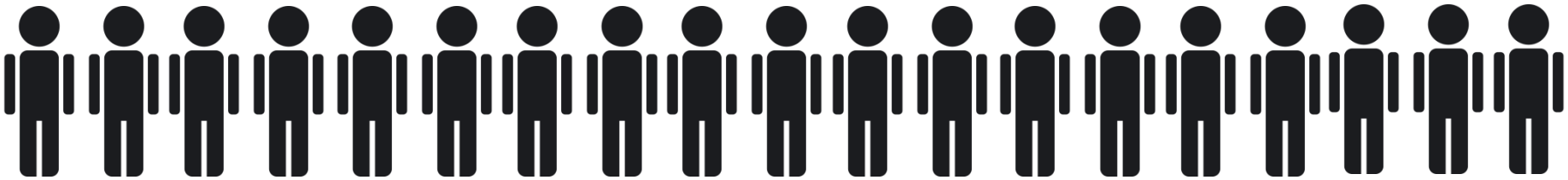
Focus: health and treatment of individual patients

- Test results obtained more rapidly
- May require less technical expertise to perform
- Overall reduces cost
- Detect a wider range of pathogens
- Potential for easier collection of specimen
- Better sensitivity, especially when antimicrobial drugs have been administered before specimen collection
- Detection of multiple infections via multiplex panels

PROs: CIDT in public health

Focus: health of the population

- Improve estimates of disease burden
  - May be more sensitive than culture
  - Ease of use may increase number of patients tested
  - Enables detection of organisms for which there is no practical test
  - Increase ability to detect polymicrobial infections



IMPLICATIONS FOR PUBLIC HEALTH

Case investigation and disease control

- Increase number of cases ascertained
- Culture-independent methods with low specificity may produce false-positive results
  - Could result in unnecessary case investigation, follow-up testing, and restriction from work or childcare
- Creates need for new public health testing algorithms for safe return to work or childcare
- Requires updated case definitions that incorporates culture-independent methods

Assessment of disease burden and trends

- Artefactual increases or decreases in reports due to variations in test performance or the use of new tests for different clinical indications
- Need to reassess multipliers used to estimate total illnesses
- Loss of strain differentiation
- Need to modify surveillance case definitions

Case definition example: Vibriosis

Clinical Description

- An infection of variable severity characterized by watery diarrhea, primary septicemia, or wound infection. Asymptomatic infections may occur, and the organism may cause extra-intestinal infection.

Laboratory Criteria for Diagnosis

- Supportive laboratory evidence: Detection of a species of the family *Vibrionaceae* (other than toxigenic *Vibrio cholerae* O1 or O139) from a clinical specimen using a CIDT.
- Confirmatory laboratory evidence: Isolation of a species of the family *Vibrionaceae* (other than toxigenic *Vibrio cholerae* O1 or O139, which are reportable as cholera) from a clinical specimen.

Case Classification

Probable

- A case that meets the supportive laboratory criteria for diagnosis, or
- A clinically compatible case that is epidemiologically linked to a case that meets the supportive or confirmatory lab criteria for diagnosis.

Confirmed

- A case that meets the laboratory criteria for diagnosis.

Outbreak detection

- Loss of ability to utilize Pulsed-field Gel Electrophoresis (PFGE) and Whole Genome Sequencing (WGS) for cluster detection
- Low specificity of CIDT may result in inappropriate outbreak response

