The Role, Challenges, and Support of PulseNet Laboratories in Detecting Foodborne Disease Outbreaks

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SYNOPSIS

In recent years, there have been several high-profile nationwide foodborne outbreaks due to enteric organisms in food products, including *Salmonella* Typhimurium in peanut products, *Salmonella* Saintpaul in peppers, and *Escherichia coli* O157:H7 in spinach. PulseNet, the national molecular subtyping network for foodborne disease surveillance, played a key role in detecting each of these outbreaks.

PulseNet laboratories use bacterial subtyping methods to rapidly detect clusters of foodborne disease, which are often the first indication that an outbreak is occurring. Rapid outbreak detection reduces ongoing transmission through product recalls, restaurant closures, and other mechanisms. By greatly increasing the sensitivity of outbreak detection, PulseNet allows us to identify and correct problems with our food production and distribution systems that would not otherwise have come to our attention. Annually, millions of potentially preventable cases of foodborne illness result in billions of dollars in lost productivity and health-care expenses. We describe the critical role PulseNet laboratories play in the detection of foodborne outbreaks and discuss current challenges and potential improvements for PulseNet laboratories to more rapidly identify future foodborne outbreaks.

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PulseNet is a network of local, state, and national public health and regulatory agency laboratories that use pulsed-field gel electrophoresis (PFGE) with standardized protocols for molecular subtyping of case isolates to identify clusters of illness. PulseNet comprises public health laboratories (PHLs) from all 50 U.S. states, 17 county and city laboratories, as well as laboratories from regulatory agencies.

PulseNet was formed in 1996 and is coordinated by the Centers for Disease Control and Prevention (CDC). The Association of Public Health Laboratories (APHL) provides support for state and local laboratories that participate in PulseNet through training, technical meetings, advocacy, research grants, information dissemination, and capability assessment. Isolates of *Salmonella, Escherichia coli* (*E. coli*) O157:H7, *Shigella sonnei* (*S. sonnei*), and *Listeria monocytogenes* (*L. monocytogenes*) are submitted to PHLs from clinical laboratories and are subtyped by PulseNet laboratories. Some PulseNet laboratories also perform PFGE using PulseNet-developed protocols for *Campylobacter jejuni*, *Vibrio cholerae*, and *Yersinia pestis*; however, routine subtyping of these organisms by all PulseNet laboratories is not universal due to resource constraints.

PHL participation in PulseNet is funded partly by the states and partly by grants from the Department of Health and Human Services administered by CDC. CDC houses the national database of PFGE patterns and acts as the curator of the database. CDC also writes standard operating procedures and provides a leadership role to the states for training and monitoring of PFGE pattern quality. The CDC PulseNet Methods Development Laboratory looks for new technologies and procedures for subtyping organisms that might supplement or eventually replace PFGE.

PulseNet is used to identify groups of isolates that have the same PFGE pattern, which may indicate that the isolates have a common origin. Isolates of *Salmonella*, *E. coli* O157:H7, *S. sonnei*, and *L. monocytogenes* are sent to the PHL by clinical laboratories for confirmation, serotyping, and PFGE subtyping. PFGE is performed on isolates and the patterns are compared with the local database each PulseNet laboratory maintains. The local database contains a library of patterns from isolates previously subtyped in their jurisdiction. Clusters of isolates with matching patterns are reported to their foodborne disease epidemiologist. PFGE patterns are uploaded to the national database, which is monitored daily by CDC to identify clusters of isolates with matching PFGE patterns on a national level. Included in the national database are patterns from food isolates submitted by regulatory agencies.

PulseNet has been responsible for identifying scores of outbreaks since its inception. Because it so effectively allows investigators to focus their efforts, PulseNet represents a significant advance in epidemiology and public health.[1] This article describes the actions and relationships required by state PHLs, which are critical in detecting foodborne outbreaks, and ways in which state processes may be improved to increase the effectiveness of foodborne disease surveillance.

EXPECTATIONS OF PULSENET LABORATORIES

Standardization is crucial for producing PFGE patterns for inter-laboratory comparison; thus, it is essential that PFGE protocols are strictly followed.[2] Individuals and laboratories must demonstrate their ability to perform PFGE according to established PulseNet protocols and guidelines and must periodically show their proficiency in the established methods. Initially, new laboratorians must become certified before they can participate in PulseNet. Certification tests an individual's ability to perform PFGE on specified organisms using standard protocols, perform pattern analysis, and upload the patterns to the national database. Once an individual is certified for an organism, he or she must participate in an annual proficiency-testing program that measures the laboratorian's ability to produce high-quality PFGE gels and analyze PFGE patterns.

