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PulseNet News™

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

PulseNet Surveillance of Methicillin Resistant *Staphylococcus aureus*

David C. Dixon Ph.D. Microbiologist, Michigan Department of Community Health, Upper Peninsula Laboratory.

Methicillin resistant *Staphylococcus aureus* (MRSA) has been a growing public health concern since the early 1960ís. Although resistance is currently tested with oxacillin rather than methicillin and these organisms are more correctly referred to as oxacillin resistant (ORSA), the abbreviation MRSA has persisted with health care professionals. These organisms are no more virulent than susceptible *S. aureus*; however, because they are resistant to many antibiotics, they may be more difficult to treat. ORSA is estimated to infect as many as 80,000 patients a year after they enter the hospital, accounting for 50 percent or more of the *S. aureus* nosocomial infections in intensive care units of some US hospitals.

In an effort to better understand and track ORSA outbreaks in the United States, the CDC is currently building a national database to correlate epidemiological information with genetic fingerprints of ORSA isolates. The ORSA database is modeled and built upon the existing infrastructure of PulseNet. Like the other databases that have been established within PulseNet, ORSA on PulseNet is expected to become a very powerful epidemiological tool for comparing the DNA fingerprints of ORSA isolates within an outbreak. ORSA on PulseNet may give insights into the temporal and geographical origins of outbreak strains, may provide correlations between genetic fingerprints and the susceptibility of specific demographic sub-populations, and may show relationships associated with increased virulence or pathogenicity. Long term, information in the database may increase our understanding of the basic biology of *S. aureus* helping to answer questions about mutation rates and how resistance genes move within a varied genetic population of *Staphylococcus* organisms.

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Farewell from the editor

Dear readers, this is the last PulseNet newsletter I will produce as editor as I am leaving the CDC and returning to the UK early next year. At this time I would like to take the opportunity to thank you for all your contributions, articles and support which have made the PulseNet News newsletter a resounding success. This is your newsletter and so, for one last time, I ask for your support, WE NEED YOUR ARTICLES, the deadline for the spring issue is 12th February 2003, with that said I bid you all farewell have a Happy Christmas and prosperous New Year.

Lindsay Sails

WHO Sponsors visiting scientist to work with CDC PulseNet task force.

Dr. Peter Gerner-Smidt, a visiting scientist from Denmark, is working with the PulseNet Task Force at the CDC in Atlanta for a year beginning August 1, 2002 to help implement PulseNet in Europe. Dr Gerner-Smidt is an MD and a specialist in clinical microbiology; he received his DMSc in Microbiology in 1994 on a thesis on the epidemiology and typing of *Acinetobacter* spp. Since 1996, he has been in charge of the Danish reference center for bacterial enteric pathogens and *Listeria.* He serves on the Steering Committee of the European Public Health Surveillance Network for *Salmonella* and STEC, EnterNet and he is the chairman of the PulseNet Europe working group of that network. The World Health Organization (WHO) is supporting Dr Gerner-Smidtís visit at the CDC.

Merry Christmas and Happy New Year

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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)

DEPARTMENT OF HEALTH & HUMAN SERVICES
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To receive regular copies of the PulseNet News, send your request to: PulseNet
c/o Lindsay Sails (ztu1@cdc.gov)
1600 Clifton Road, NE, Mailstop C03
Atlanta, GA 30333
Tel: 404-639-4558
Fax: 404-639-3333

The PulseNet News editorial committee:
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CDC PulseNet Task Force new members.

Database Administration Team - Kelley B. Hise is the data coordinator for the database team; in addition, she has primary responsibility for the *Listeria* and *Shigella* databases. **I, Joi Hudson** performs cluster identification and analysis of various serotypes of *Shigella sonnei* and other species. **Jennifer A. Kincaid** performs cluster identification and analysis of the *Escherichia coli* database. **Adam Beall** performs cluster identification and analysis of *Salmonella* serotypes.

PulseNet Methods Development / Validation Laboratory - Lynn Mauro, works in the *Listeria* laboratory on PFGE, Ribotyping, and Serotyping of *Listeria monocytogenes.* **Brenda Bowersox** and **Kimberly Hutcheson** perform routine PFGE analysis of foodborne bacterial pathogens, develop and evaluate new subtyping methods, and the coordinate the proficiency testing program.

