# PULSENET ON THE FRONT LINES OF FOODBORNE DISEASE SURVEILLANCE

## National Molecular Subtyping Network for Foodborne Pathogens

**Illnesses caused by foodborne pathogens continue to be a major burden to the health of Americans. New Centers for Disease Control and Prevention (CDC) estimates released in 2011 highlight 31 major pathogens causing nearly 9.4 million foodborne illnesses and contributing to more than 1,300 deaths in the United States annually.**

*Salmonella* ranks among the top four pathogens associated with foodborne illness, causing an estimated 1.0 million illnesses and a leading cause of hospitalizations per year.[1] Moreover, estimates by Scharff suggest the economic burden due to these illnesses is enormous, ranging from $51.0 billion to $77.7 billion annually.[2]

Public health departments across the country continue to heavily invest time and resources in investigating multistate outbreaks. In a typical year, over 1,500 local disease clusters and 250 national or multistate clusters are investigated. In 2010, multistate outbreak investigations resulted in the identification of diverse products contaminated with disease-causing microorganisms, such as shell eggs (500 million recalled), alfalfa sprouts, cheese, frozen entrees, frozen imported mamey fruit, shredded lettuce, beef, and red and black pepper on salami products. In addition, other hazards were identified as important causes of human illness, such as contact with pet African water frogs and reptiles fed with contaminated frozen rodents.[3] Due to the nature of these diffuse outbreaks and wide distribution of cases within the United States, it is likely that many of these outbreaks would have gone undetected using traditional epidemiological and laboratory tools. In these instances, a real-time laboratory surveillance network, PulseNet, was critical in the early detection of the clusters which resulted in the recall of contaminated products from the marketplace. More importantly, by detecting outbreaks and finding the gaps in our food safety systems, the food industry, regulators, and the public get critical information needed to improve the safety of our food supply.

PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance is a national early warning detection system for outbreaks caused by pathogens such as Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella*, *Listeria monocytogenes*, *Shigella* and *Vibrio spp*. Established in 1996 by the CDC, four public health laboratories, the US Department of Agriculture (USDA), and Association of Public Health Laboratories (APHL), PulseNet has since grown to over 85 laboratories consisting of local and state public health laboratories, state agricultural laboratories, and US Food and Drug Administration (FDA) and USDA food regulatory laboratories. PulseNet uses DNA fingerprinting to track and detect clusters of foodborne pathogens. Pulsed-field Gel Electrophoresis (PFGE) is the standardized subtyping method used by all participating PulseNet laboratories.  The power of the network is in its ability for the participants to share and compare patterns and to communicate findings in real-time. In 2010, the PulseNet network detected over 1,800 clusters of foodborne pathogens.

The Association of Public Health Laboratories (APHL) is a member based non-profit organization which represents local, state, agricultural, and environmental laboratories. APHL works to provide its membership and the public health laboratory community with information, resources and training to provide a solid public health infrastructure that works to prevent and control diseases domestically and abroad. In 2011, APHL administered a survey to assess the capability and capacity of the PulseNet network during the 2010 calendar year and to determine changes in laboratory practices since the last PulseNet network assessment in 2008. This *Issue Brief* presents the survey findings.

## METHODS

In May 2011, APHL conducted a survey to assess member laboratories' capacities and capabilities for conducting molecular subtyping (PFGE) through the PulseNet network for the 2010 calendar year. The survey was sent to 68 public health, agriculture, chemistry and veterinary laboratories-which included 54 state and territorial public health laboratories, 9 local public health laboratories, 4 state agricultural or veterinary laboratories and one state chemistry laboratory. Of those surveyed, APHL received 60 responses—comprised of 47 state and territorial public health laboratories, 9 local public health laboratories, 3 state agricultural or veterinary laboratories and one state chemistry laboratory. A similar survey was conducted in 2009 to assess activities for the 2008 calendar year. Some comparisons are included in this report for questions that were largely unchanged between 2008 and 2010. The survey was administered through SPSS mrInterview (version 4.5), a web-based repository and survey tool. Descriptive analyses were conducted, and responses were grouped into three main categories: surveillance, cluster detection and laboratory workflow.
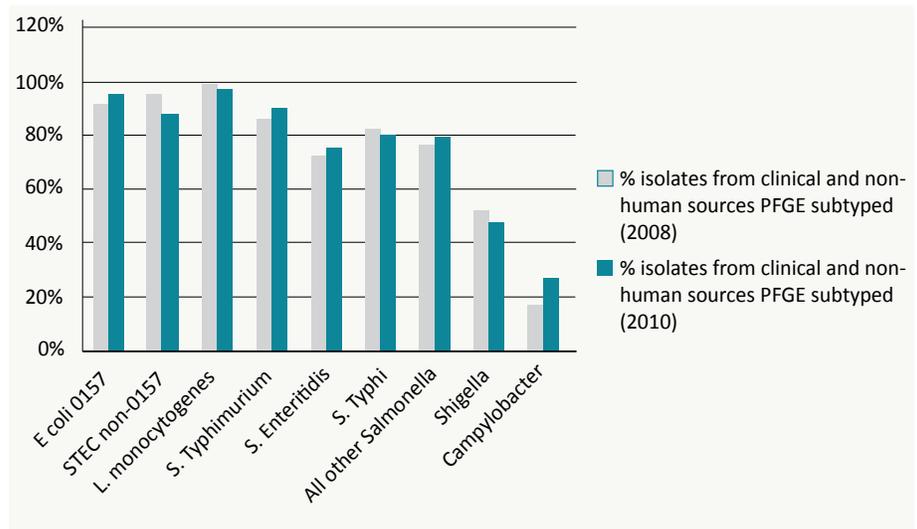
## PULSENET SURVEILLANCE

