PulseNet News™
The National Molecular Subtyping Network for Foodborne Disease Surveillance
State & Local Public Health Laboratories in the United States
PulseNet Canada

Volume 1, Issue 4, Summer 2002

Michigan hosted the 6th PulseNet Annual Update meeting.

By Stephen Dietrich, Michigan Department of Community Health

The 6th Annual PulseNet Update Meeting was held April 8-10 in Ann Arbor, Michigan. The Michigan Department of Community Health hosted the meeting. Attendance reflected the rapid growth of PulseNet, especially it's international expansion. Among the one hundred and fifty participants were representatives from 47 U.S. states, several cities and counties, the United State Department of Agriculture, (USDA) Food and Drug Administration, (FDA), Center for Disease Control and Prevention (CDC) and 8 foreign countries. International developments were also presented by members of PulseNet Canada (formally known as PulseNet North), the newly established PulseNet Europe, and the emerging PulseNet laboratory in Hong Kong.

Bala Swaminathan (Chief, Foodborne and Diarrheal Diseases Laboratory Section, CDC) began the sessions by describing the progress made in meeting PulseNet's goals for 2001 and then described the goals for 2002. Through the hard work of the CDC PulseNet staff and Applied Maths, several important goals have been or soon will be met. The national databases for *Salmonella* and *Listeria* are now ready for conversion to on-line BioNumerics databases. This development will provide more rapid and efficient data sharing and will help to realize PulseNet's full potential. Dr. Swaminathan described actions set in place to develop the next generation of typing methods that may replace Pulsed field gel electrophoresis (PFGE) in the future. These methods must produce results that are more informative, are less ambiguous, and have increased discriminatory power. Dr Swaminathan also re-emphasized the need for timely subtyping and pattern submission, which are critical for fulfilling the mission of PulseNet. This goal was not fully met in 2001 and labs are to strive for better performance in 2002. PulseNet goals for 2002 include: having on-line access to four databases by December, eliminating the backlog of certification, conducting a second round of proficiency testing, drafting the 2001 PulseNet annual report, and to begin archiving of isolates with unique PFGE patterns.

Delegates of the 6th Annual Update Meeting
Photographs provided by Michael Robeson II, Florida Department of Health

The theme of the meeting was the role of PulseNet in the public health response to bioterrorism. Dr. Swaminathan described issues involving bioterrorism and the food supply and said that a report defining the role of PulseNet in bioterrorism events will be prepared this year. Several speakers discussed the public health response to the anthrax attacks in 2001 and the impact the investigations had on the laboratory workload. Rick Roman (senior emergency coordinator, CDC) explained the organization and functions of federal and public health laboratories in the Laboratory Response Network (LRN) and the role of the LRN in investigating anthrax infections.

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Innovations in American Government Award

In 1999, the PulseNet program won the Innovations in American Government Award. During a reception in April of this year PulseNet was celebrated as one of the 15 most significant government initiatives to have ever won the innovations award.

Over the past fifteen years, the Innovations in American Government Program has received over 23,000 applications, which means that the PulseNet Program is considered among the top .0007% of all programs that have applied. To mark this achievement the PulseNet program was awarded a crystal trophy, which will be displayed in the PulseNet exhibit in the Global Odyssey Museum at the Roybal Campus, Centers for Disease Control and Prevention (CDC).

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To receive regular copies of the PulseNet News, send your request to:
PulseNet News
c/o Lindsay Sails (ztu1@cdc.gov)
1600 Clifton Road, NE, Mailstop C03
Atlanta, GA 30333
Tel: 404-639-4558
Fax: 404-639-3333
or visit the Web site at
http://www.cdc.gov/pulsenet

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Call For Contributions.

The editor welcomes any contribution for the "PulseNet News" Newsletter in the form of short articles, news of recent publications, conference abstracts, news and anything else related to PulseNet. Please direct all submissions to the editor (ztu1@cdc.gov)

PulseNet Logo

The PulseNet logo is now officially trademarked and has undergone a make-over. The new logo can be found on the WebBoard under the topic "General PulseNet Information". If you require the logo in an alternative format please email Lindsay Sails at ztu1@cdc.gov, or call 404-639-0574.

Farewells to member of the CDC PulseNet Task Force.

Anna Rae Ong came to CDC in June 1999. For 3 years she worked as a microbiologist and was a tremendous asset to our Foodborne Diarrheal Diseases Laboratory Section (FDDLS). She became an expert in performing pulsed-field gel electrophoresis on *Listeria* species and other bacteria. Anna Rae also took on the responsibility of the PulseNet *Listeria* database. Her last day with FDDLS was July 1, 2002. This fall she is going to study medicine at the Mercer University School of Medicine. We wish her the very best in her professional and personal life.