Microbiologic characterization of reported infection

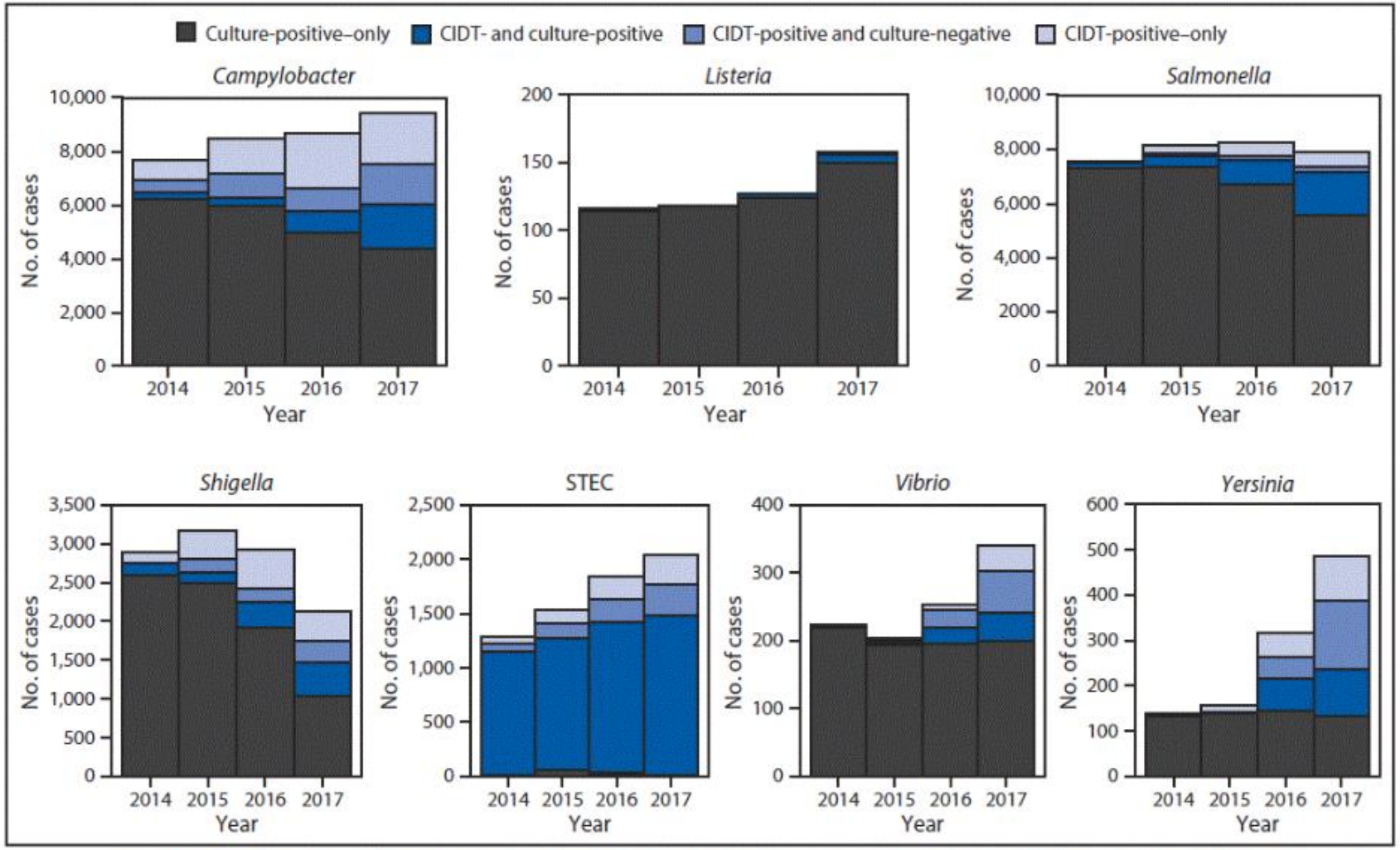
- Loss of ability to monitor antibiotic susceptibility at the population level
- Loss of subtyping information that can help attribute an outbreak to a food item

TRENDS

National trends

- The number of CIDT positive-only infections has been increasing markedly since 2013 as more clinical labs adopt CIDT
- Increase in STEC is driven by the increase in STEC non-O157 which is not typically included in routine stool culture testing

FIGURE. Number of infections diagnosed by culture or culture-independent diagnostic tests, by pathogen, year, and culture status — FoodNet sites,* 2014–2017[†,§]



Abbreviations: CIDT = culture-independent diagnostic test; FoodNet = CDC's Foodborne Diseases Active Surveillance Network; STEC = Shiga toxin–producing *Escherichia coli*.

* Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and selected counties in California, Colorado, and New York.

CASE STUDY #1

Report to public health

- On November 10th, a local health department received a report of non-bloody diarrheal illness in a 6 month old infant who attended a daycare center.
- Diarrhea was reported in four additional daycare center attendees and three family members of the index patient.
- A stool specimen of the index patient tested positive for Stx by EIA at a clinical diagnostic laboratory.

Control measures

- Index case was excluded from the daycare pending receipt of two STEC-negative stool cultures, collected at least 24 hours apart.
- Same control measures enforced for other four ill children.

Laboratory testing

- Enrichment broth sent from clinical microbiology lab to state laboratory
  - Neither STEC O157 nor non-O157 were isolated
- Enrichment broth sent to CDC
  - Again tested positive for Stx by EIA
  - Negative by PCR for stx1 and stx2
- The state lab performed additional testing on stool specimens from five ill individuals, including the index case.
  - All were positive for norovirus by RT-PCR.

Discussion

- Illustrates the importance of rapid culturing of all Stx-positive broths and specimens.
- Public health action was based on the initial, preliminary information available.
- Resulted in unnecessary burden on children, daycare center staff, and public health officials.