Overall, PulseNet laboratories are maintaining their capabilities and capacity to perform PFGE testing within the network. During 2010, a total of 51,785 clinical and non-clinical isolates (Shiga toxin-producing *E. coli* (STEC), *L. monocytogenes*, *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio spp*.) were PFGE subtyped by PulseNet laboratories, which is an increase of 7% since 2008. Overall, the number of laboratories providing PFGE subtyping remained relatively constant since 2008 with the exception of *Campylobacter*, which increased from 24 laboratories in 2008 to 33 laboratories in 2010. Collectively, over 13,300 PFGE gels were processed by member laboratories in 2010.

Figure 1. Comparison of Isolates from Clinical and Non-human Sources PFGE Subtyped in 2008 and 2010, by Pathogen



Legend:
- % isolates from clinical and non-human sources PFGE subtyped (2008)
- % isolates from clinical and non-human sources PFGE subtyped (2010)

Secondary enzyme testing increases the discriminatory power of the subtyping method and can be useful during an outbreak investigation. In 2010, secondary enzyme testing in public health laboratories varied markedly among pathogens. Among STEC and *L. monocytogenes,* over 87% of clinical isolates received into the public health laboratories were routinely subtyped with a secondary enzyme; whereas, only 14% of *Salmonella* isolates were routinely subtyped with a secondary enzyme. Moreover, approximately 66% of *Salmonella* isolates were only subtyped with a secondary enzyme when a cluster or multistate outbreak had been detected and 79% when second enzyme testing was requested by epidemiologists.

Although the majority of laboratories have the capability to perform secondary enzyme testing, such additional routine testing on large volume of isolates can strain already scarce resources. However, requesting secondary enzyme testing during an outbreak investigation may delay the response time of an investigation and subsequent recall of a product from the market. Only 10 laboratories (17%) reported being able to respond immediately to requests for second enzyme testing. Twenty-three laboratories (38%) reported responding to secondary enzyme requests within 5 days.