Frank W. Virgin Jr., Wade Ivy, III., and **Kimberly McGill** all joined the PulseNet Database Administration team in September 2001. Frank was responsible for the *Salmonella* database. He will continue his education at the Medical College of Georgia in August 2002. Wade, who was responsible for the *Escherichia coli* O157 database, will continue his education at the University of Michigan, Ann Arbor in August 2002. Kimberly the *Shigella* database manager, is completing a master's of science in biology at the Georgia State University.

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Salmonella serotypes – How should we make optimal use of PFGE resources?

By: Dianna Schoonmaker-Bopp, New York State Department of Health

At the Annual PulseNet Update Meeting, Dianna Schoonmaker-Bopp (NY), Steve Dietrich (MI), and Leslie Wolf (NC) chaired a session that addressed issues that arise when performing PFGE on *Salmonella* isolates that are received for serotyping. Table 1 summaries the points that were raised. We request that interested persons respond with suggestions or questions that could be discussed during regional conference calls or at next year's meeting. S*almonella* isolates are a significant percentage of the total number of foodborne isolates that most of us receive in our laboratories. The FoodNet data for 2001 indicates that *Salmonella* has surpassed *Campylobacter* as the foodborne pathogen with the highest incidence. In many cases, the resources are not available to do PFGE on all of the *Salmonella* isolates that are received for serotyping. In New York State (as is probably the case for most laboratories in PulseNet) *S.* serotype Typhimurium and *S.* serotype Enteritidis constitute 50% of the *Salmonella* isolates. Typically, the next 8 serotypes contribute an additional 25%, and the next 10 serotypes contribute an additional 10%. Therefore, the top 20 serotypes account for 85% of the isolates received. Only one or two isolates for 47 serotypes were received in 2001. In the face of this diverse mixture of isolates received in our laboratories, how are we to decide which isolates to test by PFGE in order to best serve the health needs of the residents of our states?

One important factor cited by participants is the prevalence and diversity of some *Salmonella* serotypes in a laboratory's catchment area. It is apparent that regional differences exist. Steve Dietrich from Michigan routinely performs PFGE on all *S.* serotype Infantis isolates, and Leslie Wolf from North Carolina performs PFGE on all *S.* serotype Javiana and Muenchen isolates. These serotypes may be quite uncommon in some regions and thus not be routinely tested. Although these may not be the most prevalent serotypes in Michigan and North Carolina, isolates that belong to these serotypes have been the cause of foodborne outbreaks in their respective states and need to be monitored. The prevalence of many serotypes increases in the summer, but increases in some serotypes are more pronounced. These increases may not be identified as a common source outbreak, because they recur from one season to another.

The diversity that is present within serotypes may also vary by region and may be another important consideration in deciding which isolates to test. Some serotypes tend to be quite clonal, such as *S.* serotypes Enteritidis, Heidelberg, and Thompson. However, in some regions, the diversity within a serotype may be greater than in other regions. Other serotypes, such as *S.* serotypes Infantis, Muenchen, and Poona, have adequate diversity. The usefulness of digestion with *Avr* II (*Bln* I) for the serotype of interest is also important. If diversity with *Xba* I is not very great, but digestion with *Avr* II (*Bln* I) further subdivides the serotype, (as seen for *S.* serotype Heidelberg), then PFGE can contribute useful information to the epidemiological investigation. Criteria that our laboratories currently use to select isolates for PFGE testing are: 1) an increase in the number of isolates of a particular serotype as detected by an increase in cases by epidemiologic surveillance or an increase in isolates received in the serotyping laboratory, 2) response to a WebBoard posting (formally listserv postings) , and 3) temporal/geographic comparison of isolates received with isolates received in previous years.

The PulseNet laboratories have collectively acquired extensive experience that may be helpful when setting priorities for PFGE analysis on *Salmonella* serotypes. Many of us are looking for

Table 1

Questions raised by the participants were:	
(1)	Seasonal peaks in the frequency of various serotypes and patterns e.g. Typhimurium, Newport, Javiana represent outbreaks?
(2)	Should we test more of the isolates belonging to the "top 10" or "top 20" serotypes, perhaps at the expense of testing more prevalent serotypes with less diversity?
(3)	Is there a benefit to testing a subset of common serotypes to monitor circulating patterns?
(4)	When should very rare serotypes be tested?
(5)	Would it be possible to post common patterns for the "top 10" or "top 20" serotypes?
(6)	Would it be helpful to develop a chart, perhaps by region, with information on diversity and the usefulness of second enyzme digestion for the more common serotypes?
(7)	How well does Avr II (Bln I) discriminate common Xba I patterns of the problem serotypes that are quite clonal?
(8)	Why are more outbeaks of S. serotype Typhimurium not identified, even though it is one of the most common serotypes, if not the most common? Is this lack of identification because of incomplete testing by laboratories, failure to communicate matches because there are so many cases, or some other reason?

