The National Molecular Subtyping Network for Foodborne Disease Surveillance

PulseNet™ News

State & Local Public Health Laboratories in the United States and PulseNet Canada

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Midwest-Area PulseNet Conference Summary

John Besser, Clinical Laboratory Manager, Minnesota Department of Health, Minneapolis, MN

The first Midwest-area PulseNet conference was hosted by the Minnesota Department of Health on October 28-29, 2003. Thirty-two laboratorians, epidemiologists, and laboratory directors from the Wisconsin, Minnesota, South Dakota, North Dakota, Iowa, Nebraska, Missouri, and Kansas health departments, CDC, APHL, and NLTN met to discuss a multitude of issues associated with the improvement of foodborne disease surveillance. A system-wide approach was taken in planning the conference agenda, which addressed foodborne disease surveillance from patient illness through interstate outbreak investigations, and everything in between. The specific objectives were (1) to facilitate communication among the states and among groups within the states, (2) to develop an idealized model for PulseNet function, and (3) to identify barriers to achieving the model and recommendations for overcoming them. The format was lectures, brainstorming sessions, and group discussions. The conference began with guest presentations chosen to represent the full range of activities associated with foodborne disease surveillance and investigation. Each state then gave a brief presentation describing the foodborne disease surveillance systems in their jurisdiction, including successes and unique issues they face. Finally, brainstorming sessions and group discussions were conducted to identify ways in which national and local foodborne disease surveillance systems could be improved. The participants were urged to consider all aspects of surveillance and investigation, including scientific, technical, political, financial, communication, and operational aspects that might contribute to PulseNet's function. The activities discussed included test ordering, specimen collection and transport, testing at local laboratories, case

(Continued on page 2)

Welcome to San Diego, California

Cathy Adams, Microbiologist, San Diego County Public Health Laboratory, San Diego, California; and Lori Yasuda, Los Angeles County Public Health Laboratory, Los Angeles, California

Warm up from the winter freeze at PulseNet 2004 here in sunny Southern California! With a beautiful view of the San Diego Bay, we will be hosting the 2004 annual PulseNet meeting. Downtown, and the Bay are the most vibrant and inviting locations in San Diego, allowing for the perfect backdrop for this year's meeting. At the end of the day, take a stroll down Harbor Drive to the Embarcadero, or to the historic Gaslamp District. On behalf of the County of San Diego, Los Angeles County, and the State of California Department of Health Services, welcome to San Diego! CDC

Mid-West Conference (Continued from page 1)

reporting and interviews, isolate submission and transport, public health analyses, data handling and analysis, cluster investigation, and interstate outbreak investigation. A second half day focused on PFGE technical issues, along with a lecture on alternate subtyping methods.

The major conference findings included:

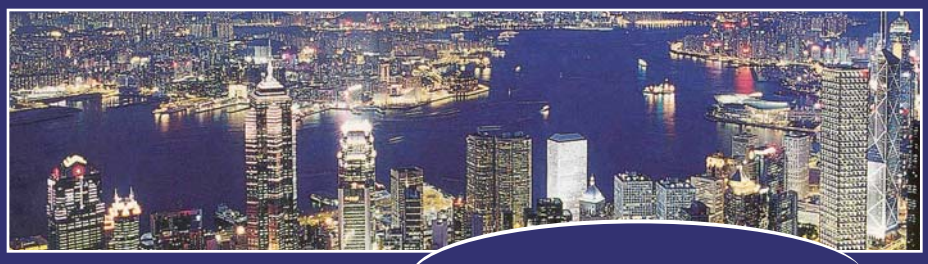
- **PulseNet's value to society:** There was general agreement that PulseNet and the disease surveillance systems that support it represent the best system available for detecting unforeseen problems in our food and water supplies, and would be the most likely mechanism by which a foodborne bioterrorism event with *Salmonella, Shigella, E. coli* O157:H7, or *Listeria* spp. would be detected. Participants believed the system's value to society would increase by enhancement of the foodborne disease surveillance infrastructure that PulseNet depends upon.
- **The ideal model of PulseNet function:** All cases of foodborne disease are detected and investigated in real time in every state. Real-time surveillance means cases are reported and isolates sent from the local facility to the Public Health Department on the day the case is identified, subtyping is conducted on a daily basis upon arrival in the PulseNet laboratory, PulseNet postings are made as soon as PFGE analyses are complete, all patients are interviewed on the day that case reports are received, and disease clusters are investigated when detected.
- **Factors limiting implementation of the ideal model:** States differ greatly in resources available for foodborne disease surveillance, both in the laboratory and epidemiology sections. Only a minority of state laboratories are able to conduct analyses in real time for all PulseNet-tracked agents, and some states must stop PulseNet activities when other issues arise that require molecular methods, such as West Nile Virus or SARS. Laboratories vary widely in the amount of Epidemiology and Laboratory Capacity (ELC) or Bioterrorism Preparedness (BT) funds they receive or are able to use for PulseNet activities. Many states only interview cases involved in recognized outbreaks and few states have sufficient resources to interview patients of PulseNet tracked diseases in real time. Many other issues were identified, such as communication issues between epidemiology and laboratory sections, political divisions between local and state public health departments, transport issues, and resistance to mandatory submission of isolates.
- **Solutions:** A variety of solutions were proposed to overcome identified barriers and technical problems. Perhaps the most important suggestions were in the area of resource allocation, which was identified as the greatest barrier. Suggestions included exploring the use of bioterrorism funds (due to the probable central role in detecting foodborne bioterrorism) and reprioritization of state public health dollars.

Based on the conference evaluation survey, the conference was a resounding success. All survey respondents felt their attendance would result in changes to their programs, and that the conference was worth the time invested. We learned the following lessons from the experience:

- Having representatives from both laboratory and epidemiology groups was essential for examining the interdependent systems involved in foodborne disease surveillance.
- The brainstorming and group discussions were key to reaching the conference goals, and could have taken up a greater proportion of the available time.
- The presence of representatives from multiple states was important for cross-fertilization of ideas. In the future, we will have each state prepare detailed presentations in advance
- The small conference size allowed people to feel more comfortable discussing ideas and to get to know each other.

- **Future plans:** The Minnesota Department of Health would like to have a Midwest-area conference at least every other year. We will also be sending a survey to all of the participants to determine what tangible changes (if any) have been made at the participating health departments. CDC

Editor's note: *APHL and CDC are willing to assist with the logistics of holding similar meetings involving PulseNet laboratory staff, foodborne epidemiologists, and Laboratory Directors in the other PulseNet areas. Funding for such meetings should come from area laboratory allocations appropriated through the ELC grants. Interested area laboratories can contact Shari Rolando at APHL.*



BE ON THE LOOKOUT FOR A *SPECIAL ISSUE* OF PULSENET NEWS HIGHLIGHTING PULSENET ASIA PACIFIC!

A few articles that will be featured:

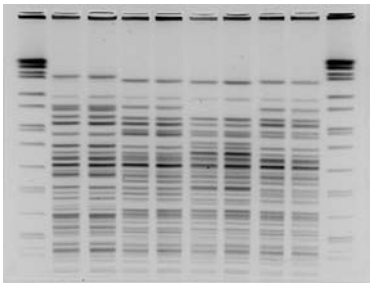
- The importance of PulseNet Asia Pacific to global infectious disease
- PulseNet Asia Pacific workshop: held in Hong Kong in March 2004
- PulseNet in Japan
- PulseNet in Taiwan

Updates on PFGE protocols for the typing of *Yersinia pestis* and *Francisella tularensis*

Kristy A. Kubota, MPH, Research Microbiologist, PulseNet, Centers for Disease Control and Prevention, Fort Collins, CO

The Bacterial Zoonosis Branch, in collaboration with the Foodborne and Diarrheal Diseases Branch (FDDB) at CDC, has begun efforts to include *F. tularensis* and *Y. pestis* PFGE subtyping in PulseNet. For the past year, Kristy Kubota has been working with the Bacterial Zoonosis Branch in Fort Collins, CO, to develop PulseNet PFGE protocols for these organisms.