Figure 2. Number of Clinical Isolates PFGE Subtyped by Pathogen in 2008 and 2010



During 2010, public health departments reported a total of 85,438 clinical cases of Shiga toxin-producing *E. coli* (STEC), *L. monocytogenes*, *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio spp*. Of reported cases, public health laboratories received an estimated 68,663 (80%) clinical isolates for laboratory testing. Of these, 50,226 (73%) of isolates were PFGE subtyped. Among specific pathogens, most *L. monocytogenes* (97%) isolates which were received in the public health laboratories were PFGE subtyped followed by O157 STEC (95%) and non-0157 STEC (88%), and all *Salmonella* spp. (79%). Lower percentages were found among *Vibrio cholerae* (55%), *Shigella* (48%), and *Campylobacter* (27%) isolates. The number of laboratories which received isolates from non-human sources (i.e., food) for PFGE subtyping remained relatively constant, increasing from 38 laboratories (67%) in 2008 to 41 laboratories (68%) in 2010. However, PFGE subtyping of non-clinical isolates increased overall since 2008. In total, 1,559 non-human isolates were PFGE subtyped in 2010 compared to 780 isolates in 2008. The number of non-human isolates varied greatly among pathogen. PFGE testing of non-human *L. monocytogenes* isolates increased from 146 isolates in 2008 to 259 isolates in 2010. Testing of non-human *Salmonella* isolates increased from 498 in 2008 to 1,089 in 2010. Likewise, PFGE subtyping of *Campylobacter* isolates increased from 12 isolates in 2008 to 86 isolates in 2010. Increases in testing were seen among non-O157 STEC from 5 isolates in 2008 to 69 isolates in 2010. Conversely, PFGE testing of non-human isolates for O157:H7 STEC decreased almost by half, from 100 isolates in 2008 to 51 isolates in 2010.
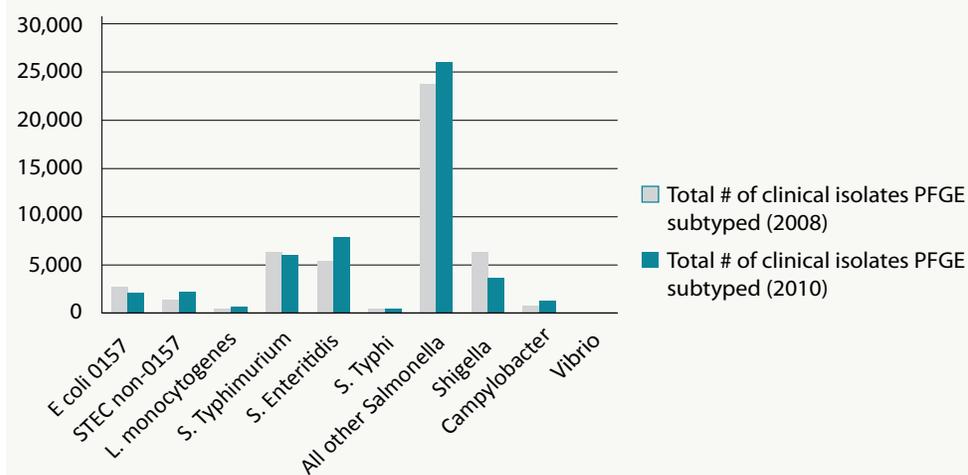
Although PFGE has utility in detecting clusters of foodborne pathogens, newer methods, such as Multiple-locus Variable-number tandem repeat Analysis (MLVA), have proven to be useful in confirming clusters for *E. coli* O157 and *Salmonella*.[4-5] Laboratories within the PulseNet network assist with evaluation and implementation of new molecular subtyping tools, such as MLVA. Twelve of the 59 laboratories (20%) reported performing subtyping methods other than PFGE. Eight laboratories (13%) performed MLVA testing and the remaining 4 laboratories performed a third method MLST (Multiple-Locus Sequence Testing) or other methods. This was an increase from 2008, in which 12% of laboratories reported performing subtyping methods other than PFGE on isolates. With the ability of more local and state laboratories performing other subtyping methods, such as MLVA, laboratories have the capability to respond more rapidly to foodborne outbreaks.

A total of 30 laboratories reported performing in-house characterization tests other than PFGE or MLVA, e.g., toxin/virulence gene PCR and molecular serotyping. Of these, 27 laboratories reported toxin/virulence PCR testing, 8 laboratories provided susceptibility testing, 5 laboratories provided molecular serotyping and 3 laboratories provided other PCR testing on isolates. Toxin/virulence PCR tests were commonly performed on STEC isolates. Twenty-six of 30 laboratories (87%) performed in-house toxin/virulence PCR tests on non-O157 STEC whereas, 23 laboratories (77%) performed toxin/virulence PCR testing on O157 STEC isolates.

## PFGE CLUSTER DETECTION AND EPIDEMIOLOGIC FOLLOW-UP

In 2010, a total of 1,842 local and state foodborne illness clusters were detected by PFGE subtyping. This is an increase of 327 clusters (or 22%) since 2008, when 1,515 clusters were detected. Overall, approximately 76% of PFGE clusters detected in 2010 were followed up with an epidemiological investigation.

The majority of these clusters, 1,442 (78%), were attributed to *Salmonella* and of these, 75% were followed up by epidemiologists. Ninety-one percent (n=174) of all STEC clusters were followed up by epidemiologists, compared to 80% (n=54) of clusters for *L. monocytogenes*.