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PulseNet and NARMS: Why we should link-up.

By: Jennifer McClellan, NARMS, Centers for Disease Control and Prevention (CDC)

The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS-EB) was established in 1996 within the framework of the CDC's Emerging Infections Program's (EIP) Epidemiology and Laboratory Capacity Program. NARMS collaborators include CDC, state and local health departments, and the U.S. Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM). NARMS has expanded and continues to expand its surveillance network.

In 2001, 15 state and 2 local public health laboratories (New York City and Los Angeles County) began participating in NARMS. In January 2002, 11 new state health departments joined NARMS, bringing the total number of sites under surveillance to 28 sites. NARMS now monitors 156 million people (56% of the U.S. population). Beginning in 2003, NARMS will be nationwide.

NARMS is an active surveillance system whose primary objective is to monitor antimicrobial resistance among *Salmonella*, *E. coli* O157:H7, and *Shigella*. Presently, after serotyping their isolates, participating sites forward every tenth nontyphoidal *Salmonella*, every *Salmonella* Typhi, every fifth *E. coli* O157:H7, and every tenth *Shigella* isolate to CDC. In 2003, when we expand nationwide, this sampling scheme will change to every twentieth nontyphoidal *Salmonella, Shigella*, and *E. coli* O157:H7 isolates, and all *S.* Typhi and *Vibrio* isolates.

Once the isolates arrive at CDC, analysts test them for antimicrobial susceptibilities against 17 antimicrobial agents using the Sensititre System. Because sensititre uses broth microdilution it can determine partial-range Minimum Inhibitory Concentration (MICs). Results are analyzed and published in an annual report, which is available from our Web site (www.cdc.gov/narms). Though this report is a summary of national trends, we anticipate developing region-specific annual reports in the future. Additionally, we hold quarterly conference calls where we provide feedback on emerging resistances and other ancillary research related to NARMS participants.

Over the past year, NARMS and the Foodborne Diseases Active Surveillance Network (FoodNet) have formed a partnership to link their data, thus combining susceptibility data from NARMS with patient data from FoodNet. We would like to extend this linking exercise to PulseNet so that we can better define resistance originating among isolates included in the National Molecular Subtyping Network for Foodborne Disease Surveillance. An example of the importance of such linking is tracking the emergence of multidrug-resistant *S.* Newport. For PulseNet and NARMS data to be linked, each isolate must have a unique identifier, which will be your state lab ID. We urge PulseNet microbiologists to communicate with the NARMS microbiologist in your state to make sure that PulseNet and NARMS isolates from the same patient be identified by the same state laboratory ID. If you have questions, please contact Jennifer McClellan **zcn6@cdc.gov** (404-371-5409), Efrain Ribot, PulseNet Methods Development and Validation Laboratory, CDC, **ery4@cdc.gov** (404-639-3521) or Susan Hunter, PulseNet Database Administration Team, **sbh1@cdc.gov** (404-639-1749).

PULSENET CANADA

New name, same people:

By Cynthia Misfeldt, PulseNet Canada Team

Things are changing at PulseNet North. Due to increased participation in molecular typing networks at an International level, the steering committee of PulseNet North has decided to change our name to PulseNet Canada.

To increase communication across Canada, PulseNet Canada (PNC) is developing a newsletter and Web site to keep all provinces informed of the efforts and accomplishments of PNC members. International participation in either of these endeavors is always welcome. We also have a new e-mail address for PulseNet Canada PN_Canada@hc-sc.gc.ca.

Continued Salmonella serotypes page 2.

information to help us make decisions that will make the best use of our resources. Combining our experience can benefit all of the laboratories, raise the level of public health, and better enable us to deal with foodborne illness in the future. If you have contributions, questions, or requests, please forward them to either Dianna Schoonmaker-Bopp, New York State Dept of Health, djs03@health.state.ny.us; Sharon Rolando, APHL, SRolando@aphl.org; or Efrain Ribot, CDC, eyr4@cdc.gov.