CDC PulseNet Task Force Farewells

Michele Bird, who was a laboratory fellow in the PulseNet Methods and Validation Laboratory has accepted a full-time employee microbiologist position with the Epi Investigations Bioterrorism Response Unit at CDC. She will be part of a team traveling to specific third world countries evaluating diagnostic methods for rapid detection of cholera, dysentary and typhoid fever as part of CDC preparedness and response mission to bioterrorism.

Proficiency testing Continued from page 5

the PulseNet National Database, and their ability to correctly identify the restriction fragments within the PFGE patterns compared to the fragments in the consensus pattern. The PFGE pattern obtained by the majority of laboratories that submitted their proficiency results during the 2001-2002 year was chosen as the consensus pattern. All laboratories successfully passed.

The consensus pattern will be available for laboratories to review once the results from both rounds of PT testing for the year are completed. If you have any questions, please feel free to contact Susan Van Duyne (mdv9@cdc.gov) or Susan Hunter (sbh1@cdc.gov).

State, County and City Health Department

From around the nation, we also welcome:
Elizabeth Anderson, Minnesota Department of Health
Kali Erickson, North Dakota Department of Health
Sonya Flores, New Mexico Department of Health
Melissa Gosuico, City of Houston Dept. of Health and Human Services
Kris Hardin, Iowa Department of Public Health
Kim Laurie, Indiana State Department of Health
Debra Sizemore, Rhode Island Department of Health
Kimberley Holmes Talbot, Connecticut Department of Public Health
Jenny Wagner, Utah Department of Health
Simone Warrack, Arizona Department of Health Services
Lori Yasuda, Los Angeles County Public Health Laboratory

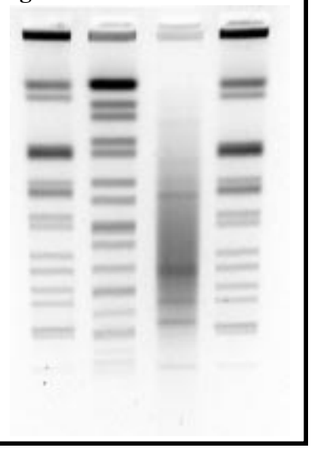
Evaluation of HEPES Buffer for ìnontypeableî strains of *Salmonella* and *E. coli*

Ravi Pallipamu, microbiologist, Washington State Department of Health Public Health Laboratories and Mary Ann (Lambert) Fair, research microbiologist, CDC PulseNet Laboratory

A recent publication in the Journal of Clinical Microbiology (JCM **40**:3497-3498) described the prevention of DNA degradation in PFGE gels with HEPES buffer instead of the addition of thiourea to the 0.5X TBE buffer used in the PulseNet standardized PFGE protocols. Both the Washington State Department of Health and the CDC investigated using HEPES buffer for subtyping ìnontypeableî strains of *E. coli* and/or *Salmonella* (See PulseNet News, Fall 2001 issue for additional information about nontypeable [also referred to as ìuntypeableî] strains and the use of thiourea.).

At CDC, the buffer and electrophoresis conditions described in the paper by Koort, et al were duplicated except the PulseNet conditions for *E. coli* and *Salmonella* were used (1% Sea Kem Gold agarose, 2.2 s ñ 54.2 s for *E. coli*; 2.2 s ñ 63.8 s for *Salmonella*). The voltage gradient was reduced to 4 V/cm as described. With both gels, the milliampere (mA) reading was ª 512, an extremely high value. The *E. coli* gel ran very short and the bands in the nontypeable *E. coli* strain were not as clear as the standards and strains in the other lanes. A gel with two am01144 and one H9812 standard and a nontypeable strain of *S.* ser. Saintpaul (Figure A, lane 3) showed similar results even though it was run for 23 hours. In addition, a thick precipitate of salts was seen on the lower electrodes after just two runs, even though the HEPES buffer was removed and the chamber was rinsed with water between runs.

Figure A



The Washington State Department of Health had similar results initially with the HEPES buffer; however, after running 10 gels using different conditions, they approximated the results seen with the *Salmonella* PulseNet conditions with two nontypeable (Figure B, lanes 2 and 3) and other *Salmonella* strains. In order to do this, the voltage gradient was changed to 4.8 and the agarose concentration to 0.8%. Because of the high voltage generated (ª507 mA), a Chef Mapper had to be used for the experiments; the Gene Path could not be used because it has a voltage ceiling of 366 mA.