In August, PulseNet laboratories in Colorado, Wyoming, and New Mexico's Los Alamos National Laboratory participated in the evaluation of the *Y. pestis* PFGE protocol. Each laboratory was given the protocol along with a set of PFGE plugs of both *Y. pestis* and *Salmonella* Braenderup H9812 standard strain. Laboratories were instructed to make plugs of an attenuated strain of *Y. pestis* and H9812, which the laboratories already had, and restrict them with the plugs that were sent to them. All *Y. pestis* plugs were restricted with



strains of Y. pestis

*Asc*I enzyme, and *Xba*I was used for the H9812 strain. The protocol worked well for the laboratories with minimal problems. As a follow-up to this study, Kristy trained three laboratorians at Los Alamos National Laboratories in PFGE and BioNumerics in December. Additionally, the Bacterial Zoonosis Branch currently is working with Applied Maths for customization of scripts for a *Y. pestis* database.

Work has begun on the development of a PFGE typing protocol for *F. tularensis*. Currently, we are evaluating *Bln*I, *Pme*I, and *Bam*HI enzymes as possible candidates for an *F. tularensis* PFGE database. It appears from initial work that *F. tularensis* has quite limited genetic diversity, as seen with a clonal PFGE pattern of type-B isolates. However, *F. tularensis* type-A PFGE patterns seem to be a little more diverse, as several patterns are seen throughout the United States. Our lab is in the process of evaluating more enzymes and associated electrophoresis conditions. Additionally, we plan to evaluate multi-locus variable number tandem repeat analysis (MLVA) to determine if more polymorphisms are seen with this technique. If anyone is interested in evaluating the PFGE protocol for *F. tularensis*, please contact Kristy Kubota at 970-266-3559 or e-mail at kkubota@cdc.gov. CDC

South Carolina

Molecular Epidemiology Group

Lucy Scarborough, Dr. Adam Leaphart, Dr. Jennifer Fraylick-Meredith and Dr. Art Wozniak, South Carolina Department of Health and Environmental Control, Columbia, South Carolina

Within the South Carolina Department of Health and Environmental Control, the Bureau of Laboratories serves as the state public health reference laboratory and is responsible for providing laboratory support essential for monitoring infectious disease outbreaks and emerging infections. Under the Division of Diagnostic Microbiology, the Molecular Epidemiology Laboratory is charged with providing rapid and effective molecular-based laboratory testing for surveillance and response to emerging infectious diseases and other public health or bioterrorism threats.

The Molecular Epidemiology Lab began its participation in PulseNet in 1998, and is responsible for the molecular typing of all foodborne and antimicrobial-resistant, nosocomial pathogens in the state. We routinely use pulsed-field gel electrophoresis (PFGE) to analyze isolates referred by our Bacteriology Reference Laboratory and constituent healthcare facilities. During fiscal year 2003, the number of isolates submitted for molecular typing by PFGE, including foodborne and nosocomial isolates, increased from 904 isolates in 2002 to a total of 1,936. This 47% increase in PFGE workload was the result of an 11% increase in the total number of *Salmonella* serotype strains typed, and a 43% increase in the total number of antibiotic resistant strains typed from fiscal year 2002 to fiscal year 2003.