Case study from: Centers for Disease Control and Prevention (CDC). Importance of culture confirmation of shiga toxin-producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis—New York and North Carolina, 2005. MMWR. Morbidity and mortality weekly report. 2006;55(38):1042.

CASE STUDY #2

Report to public health

- On April 6th, a local board of health noticed an increase in the number of *E. coli* O157:H7 cases in their jurisdiction
- Case interview suggested that a restaurant and food truck operation was the common link among cases.
- On April 12th, all operations by the restaurant were closed.

Outbreak investigation

- A total of 12 confirmed cases of *E. coli* O157:H7 were ultimately identified with indistinguishable PFGE patterns
  - Additional 4 probable cases with no laboratory testing
- Cases reported dining at the establishment March 26-30th, and subsequently becoming ill March 30-April 4th.
- Multiple inspections took place at the facilities
- Food samples were collected on April 11th.
- Stool specimens were collected from 97 food handlers, who were restricted from work until submitting two STEC-negative stool specimens produced at least 24 hours apart.

Laboratory testing

- Employee stool specimens were tested for Shiga toxin and *E. coli* O157:H7 at the state public health lab using the Luminex Gastrointestinal Pathogen Panel, the Meridian STEC EIA, and culture.
- Eight stool specimens (collected April 12-14th) enriched in Mac broth were positive by EIA for shiga toxin.
  - All were negative by EIA performed on stool, GPP, and culture.

Discussion

- In terms of the outbreak, what does this mean?
- Considerations:
  - All foodhandlers denied illness
  - Foodhandler testing took place 2.5 weeks after first ill patron

CASE STUDY #3

Report to public health

- On September 13th, notified by PFGE Lab of a single genetic match to a multi-state cluster of *S*. I 4, [5],12:b-
- Case interviews nationally signaled Asian ethnicity and Asian food consumption

Outbreak investigation

- On September 13th, Epi interviews 1st case with CDC questionnaire
- On November 13th, notified by CDC of a single genetic match to *S*. Newport identified in a coconut sample from a Restaurant A location in NY.
- On November 13th, LBOH notified of 2nd case
- On December 13th, DPH Food Protection Program (FPP) and LBOH inspected and sampled local Restaurant A

Laboratory testing

- On December 26th, one sample of frozen, shredded coconut from Rest. A, was positive for *Salmonella* Hillingdon (not OB strain nor what was found in NY) and not seen previously in PulseNet!

- On January 2nd, subsequent sampling of 10 bags of frozen shredded coconut collected

- On January 10th, 9/10 samples positive for multiple serotypes of *Salmonella* including the original OB strain!

Discussion

- On January 3rd, Voluntary Recall initiated by Importer due to positive findings in an unopened bag
- This outbreak wouldn't have been solved if only CIDT was used
- Prevented at least 40 additional cases in MA from this one establishment

WHAT TO DO?

Short-term

- Encourage clinical labs to continue culturing specimens with positive CIDTs
- Consider ways to make follow-up cultures easier and cheaper for clinical laboratories
- Encourage companies that make CIDTs to design the tests in a way that keeps the bacteria alive so they can be cultured if test is positive
- Modify surveillance systems to include infections diagnosed only by CIDT

Long-term

- Develop advanced testing methods that will not require bacterial isolates to provide needed information to public health officials

THANK YOU

- APHL Position Statement: Use of Non-Culture Assays to Detect Foodborne Infectious Agents. February 2012.
- APHL Position Statement: Establishing Legal Requirements for the Submission of Enteric Disease Isolates and/or Clinical Material to Public Health Laboratories. February 2015.
- CDC. Importance of culture confirmation of shiga toxin-producing Escherichia coli infection as illustrated by outbreaks of gastroenteritis--New York and North Carolina, 2005. MMWR Morb Mortal Wkly Rep. 2006 Sep 29;55(38):1042-5
- Conquist et al. Impacts of culture-independent diagnostic practices on public health surveillance for bacterial enteric pathogens. Clin Infect Dis. 2012 Jun;54 Suppl 5:S432-9
- Imdad et al. Impact of Culture-Independent Diagnostic Testing on Recovery of Enteric Bacterial Infections. Clin Infect Dis. 2017 Dec 26.
- Langley et al. Effect of Culture-Independent Diagnostic Tests on Future Emerging Infections Program Surveillance. Emerg Infect Dis. 2015 Sep; 21(9): 1582–1588.
- Marder et al. Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food and the Effect of Increasing Use of Culture-Independent Diagnostic Tests on Surveillance — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2013–2016. MMWR Morb Mortal Wkly Rep 2017;66:397–403..