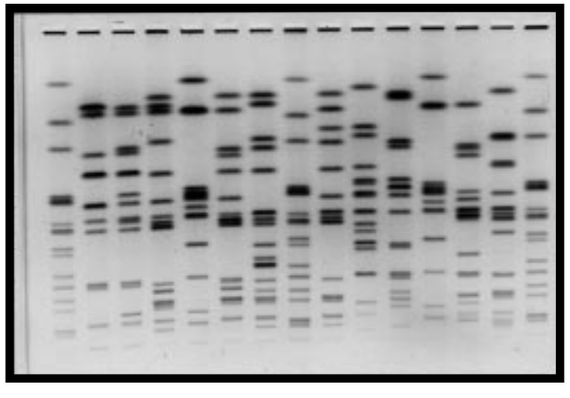


Figure B

Salmonella using HEPES with out thiourea, lane 2 & 3 ìnontypeableî isolates

For several reasons, both the Washington State Health Department and CDC conclude that HEPES buffer is not a viable substitute for running nontypeable strains of organisms tracked by PulseNet. These include: 1) Major changes have to be made to the PulseNet standardized conditions and, even then, the gel images may not be comparable with those run with 0.5X TBE; 2) The HEPES buffer as described in the Koort manuscript is not commercially available; it is difficult to make and is approximately three times more expensive than TBE; 3) Extremely high current is generated with unknown long term effects on the electrodes, electrophoresis chamber, and lines. 4) At CDC, the bands seen with the nontypeable strains were not as clear and crisp as those seen when these strains are run with thiourea in the buffer.

Although thiourea is a suspected carcinogen, there is minimal exposure to lab personnel if it is added to the 0.5X TBE buffer in the electrophoresis chamber (instead of adding it to the agarose gel or container used to make the buffer, which contaminates the comb, gel rig and glass- or plastic-ware). Since stock solutions of thiourea (10mg/ml in sterile water) are stable for at least 18 months, there is infrequent exposure to handling and weighing the chemical. Dedicating one machine to gels that have to be run with thiourea, limiting the use of thiourea only when necessary, and proper disposal of the waste chemical will minimize adverse exposure. It is important to remember that ethidium bromide, another carcinogen/mutagen, is used on a daily basis in most labs, and should be considered more of a bio-hazard than the occasional use of dilute solutions of thiourea.

The PulseNet proficiency testing program - A year in review

Michele M. Bird, Research Microbiologist, PulseNet, Center for Disease Control and Prevention.

The PulseNet Proficiency Testing (PT) Program began October 2001. The goal of this program, as stated in the Quality Assurance / Quality Control Manual for PulseNet Standardized Pulsed-Field Gel Electrophoresis is: ì To provide an on-going program of external testing to ensure laboratories participating in PulseNet maintain a satisfactory level of performance for pulsed-field gel electrophoresis (PFGE) or other molecular subtyping methods.î The PT program tests on an annual basis the quality of gels being produced by laboratories involved in PulseNet.

The proficiency ìchallengeî was divided into two groups. One group received isolate(s) in the fall of 2001; the second group received isolate(s) in the spring of 2002. The participating laboratories received a proficiency isolate for only the organism(s) for which they were currently certified.

During this first year of the PT program, 31 labs participated. Seventeen laboratories completed proficiency testing for both *E. coli* and *Salmonella*. Three labs completed only the *Salmonella* proficiency challenge and eleven laboratories completed only the *E. coli* proficiency challenge. Laboratories were evaluated on the quality of the gels submitted, their ability to successfully report their results to

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Staphylococcus aureus continued from page 1

Traditionally, bacteriophage typing has been used to characterize *S. aureus* isolates. However, at a molecular level, Pulsed-Field Gel Electrophoresis (PFGE) is currently one of the best methods for discriminating genetic differences and making correct associations between epidemiologically related organisms. Additionally, PFGE is amenable to the high levels of standardization that are required to build a functional national database with data submitted from laboratories across the country. As the current ìGold Standardî for genotyping bacterial pathogens, PFGE was selected by the CDC for the genetic characterization of ORSAs.