The most common foodborne pathogens analyzed by molecular strain typing included *Salmonella* (24 serotypes), *Shigella sonnei, Escherichia coli* O157:H7, and *Listeria monocytogenes*. The dramatic increase in the number of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) isolates submitted for PFGE analysis was likely the result of an increase in the number of medical facilities submitting isolates. The increase indicates the continued strengthening of antimicrobial resistance surveillance in the state. Additionally oxacillin-resistant *Staphylococcus aureus* (ORSA) fingerprint patterns continued to be submitted to the CDC ORSA on PulseNet (OPN). This effort is of particular importance as the Bureau of Laboratories continues to partner with South Carolina hospitals in investigations of ORSA. In addition to foodborne and antibiotic-resistant pathogens, other organisms less commonly studied by molecular strain typing included *Enterococcus spp., Campylobacter jejuni*, Group A *Streptococci, Neisseria meningitidis, Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens, Streptococcus pneumoniae*, and *Yersinia enterocolitica*, totaling 63 isolates.

Polymerase chain reaction (PCR) technology was introduced to the lab in 1995, and the capability for performing reverse transcriptase PCR (RT-PCR) and DNA sequence analysis was introduced shortly thereafter. These methodologies were first used in 2001 for the identification of Norovirus infection in cases of gastroenteritis where no bacterial pathogen could be detected. Several Norovirus outbreaks have subsequently been identified. Currently we utilize real-time RT-PCR for seasonal Arboviral surveillance of mosquito pools, birds, horses, and humans. The use of this technology has resulted in the detection and characterization of West Nile Virus, Eastern equine encephalitis, St. Louis encephalitis, LaCrosse, and California serogroup viruses in South Carolina. Fastidious or biochemically inert bacteria and slow-growing, non-fermenting mycology isolates are also identified in a more expeditious manner by the DNA sequencing of a portion of the 16S and D2 genes, respectively. Another important gene used to characterize bacterial strains is the *emm* gene of *Streptococcus pyogenes*, which encodes the M protein that is thought to be responsible for the M serotypes of *S. pyogenes*. We have found that genotypic *emm* sequence typing is an important epidemiologic tool for distinguishing the strains of *S. pyogenes* that cause Group A streptococcal invasive disease (GAS).

The Molecular Epidemiology section has proven invaluable in the mission of public health laboratory preparedness for and response to bioterrorism, and continues to conduct validation studies of the Laboratory Response Network PCR protocols for agents of bioterrorism.

There are currently five full-time technologists in the Molecular Epidemiology Laboratory (Lucy Scarborough - Program Manager, Kay Passauer, Melissa Langmo, Amanda Moore, and Lynn Lane). Adam Leaphart, Ph.D., and Jenny Fraylick-Meredith, Ph.D., also collaborate with the laboratory on special projects.

PulseNet™ News

PulseNet Latin America Gets Off to a Flying Start

Bala Swaminathan, Ph.D. and Efrain Ribot, Ph.D., Centers for Disease Control and Prevention; and Sharon Rolando, MHS, MT(ASCP) PulseNet Program Manager, Association of Public Health Laboratories, Washington, DC

Argentina Meeting Participants (above) Teams hard at work during a Breakout session (below)

A meeting in Buenos Aires, Argentina, on December 1 and 2, 2003, to explore regional interest in establishing a laboratory subtyping-based network for foodborne disease surveillance modeled after CDC's PulseNet.

At the request of CDC, *Instituto Panamericano de Protección de Alimentos y Zoonosis, Argentina* (INPPAZ: Dr. Claudio Almeida, Director, and Dr. Enrique Pérez-Gutiérrez) and *Instituto Nacional de Enfermedades Infecciosas ANLIS "Carlos G. Malbran"* (Institute Malbran: Dr. Norma Binzstein and Dr. Marta Rivas) organized a meeting in Buenos Aires, Argentina, on December 1st and 2nd, 2003, to explore regional interest in establishing a laboratory subtyping-based network for foodborne disease surveillance modeled after CDC's PulseNet. Ten South American countries and three Central American countries were represented at the meeting. Dr. Lisa Indar represented the Caribbean Epidemiology Centre at the meeting on December 2nd, and Dr. Christopher Braden, Dr. Efrain Ribot, Richard Skibicki, Dr. Bala Swaminathan, and Jennifer Stevenson represented CDC. On the first day, the CDC representatives, Sharon Rolando (APHL) and John Besser (Minnesota State Public Health Laboratory) presented information on how foodborne disease surveillance is set up in the United States and described the central role played by PulseNet in outbreak detection and investigation. The U.S. team also answered questions from the attendees. This was followed by discussions on the status of foodborne disease surveillance in Latin American countries and of epidemiologic and laboratory capacities in different countries in the region.