Continued PulseStar Awards page 4.

Reference: Proctor ME, Kurzynski T, Koschmann C, Archer JR, Davis JP. 2002. Four strains of *Escherichia coli* O157:H7 isolated from patients during an outbreak of disease associated with ground beef: importance of evaluating multiple colonies from an outbreak-associated product. J. Clin. Microbiol. **40**:1530-3.

State Laboratory Profile: Massachusetts

The Massachusetts PFGE Laboratory at the State Laboratory Institute has been a PulseNet regional laboratory since the inception of PulseNet. We routinely run PFGE on over 2,000 isolates per year, including E. coli O157:H7; shiga toxin-producing, non-O157:H7 E. coli; Salmonella serotypes; Shigella sonnei; Listeria monocytogenes; Neisseria meningitides; and Bordetella pertussis, all of which are isolated and typed by the enteric and reference laboratories. In addition, we test isolates associated with nosocomial infections on a case-by-case basis. This testing often involves Group A Streptococci, Streptococcus pneumoniae, or Oxacillin-resistant Staphylococcus aureus (ORSA). The Massachusetts Antimicrobial Resistance Surveillance (MARS) Laboratory, which began in 1999, was recently incorporated into the PFGE laboratory to consolidate testing and data management and to facilitate the flow of information to state epidemiologists and to CDC. It performs susceptibility testing on select enteric pathogens using the NARMS custom Sensititre sensitivity plate and on invasive S. pneumoniae using the Dade Microscan MICroStrep plate. The laboratory tests approximately 500 enteric isolates and 400 invasive S. pneumoniae isolates per year.

Room 412

We currently have four full-time bacteriologists in the PFGE laboratory (Ali Stout, Traci Stiles, Lee Kenny, Janet Sennott) and one full-time bacteriologist (Mark Tyndall) in the MARS Laboratory. Sandra Smole, Ph.D., an APHL/CDC EID fellow, also collaborates with the laboratory on research projects. Other personnel include Nimisha Calia, an APHL/CDC EID fellow, and Caleb Mulu, a bacteriologist.

***Left to right:** Massachusetts PFGE Laboratory. Nimisha Kalia, Dr. Sandra Smole, Caleb Mulu, Mark Tyndall, Lee Kenny, Dr. John Fontana, Janet Sennott, Alison Stout.*

Highlights & New Projects:

S. Newport MDR-Amp C
All S. Newport isolates are tested for antibiotic susceptibility, and a sharp increase in the number of S. Newport MDR-Amp C (resistant to 9 or more antimicrobials including extended spectrum cephalosprins; MMWR 51 (25) 545-548, 2002) was detected in Massachusetts in 1999. These isolates are being analyzed by PFGE, automated ribotyping, and plasmid analysis.

Oxacillin resistant staphylococcus aureus (ORSA) PFGE
The PFGE Laboratory has been certified by CDC to participate in ORSA on PulseNet (OPN). To determine the feasibility of offering ORSA testing to hospitals in Massachusetts, the Massachusttes laboratory collected isolates from two Boston area hospitals over a period of 2 months. Of the 138 isolates submitted to the laboratory, 67 had unique patterns. There were three distinct clusters, comprising 12, 16, and 18 isolates with indistinguishable PFGE patterns. One of the clusters represented isolates from a neonatal intensive care unit at one of the participating hospitals. Two employees had PFGE patterns indistinguishable from those of 11 patients. These results allowed the hospital staff to implement appropriate infection-control measures to limit patient exposure.

Norwalk like virus outbreak
With new support from CDC through the Epidemiology and laboratory capacity enhancement (ELC) cooperative agreement, Polymerase chain reaction (PCR) testing for the presence of Norwalk-like virus (NLV) has been implemented in clinical stool specimens where foodborne illness is suspected and no enteric bacterial pathogens have been found. Recently, an NLV outbreak was identified. In early May, four stool specimens from an outbreak characterized by severe vomiting and diarrhea involving over 200 people were positive by NLV testing at SLI and were confirmed by CDC 's Viral Gastroenteritis Section. The investigation is ongoing, and we expect more test results to follow.

Riboprinter studies
The Riboprinter has been used on two occasions for rapid species identification of bacteria derived from spores found in biological threat letters after Bacillus anthracis has been ruled out by conventional microbiological methods. We are also evaluating a protocol using the Riboprinter with custom antibiotic resistance gene probes to characterize chromosomal and plasmid DNA in S. Newport MDR-Amp C.