The ORSA on PulseNet program was announced March 29, 2001. Since then, more than twenty-two laboratories have been certified or are in the process of certification to participate in the program. The database is already showing some interesting correlations between the PFGE patterns of ORSA isolates and epidemiological data. The isolates are currently organized by computer analysis into seven pulsed-field types (PFTs) or lineages, based upon similarities in their fingerprint patterns. Preliminary analysis with limited patient data indicates that isolates from hospital-acquired infections usually have PFGE patterns that are more closely related to each other than to ORSA isolates from community sources. Most of the hospital-acquired isolates from the U.S. share a single genetic lineage. Similarly, isolates from community-acquired infections appear to be related and are grouped together in several other lineages. One interesting lineage contains isolates from Native American populations and Australian Aboriginals.

As a participant in the ORSA on PulseNet program, the Michigan Department of Community Health (MDCH) is working to implement this powerful epidemiological tool for investigating ORSA outbreaks within the state of Michigan. Clearly for the program to be effective, PulseNet member laboratories must establish a close working relationship with the healthcare facilities that they serve. For the past several years, MDCH has worked closely with local health services laboratories throughout our state. For example, several hospital laboratories in the state are currently enrolled in active surveillance with MDCH for vancomycin-resistant enterococci and penicillin-resistant pneumococci. Additionally, programs such as visits for onsite training in microbiology, parasitology and mycology, the establishment of laboratory advisory groups and the creation of outreach programs for quality assurance of susceptibility testing have helped build and sustain quality working relationships.

Building upon our past successes, we are currently exploring ways to get Michigan health care facilities that are interested in augmenting their infection control practices involved with us in the PulseNet program. We are presenting seminars to familiarize infection control officers and laboratory staff members with PFGE bacterial subtyping technology, explaining how this tool can be used in their facilities and inviting them to become participants with MDCH in the PulseNet program. Additionally, we have also published short articles (similar to this one) about the ORSA on PulseNet program in *LabLink*, the MDCH Bureau of Laboratories Newsletter. We plan future publications in the Michigan Society for Infection Control Newsletter. Further, we plan to survey infection control officers working within the state to help us assess the specific needs of Michigan health care facilities and define the challenges we must address in developing an improved MRSA surveillance program within the state. We hope to use information gathered from the survey and the seminars to develop an effective MRSA on PulseNet program that has the flexibility to establish epidemiological relationships of MRSA isolates whether they are occurring within a single health care facility or scattered across the state of Michigan. At same time, we hope our program will help meet the objectives of the ORSA on PulseNet program and contribute toward a broader epidemiological understanding of MRSA on a national level.

Laboratories that are not yet participants in the CDCís ORSA on PulseNet program are encouraged to participate. Participation requires the submission of a certification gel that has been run with *S. aureus* isolates with defined PFGE patterns and analysis of the gel with BioNumerics software. For further information about the program or to become a participant, contact StaphPFGE@cdc.gov.

PulseNet The next generation:

An update on the development of DNA sequencing-based subtyping methods for implementation within the PulseNet network.

Participants in the PulseNet network have been conducting research towards the development of the next generation of subtyping methods for implementation within the PulseNet network. In this article we review the progress achieved by the two state public health laboratories that have been performing the research.

The Massachusetts Department of Public Health/ State Laboratory Institute (MDPH/SLI) has been investigating new molecular methods for subtyping *E. coli* O157:H7. Sandra Smole of MDPH/SLI has been collaborating with Paul Keim of the Northern Arizona University to compare the discriminatory capacity of a newly developed typing method his group has been developing for *E. coli* O157:H7. This method called multiple locus variable tandem repeat analysis (MLVA) is based on rapidly evolving variable-number tandem repeat (VNTR) loci. MLVA utilizes several marker loci with fluorescently labeled PCR primers facilitating automated detection and size determination of the fragments using an automated DNA sequencer. The performance of this novel new method is currently being compared to the current ëgold standardí, pulsed field gel electrophoresis currently employed by the PulseNet network. Although data has not yet been analyzed, the groups are very impressed with the technical aspects of MLVA and its potential to streamline the typing process. They are very close to bringing the project to a point at which a comparison can be made between these two techniques on a carefully selected set of organisms from the PulseNet system.