The next morning, the representatives were divided into three groups and members of each group participated in two brainstorming sessions – one on the advantages of establishing a PulseNet-compatible network in the region, and the other on identifying barriers to the establishment of such a network. After the group brainstorming sessions, all participants convened in plenary sessions in which each group presented a summary of its deliberations and outcomes. From the plenary session, the following findings emerged:

1. It would be beneficial and cost-effective for the countries in the region to establish a PulseNet-compatible network for foodborne disease surveillance. Such a network would have a direct positive impact on improving the safety of foods produced in the region and would also positively impact tourism.
2. Countries in the region vary in their epidemiologic and laboratory capacities and can be divided into three categories:
   Category A: Countries with national laboratories that have adequate laboratory and epidemiologic capacities to immediately begin implementing a subtype-based surveillance system.
   Category B: Countries with national laboratories that need some enhancement to their epidemiologic and laboratory capacities.
   Category C: Countries with national laboratories that need significant enhancements to their epidemiologic and laboratory capacities before they can participate in a subtype-based surveillance network.
3. External funding sources need to be identified to assist countries in categories *B* and *C* above to enhance their epidemiologic and laboratory capacities. Also, funding needs to be identified for establishing a central server system for the network and maintaining the databases.

Based on these findings, the participants developed an action plan for the establishment of PulseNet Latin America. CDC will assist INPPAZ in identifying potential sources of funds for item 3 above. Countries that fell in category *A* will begin the process of establishing a PulseNet-compatible subtyping system in their countries by attending a PulseNet workshop in Buenos Aires in July 2004 (to be jointly conducted by CDC and Malbran Institute under the coordination of INPPAZ/OPS-OMS) and working toward obtaining PulseNet certification. Representatives from these countries would become trainers for the other countries when they are ready to join the network.

In summary, the results of the two-day meeting exceeded our highest expectations. By the time the meeting was concluded, the representatives from the Latin American countries were enthusiastic about participating in the PulseNet Latin America network and promised to lobby their administrators for funding. INPPAZ is poised to play a vital coordinating role in the project and will work closely with CDC and Malbran Institute to facilitate the establishment of the network. CDC

PulseNet and BioNet:

Joining Forces to Enhance Bioterrorism Response

Susan B. Hunter, M.S. (Foodborne and Diarrheal Diseases Branch) and David Bray (Bioterrorism Preparedness and Response Program, BPRP), Centers for Disease Control and Prevention, Atlanta, GA

In 1999, the Laboratory Response Network (LRN) was established to ensure state-of-the-art detection, diagnosis, and capacity to deal with select agents was in place. Recently, BioNet, a joint collaboration between the LRN and PulseNet, was begun.