A PulseNet laboratory needs to follow mandatory requirements for inclusion in the system. It is required that every PulseNet laboratory has at least one PulseNet-certified individual. PulseNet laboratories must (*1*) perform PFGE on PulseNet-tracked organisms as requested by CDC or state epidemiologists, (*2*) submit all PFGE patterns and corresponding information to the PulseNet national database within 24 hours of being generated, (*3*) adhere to the protocols and requirements of the PulseNet quality assurance/quality control manual, (*4*) send at least one representative to the Annual PulseNet Update Meeting, and (*5*) store isolates that have been subtyped by PFGE for at least one year.

COMMUNICATION IN PULSENET

PulseNet was designed to facilitate the sharing of information and data with a wide range of groups. While the standardization of protocols across the nation allows data to be shared across state lines, the means by which those data are shared is essential to the vitality of the program. CDC has provided all PulseNet laboratories with the necessary equipment and protocols to perform PFGE testing. Equally important, CDC has constructed standardized methods of communication that allow

for the exchange of results and information among laboratories.

Cluster investigations conducted close to the case reporting dates are more likely to lead to the identification of a common source. Therefore, it is important for laboratorians to rapidly inform their epidemiologists of PFGE results and newly identified clusters. Some states create reports that are automatically generated and sent to the epidemiologist. These reports may highlight new clusters and calculate historical prevalence of the cluster pattern in their jurisdiction to help interpret cluster significance. Historical pattern data enable epidemiologists to interpret the significance of the cluster. Communication among the groups is typically conducted via e-mail, phone calls, or laboratory-generated reports. Continuous communication between PulseNet laboratorians and foodborne disease epidemiologists is essential to avoid any delay in the identification and investigation of clusters.

CDC Team

One important method of communication among PulseNet participants is a restricted online forum called CDC Team. CDC Team allows certified PulseNet laboratorians and foodborne disease epidemiologists nationwide to communicate information about clusters. Included in a typical posting is information about the agent, the number of isolates involved in the cluster, the suspected outbreak source, and a bundle file that contains an image of the pattern of interest. This file can be downloaded by states and used to compare the cluster pattern with their local database. CDC adds information to the posting, such as recent pattern distribution, historical frequency of the pattern, national pattern designation, and cluster designation as assigned by CDC, as well as information regarding the possible source of the cluster. PulseNet laboratories compare all posted clusters with their local databases and respond to CDC Team if they have recent pattern matches.

CDC Team is also used to communicate new standard operating procedures, troubleshooting and quality control issues, and any other information that may be important to PulseNet laboratories. PulseNet participants can receive e-mails notifying them of any new postings to CDC Team. CDC Team allows important information to be rapidly communicated to PulseNet participants in all 50 states.

Effective communication via PulseNet was demonstrated in a 2003 *E. coli* O157:H7 outbreak associated with needle-tenderized steaks.[3] In this outbreak, two isolates from two cases in a single state shared the same rare PFGE type. Food history interviews revealed that both of these cases had consumed needle-tenderized steaks purchased from a door-to-door vendor shortly before onset of illness. A search of the PulseNet *E. coli* database determined that there were two additional, recent isolates with the same pattern from two nearby states. The cluster information was added to CDC Team (at that time it was called the PulseNet Webboard) and communicated to the epidemiologists in the involved states. It was subsequently determined that all four cases had consumed needle-tenderized steaks, and testing of the suspect product identified a matching strain of *E. coli* O157:H7. Identification of this outbreak prompted a nationwide recall of 739,000 pounds of needle-tenderized steaks, which likely prevented multiple cases of human illness. The effective use of PulseNet data and communication methods is a powerful tool for rapidly identifying the source of foodborne disease outbreaks.

Area laboratories

Area laboratories can facilitate communication among states. PulseNet USA is divided into eight regions, with each region having a designated area laboratory. Area laboratories function as a liaison between the states in that region and CDC. Area laboratories can assist states in their area with troubleshooting requests and can provide PFGE surge capacity during large outbreaks or staffing shortages. Area laboratories facilitate regular regional conference calls to discuss issues regarding PFGE and foodborne disease surveillance. Area laboratories host a Regional PulseNet Conference every few years with a variety of participants involved in food safety. These conferences have been an effective method for identifying improvements to the foodborne disease surveillance system at the state, regional, and national levels.

PulseNet USA holds an annual update meeting for PulseNet laboratorians from all PulseNet laboratories. Update meetings have played a critical role in promoting collaboration among PulseNet laboratories and providing training and information critical to maintaining standardization throughout PulseNet.