Kristin Pederson of The Minnesota Department of Health has been developing MLVA-based approaches for the epidemiological subtyping of *Salmonella* Typhimurium and *S.* Enteritidis. Preliminary analysis of fifty-five *S.* Typhimurium strains with four VNTR loci indicated that MLVA provided a similar level of discrimination as PFGE in distinguishing outbreak strains from temporarily associated sporadic isolates. Future work will include identifying additional VNTR loci for *S.* Typhimurium and VNTR loci for *S.* Enteritidis. These additional VNTR loci will be used to investigate the utility of MLVA on a larger panel of isolates. The Minnesota group are also attempting to identify regions in the *S.* Enteritidis genome sufficiently polymorphic to be useful as targets in a multilocus sequence typing (MLST) approach. Ran

PulseNet 7th Annual Update Meeting

Hosted by the Texas Department of Health
Wednesday, April 30 through Friday, May 2, 2003
St. Anthony Hotel in San Antonio, Texas.

Ideas and suggestions for agenda items, workshops and discussion groups are welcome, and if you would like to volunteer as moderator for any of the sessions then please contact Shari Rolando, APHL at SRolando@aphl.org or (202) 822-5227 , Ext 205.

PulseStar Awards

Please begin to consider possible candidates to be nominated for the PulseStar Awards that are presented annually by FDDLS/CDC and APHL. The award consists of a plaque and a check for $500 provided by APHL. Nomination forms and criteria will be posted on the WebBoard early in the new year.

State laboratory profile: Idaho

(Photo: Michelle Wilkin left and Tricia L. Hosch-Hebdon right.)

Michelle Wilkin, medical microbiologist and Tricia L. Hosch-Hebdon, molecular microbiologist, State of Idaho bureau of laboratories.

The Idaho PFGE laboratory began back in 1997, when it was primarily responsible for strain typing nosocomial infections and known foodborne outbreaks. In the spring of 2000, the Idaho laboratory began typing organisms for routine PulseNet surveillance. The Idaho laboratory routinely runs between 300 ñ 500 isolates per year, including *E. coli O157: H7* and all other shiga-toxin producing *E. coli,* all *Salmonella* serotypes, *Shigella* species, *Listeria monocytogenes, Neisseria meningitidis*, *Bacillus cereus* and *Bordetella pertussis* which are isolated by our medical microbiology laboratory. In addition, the PFGE laboratory is part of a local effort to identify and monitor nosocomial infections in our medical institutions and the community at large. Our testing mainly involves oxacillin resistant S*taphylococcus aureus* (ORSA) and Vancomycin resistant Enterococcus (VRE).

Our PFGE laboratory is just one part of our molecular microbiology and medical microbiology laboratories. Currently we have one full-time person in our PFGE laboratory, with an additional person fully trained and certified as a backup. In total, our medical and molecular laboratories have 3 full-time persons that conduct all of the testing that goes on in these laboratories.

Highlights and new projects:

Oxacillin Resistant ORSA PFGE
The PFGE laboratory is currently working on completing our certification sets for the CDC ORSA program (OPN). Idaho currently offers ORSA testing to all hospitals that request the service and we are planning on working with laboratories in the state to begin community surveillance of ORSA.

RADAR/ IMPART Study
We are working for Qualis Health, formerly Pro-West, on a project investigating antimicrobial resistance in rural Idaho. The project involves testing *E. coli* in meat purchased at local stores, stools from healthy citizens, and clinical isolates collected at the hospital, in an effort to determine possible transmission of antimicrobial resistant *E. coli* from meat products to humans that have not been taking antibiotics. This has been the first year of a three year grant and we look forward to the continued work with Qualis Health and the publication of their results.

EHEC / STEC surveillance
As of spring 2002, we have offered enterohemorrahgic *E. coli* screening, using the Meridian EHEC kit, to all Idaho laboratories that perform stool cultures and do not identify any enteric pathogens. Since beginning this program we have isolated *E. coli* O26:H11, O111:NM, and other shiga-toxin producing *E.coli* from throughout our state. We also plan on attending the STX PCR training that the CDC is offering.

The Next Generation continued from page 3

dom Amplified Polymorphic DNA (RAPD) analysis is being used to identify polymorphic regions that could be targeted as MLST loci. The identification of polymorphic loci with the ability to further differentiate and subtype strains with the more common *S.* Enteritidis PFGE patterns and phage types is a research goal and would be most useful for the epidemiological analysis of this clonal pathogen.

North Carolina State Laboratory of Public Health was recently selected to conduct research into the development of DNA sequencing-based methods for the subtyping of *Listeria* monocytogenes. Leslie Wolf at North Carolina State Laboratory of Public Health plans to investigate multilocus sequence typing (MLST) and MLVA as methods for the epidemiological subtyping of *Listeria monocytogenes*. PulseNet wishes Leslie every success in this project.