## TURNAROUND TIMES AND WORKFLOW PRACTICES

There are many steps in the continuum for identifying a case of foodborne illness. Ill persons must seek care, a specimen must be collected, clinical laboratories must culture the specimen and isolate a pathogen, and transport isolated organisms to public health laboratories for further testing and characterization. Ideally, clinical laboratories should transport all clinical reportable isolates/specimens related to foodborne illness as rapidly as possible to public health laboratories. In doing so, these isolates can be further characterized and subtyped to facilitate real-time national surveillance and early detection of outbreak clusters. However, in practice and in times of limited resources, clinical laboratories are not always able to carry out this practice in real-time. Our survey asked member laboratories how often clinical laboratories batched isolates for transport to the public health laboratories. Our results showed that clinical laboratories which batched >50% of isolates was highest for *Salmonella* (27%). In contrast, 3 laboratories (5%) reported that >50% of the STEC isolates from clinical laboratories were transported in batches. Likewise, 4 laboratories (7%) reported >50% of *L. monocytogenes* isolates being transported in batches.

Measuring turnaround times in the laboratory is an important metric to determine the efficiency of the PulseNet system to identify foodborne disease clusters. Turnaround time is defined as the time (in working days) from the receipt of an isolate in the PulseNet laboratory to the time the isolate's fingerprint image is uploaded to the national PulseNet database. The median turnaround times for PFGE subtyping and uploading to the national databases decreased by one day, from 4 days in 2008 to 3 days in 2010 for *E. coli* O157 STEC and *Listeria monocytogenes*. *Salmonella* also

had a decrease by one day from 5 days in 2008 to 4 days in 2010. Decreases in turnaround times indicate that laboratories subtype pathogens and upload the data to the national databases more rapidly. Turnaround times for the other pathogens remained relatively the same for non-O157 STEC (4 days) and *Shigella* (5 days). *Campylobacter* saw a slight decrease in turnaround times, from 5 days to 4.5. As noted above, these improvements in laboratory testing were achieved during a time of decreasing budgets and severe fiscal cuts to public health laboratory departments.

Factors that can affect timely PFGE subtyping of isolates include simultaneous testing such as performing confirmatory, identification and PFGE testing at the same time. In 2010, more laboratories reported performing simultaneous PFGE subtype testing and confirmatory testing on O157:H7 STEC, non-O157 STEC, *L. monocytogenes*, *Salmonella* and *Shigella* isolates than in 2008. Additionally, performing PFGE testing in real-time can also significantly decrease turnaround times. When reviewing the percentage of laboratories who reported performing PFGE in real-time on ≥ 75% of their isolates, 80% of laboratories reported real-time subtyping for O157 STEC, 79% for *L. monocytogenes*, and between 53-60% for *Salmonella* (depending on the serotype). Other contributing factors accounting for decreases in turnaround time may be that 68% of laboratories reported that the PFGE and bacteriology laboratories were under the same laboratory division. This is significant given that some laboratories isolate a pathogen in one laboratory and transport isolates to another laboratory for PFGE subtyping which can result in delayed testing.
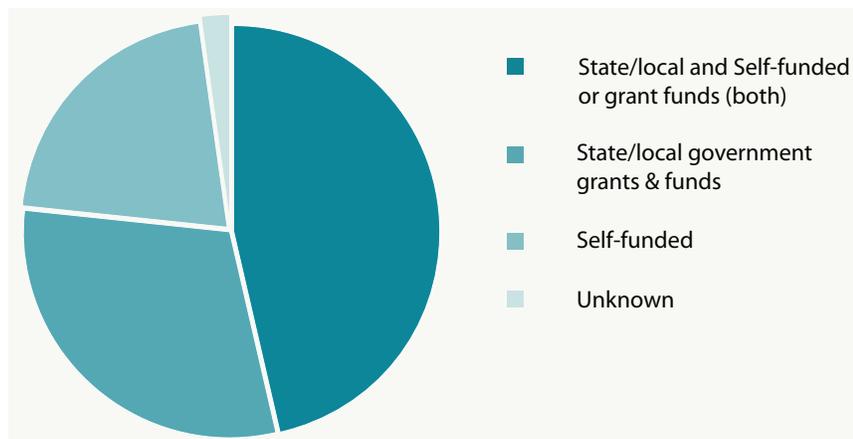
Table 1. Median PFGE Turnaround Times of PulseNet Pathogens from Clinical Sources in 2008 and 2010

|  | Median PFGE turnaround time of clinical isolates in 2008 (in working days) | Median PFGE turnaround time of clinical isolates in 2010 (in working days) |
|---|---|---|
| O157 STEC | 4 | 3 |
| Non-O157 STEC | 4 | 4 |
| *Listeria monocytogenes* | 4 | 3 |
| *Salmonella spp.* | 5 | 4 |
| *Shigella* | 5 | 5 |
| *Campylobacter* | 5 | 4.5 |
| *Vibrio* | N/A | 6 |