Foodborne disease surveillance agencies and programs

PulseNet interacts with a number of agencies and programs involved in foodborne disease surveillance activities. Some state agriculture laboratories are PulseNet-certified and perform PFGE on *Salmonella*, *Shigella*, *E. coli* O157:H7, and *L. monocytogenes* isolates from food, while others send their food isolates to their PHL for PFGE. U.S. Department of Agriculture (USDA) and Food and Drug Administration (FDA) laboratories participate in PulseNet and subtype pathogens isolated

from food. USDA and FDA use PulseNet data during outbreaks to guide tracebacks and order recalls.

NARMS. The National Antimicrobial Resistance Monitoring System (NARMS) monitors antimicrobial resistance patterns of enteric pathogens isolated from animals, retail meats, and humans. NARMS information is linked to PFGE information in the PulseNet national database.

VetNet. VetNet is a USDA-Agricultural Research Service program that uses PFGE and antibiotic susceptibility information to characterize *Salmonella* and other organisms from animals. VetNet is a separate program from PulseNet; however, the two programs use the same PFGE protocols so that PFGE patterns can be compared between the two databases.

FoodNet. FoodNet is a program that performs active surveillance for foodborne diseases and related epidemiologic studies designed to help public health officials better understand the epidemiology of foodborne diseases in the U.S. Isolates from FoodNet studies are identified in the national database.

OutbreakNet. OutbreakNet has been developed by CDC epidemiologists to enhance communication during foodborne outbreaks and allow for rapid communication among state and local partners. PFGE data are vital information in any outbreak investigation, so a strong relationship between PulseNet and OutbreakNet is necessary.

Foodborne disease surveillance is a complex activity; therefore, it is imperative that PulseNet has the capability to work with a wide variety of networks and agencies.

CHALLENGES FOR PULSENET LABORATORIES

There have been many documented outbreaks whose detection can be attributed to the PulseNet system.[3–6] As mentioned previously, the activities of state laboratories in PulseNet have been instrumental in the early detection of foodborne disease outbreaks over the years; however, more can be done to improve food safety at the state level, and significant challenges exist for state PHLs.

Insufficient funding

Perhaps the most pressing challenge to state PHLs is insufficient funding. The true strength of PulseNet is the collective and active participation of its members. However, due to funding cuts and shrinking state budgets, some states have had to scale back their PulseNet activities. According to a 2005 APHL survey (Unpublished data, Association of Public Health Laboratories. 2005 PulseNet Survey. Silver Spring (MD): APHL; 2006), only 28% of state laboratories are performing PFGE subtyping on all *Salmonella* isolates they receive. The lack of PFGE testing on all human *Salmonella* isolates throughout the country decreases our ability to identify clusters and outbreaks of *Salmonella.*

Staffing issues

Funding shortfalls in many state laboratories have led to staffing shortages. Insufficient staff levels due to a lack of funding were cited as a major issue in 64% of state laboratories surveyed in 2007.[7] Due to budgetary constraints, many states have enacted travel freezes that have prevented participation in training opportunities. The primary source of funding for foodborne disease surveillance and PulseNet activities at the state level has been CDC's Epidemiology and Laboratory Capacity Cooperative Agreement (ELC). However, while states have been facing severe cuts in ELC funding,[7] the cost of laboratory testing and foodborne disease surveillance activities continues to increase. Funding issues also affect the ability of PulseNet laboratories to perform timely subtyping. Some states wait several days for a number of isolates to accumulate before performing PFGE because larger batches are more cost-efficient per sample. However, batching increases the turnaround time to subtype the isolates and delays identification of potential outbreaks.

PulseNet laboratorians frequently are not solely dedicated to PulseNet and must perform other functions in their laboratory. Emergency situations or shifting priorities within the PHL can adversely affect a state's ability to perform PFGE. This problem was experienced during the 2001 *Bacillus anthracis* attacks and during the emergence of the West Nile virus in the U.S. in 2002. During these situations, in some states PulseNet laboratorians were needed to perform other functions in the laboratory, so PFGE was either delayed or not performed. Funding initiatives to address this need will be paramount to continued effective foodborne surveillance activities of state laboratories.

Staff turnover at the state laboratories is another significant challenge to effective foodborne surveillance activities. State laboratories are losing valuable expertise through attrition and reallocation of laboratory staff as hiring freezes become commonplace at the state level. Most of the laboratory testing methodologies employed in foodborne outbreak surveillance activities, such as PFGE and serotyping, are considered high-complexity testing, and state laboratory personnel trained in these areas are not readily replaced.