International training
During March of 2002, we hosted three laboratory scientists from Nicaragua who were trained to do PFGE, antimicrobial susceptibility testing, and plasmid analysis.

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Dr. Frances Pouch Downes (Michigan Department of Community Health) and Dianna Schoonmaker-Bopp (New York Department of Health) presented two state public health laboratory perspectives on the anthrax outbreak. They stated that although public health labs experienced a great increase in anthrax testing, their staff displayed the flexibility and desire to work together that got the job done. Sharon Rolando (PulseNet coordinator, Association of Public Health Laboratories) provided results from a survey she conducted to determine the impact that the increased anthrax testing had on PFGE testing. Over half of the responding laboratories were significantly affected, resulting in a backlog of testing and data submission.

Participants also discussed a number of technical topics related to the PulseNet Program, including the use of the new standard universal marker strain, *Salmonella* serotype Braenderup (H9812). The bands of this strain have a better size range and are distributed better throughout the pattern than the original marker standard. This strain will be used for all organisms tracked by PulseNet in the future. Susan Hunter (PulseNet Database Administration Team, CDC) presented comparisons of data based on the original *E. coli* standard strain (G5244) with data based on *S. Braenderup* (H9812). She demonstrated the compatibility of patterns normalized with either marker. BioNumerics files for using *S. Braenderup* H9812 and converting old data will soon be available. Participants also discussed carious laboratory protocols. Dr. Collete Fitzgerald (PulseNet Task Force, CDC) presented the final version of the standardized protocol for *Campylobacter*. A set of isolates for certification is available for labs that are interested, BioNumerics files will soon be available, a national Campylobacter database being developed. Rebecca Middendorf (PulseNet Task Force, CDC) described PulseNet's progress towards completing protocols for *Vibrio cholerae* and *Yersinia enterocolitica*. Laboratories will be able to become certified for *V. cholerae* this year, whereas the *Y. enterocolitica* procedure needs additional testing. Lewis Graves (PulseNet Task Force, CDC) described improvements in the *Listeria* protocol and gave tips for improving gel electrophoresis results.

This article describes just a few of the many interesting sessions presented at the 6th Annual PulseNet Update Meeting. Future editions of the PulseNet Newsletter will follow up on some of these issues. At the close of the last session, Dr. Swaminathan announced that the Texas Department of Health will host the 2003 Annual PulseNet Update Meeting. Watch the PulseNet WebBoard for the date and location of the 7th annual meeting.

A special thank you goes to Michigan Department of Community Health, especially Stephen Dietrich, Dr. Jeff Massey, Dr. Patricia Somsel, and Dr. Frances Pouch Downes for all their efforts and hospitality in making the 6th Annual PulseNet Update Meeting a success.

2002 PulseStar Awards

and the winners are…

The PulseStar awards recognize individuals for their outstanding contributions to PulseNet during the previous year, and nominations are received from co-workers, supervisors, and other PulseNet participants, winners receive a plaque from CDC and a check for $500 from APHL. The 2002 awardees were:

Wayne Chmielecki, Chemist II, Pennsylvania State Public Health Laboratory, attended PulseNet training in June 2000, returned to Pennsylvania to set up his laboratory equipment, and very shortly thereafter found himself working on an *E. coli O157:H7* outbreak. Wayne quickly honed his skills in producing quality gels as well as his troubleshooting abilities. Wayne's attention to detail has helped the Pennsylvania Department of Health Laboratory become an active and successful member of the PulseNet team.

Terry Kurzynski M.S., Advanced Microbiologist, Wisconsin State Laboratory of Hygiene, has been with PulseNet since its inception and his activities have been invaluable to the epidemiologists in Wisconsin. Recently, Terry co-authored an article on *E. coli O157:H7* involving multiple PFGE strain types, incorporated the *Campylobacter* protocol in the WI laboratory, played a major role in Bioterrorism testing efforts, and taught students from the University of Wisconsin. Terry's high-quality, reliable, and innovative services have been key to improving the public health in Wisconsin.

Leslie Wolf, Ph.D., North Carolina State Laboratory of Public Health, assumed full responsibility for the North Carolina PulseNet Program after completing her Emerging Infectious Disease (EID) fellowship in the North Carolina PFGE Laboratory. Leslie has actively worked with vendors on instrumentation issues, and she promptly relates summaries to the PulseNet participants. She collaborates in multi-state outbreak investigations, responds quickly to WebBoard postings, and presents her PulseNet experiences to both local and national audiences.

Congratulations to the three PulseStar Award recipients, and thank you to all the 2002 nominees for your excellent contributions to the PulseNet Program.

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