## SPECIMEN TRANSPORT AND FUNDING FOR SHIPPING

PulseNet surveillance relies on clinical laboratories to submit specimens and isolates to public health laboratories. Courier services are one method to provide transport of specimens between laboratories. The utilization of various courier services (FedEx, US Postal Service, privately-operated and government-operated) remained relatively the same between 2008 and 2010 with the exception of a 21% increase in the use of government-operated couriers. In 2010, more than 75% of clinical laboratories used either government funds/grants or a combination of self-funded and government funds to pay for these services. Laboratories using a combination of both self-funded and government funds increased from 38% in 2008 to 46% in 2010.

Figure 3. Distribution of Funding Sources for Courier Services in 2010



- State/local and Self-funded or grant funds (both)
- State/local government grants & funds
- Self-funded
- Unknown

## GAPS IN THE SYSTEM

- Ability for public health laboratories to obtain isolates from all clinical cases of foodborne illnesses and obtain them in real-time.

- Simultaneous serotyping (and other confirmatory testing) and PFGE subtyping of all isolates under PulseNet surveillance that are received in the public health laboratories.

- Resources for PulseNet laboratories to perform testing on all PulseNet pathogens in real-time.

- Resources for public health laboratories to expand molecular subtyping to next generation methods, e.g., MLVA.

- Resources for epidemiologists at the local and state levels to investigate all PulseNet detected clusters and interview all cases of foodborne illness.

## THREATS TO THE SYSTEM

- Maintaining funding for transport of clinical isolates to the public health laboratories for isolate confirmation and characterization, such as PFGE subtyping.

- Maintaining funding for local and state laboratories to support next generation subtyping methods, to augment PFGE subtyping for surveillance and outbreak investigations.

- Loss of basic federal funding, such as the Epidemiology Laboratory Capacity (ELC) cooperative agreements that supports foodborne illness detection and surveillance, including PFGE subtyping, in state and local laboratories.

## HOW APHL IS SUPPORTING PULSENET

- Providing assistance to laboratorians to attend CDC sponsored wet-bench and software training workshops.

- Awarding small grants to laboratories for validating and implementing MLVA protocols at state/local laboratories.

- Enhancing and facilitating communication between member laboratories, federal partners and other stakeholders.

- Organizing regional and national PulseNet meetings for public health and agricultural laboratorians, epidemiologists and environmental health sanitarians.

- Organizing regional workshops for laboratorians to gain information on guidelines for foodborne outbreak and response through Council to Improve Foodborne Outbreak and Response (CIFOR) materials.

## CONCLUSION

PulseNet laboratories at the local and state levels continue to participate in this critical network for the early detection of foodborne disease clusters. The 2010 APHL survey demonstrates that public health and agricultural laboratories have steadily increased the number of isolates subjected to PFGE subtyping, identified more clusters, and decreased the turnaround time of PFGE subtyping for some PulseNet pathogens. However, several areas still need to be addressed. It is critical that the public health laboratory system has the ability to obtain isolates from *all* reported cases, subtype *all* isolates in real-time, and maintain federal and state funding to support local and state laboratories. In order to maintain submission of isolates to the public health laboratory system, the process needs to be easy and economical for both clinical laboratory partners and the public health departments. Also, batching isolates for submission to public health laboratories can cause significant delays in identifying foodborne outbreaks. It should also be noted that loss of government funding to support courier services for the transportation of isolates threatens this successful public health program.

Investments in new subtyping methodologies in public health laboratories are critical as technology for isolate characterization is becoming more rapid and better at discriminating isolates which could even shorten the time in detecting clusters for outbreak investigations. It is hoped that these challenges and gaps will be addressed by certain provisions of the Food Safety Modernization Act which passed into law in January 2011. Language in the Act indicates that Congress is aware of the importance of rapidly receiving clinical isolates in public health laboratories for inclusion in the PulseNet database. However, the law did not contain any funding provisions to pay for new initiatives. Funding through this new legislation towards promoting and supporting national surveillance programs such as PulseNet is critical.

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## Association of Public Health Laboratories

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The Association of Public Health Laboratories (APHL) is a national non-profit organization dedicated to working with members to strengthen governmental laboratories that perform testing of public health significance. By promoting effective programs and public policy, APHL strives to provide member laboratories with the resources and infrastructure needed to protect the health of US residents and to prevent and control disease globally.

### Funders

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