Isolate submission by clinical laboratories

Submission of isolates by clinical laboratories to their PHLs presents another current challenge for state PulseNet laboratory foodborne disease surveillance activities. State laboratories rely on the receipt of bacterial isolates or clinical samples from clinical laboratories to monitor for clusters of illness. As of 2007,[7] 43% of state PHLs did not have state-mandated laws within their states requiring nongovernment laboratories to submit isolate and/or food samples to them. If information and data from these foodborne pathogens are not gathered, the sensitivity of cluster detection is reduced. Outbreak recognition may either be delayed or missed entirely, thus leading to illnesses that otherwise could have been prevented.

Communication

Communication between PulseNet laboratories and their foodborne disease epidemiologists is another challenge for effective foodborne disease surveillance. Some states have an automated, standardized, methodical system for sending PFGE results from their PFGE laboratory to their foodborne disease epidemiologist. Included in the automated PFGE report is information regarding recent PFGE clusters and outbreaks. Other states have no standardized PFGE results reporting mechanism. In addition, some states have a longstanding, close relationship between the PulseNet laboratory and their foodborne disease epidemiologists, while other states have few interactions. Efforts need to be made to improve the current laboratory/epidemiology communications system so that strong communication between the groups is the norm throughout the country.

Lack of quality exposure information

Perhaps the greatest challenge to our foodborne disease surveillance system is the lack of quality exposure information from ill cases, which limits the usefulness of PulseNet data. For outbreaks detected by pathogen-specific surveillance, exposure information obtained from case interviews is necessary to link disease clusters to specific foods or other vehicles. The more quickly thorough interviews are performed, the better the patient recall. This process is limited by resources needed to conduct interviews; lack of standard forms and methods; and suboptimal communication among the local health departments, state health departments, and federal agencies.

Future challenges

In addition to the aforementioned current challenges facing state PHL foodborne outbreak response and surveillance, multiple challenges will threaten these activities in the future. Funding will continue to be a significant issue for most or all state PHLs. Expected level or decreased future ELC funding and diminishing availability of Public Health Emergency Preparedness Cooperative Agreement funds, as they are needed elsewhere in state activities, may lead to future cuts in surveillance activities and/or staffing. Recent national initiatives to secure and bolster government funding for food safety activities in light of some recent high-profile national foodborne outbreaks hold promise for future state-level foodborne outbreak activities.

The increasing use of non-culture methods to detect foodborne pathogens in clinical and food samples by clinical and agricultural laboratories is creating a long-term dilemma for PulseNet laboratories and our foodborne disease surveillance system. Commercially available diagnostic assays continue to be developed and adopted by laboratories that do not rely on the isolation of pathogenic organisms to detect their presence in a clinical or food sample. While these assays have improved the sensitivity and specificity of pathogen detection to a greater or lesser extent, and have decreased the time from specimen receipt to test result, isolates are generally not available to PulseNet laboratories. Not receiving isolates for PFGE decreases the overall sensitivity of outbreak detection.

Non-culture-based detection methods are currently used extensively for *E. coli* O157:H7 and other shiga toxin-producing *E. coli* (STEC). Many states require clinical and/or agricultural laboratories to send a portion of the enrichment or original sample from all specimens that contain *E. coli* O157:H7 or STEC so that the state PHL can isolate and further characterize the pathogen by serotyping and PFGE. As non-culture assays for other foodborne pathogens are developed and become commercially available, it is essential that isolates can be characterized by PulseNet laboratories to ensure effective foodborne disease surveillance.

Another continued and future challenge to state PHL surveillance is the reliance of clinical laboratories on out-of-state reference laboratories for diagnostic testing. Many clinical laboratories throughout the U.S. send specimens to out-of-state reference laboratories for routine diagnostic testing to streamline their processes and cut costs. The challenge for the state PHLs has been obtaining either isolates of foodborne pathogens or clinical samples known to contain foodborne pathogens from the out-of-state reference laboratory to serotype and subtype the isolates. Failure to subtype the isolates decreases sensitivity of outbreak detection. In some states, the majority of their isolates are sent to these reference laboratories. Some states have

agreements with out-of-state reference laboratories that allow isolates to be shipped back to the state PHL of origin for surveillance testing. However, too often clinical isolates are not sent back to the PHL for surveillance testing and valuable surveillance data are lost.

CONCLUSIONS

PulseNet has been one of the most successful government programs, winning the prestigious Ford Foundation Innovations in American Government Award in 1999 as one of the 10 best programs of the year. In 2002, it was recognized as one of the 15 most significant government initiatives to have won the award. PulseNet is a model network for communication and collaboration among partners involved with food safety. However, PulseNet laboratories need to be strengthened and must improve their ability to work with their foodborne disease epidemiologists. Such improvements will allow the foodborne disease surveillance system in the U.S. to more rapidly identify outbreaks so that contaminated products can be removed from the marketplace and underlying causes of contamination can be remediated, thus preventing future illness.

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