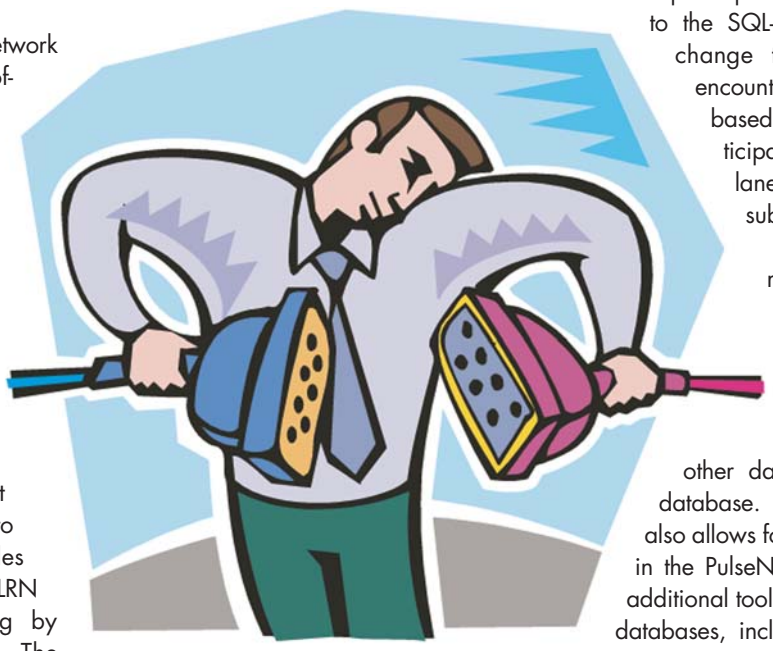
BioNet represents the collaboration between the LRN and PulseNet to further enhance the ability of the Centers for Disease Control and Prevention to rapidly detect and determine possible links between disease agents during terrorist attacks. At the heart of this collaboration is the idea that the LRN and PulseNet should be able to exchange data on similar samples received for confirmatory testing by LRN laboratories and molecular subtyping by PulseNet participating laboratories. The BioNet investigational effort will combine the rapid identification capacity of the LRN with the DNA "fingerprinting" capabilities of PulseNet. The information generated could be crucial to epidemiologists, public health officials, and criminal investigators during attacks using bacterial pathogens. The goals for BioNet are two-fold:

1. ***Increase cooperation between PulseNet and the LRN.***

2. ***Use a similar format for electronic reporting of results between the two systems to facilitate data exchange.***

For the PulseNet Database development, a contractor named Robert Long, has been recruited and is being funded by the CDC/NCID/BPRP to work with Susan Hunter to develop improved data sharing and reporting from the PulseNet databases. Robert will act as a liaison between the LRN personnel in BPRP and the Information Resources Management Office at CDC to enable PulseNet's use of the same electronic laboratory reporting format as in the LRN. Appropriate methods to share this data will be developed; special consideration will be given for the security requirements of both networks.

As a first step in the collaboration between PulseNet and LRN, funds were also made available to assist with the transition of the PulseNet *E. coli* National Database to SQL server format. The *E. coli* National Database was used as the development system and was tested with the assistance of volunteers from some PulseNet-participating laboratories during late 2003. On December 29, 2003, all of the current online databases were officially converted to SQL format. Earlier, new client scripts were sent to the PulseNet-participating laboratories so the certified participants would be ready to upload to the SQL-formatted databases. One change that certified participants encountered was the switch to TIFF-based uploading. This allows participants to upload one or all test lanes from a single TIFF in one submission.



The change to the SQL format marks a move to a common method of storing relational data. This common method will facilitate data sharing between the PulseNet database and other databases, such as the LRN database. The SQL database format also allows for a larger number of entries in the PulseNet databases and provides additional tools for the management of the databases, including the ability to import and export data in an XML format. The same technology and XML data sharing will then be used for other pathogens, including agents that may be used by bioterrorists. Efforts are underway to develop PulseNet databases for these pathogens. CDC

STATE, COUNTY AND CITY HEALTH DEPARTMENTS

State, County and City Public Health Laboratories

From around the nation, we welcome:

- **Shelley R. Stahl** earned her bachelor's degree in Biology at Indiana University in Bloomington. She worked previously in the Blood Lead Laboratory at the **Indiana State Department of Health**, and now she has joined the PFGE laboratory.
- **Carol Sandt** earned her Ph.D. in Biological Sciences at the University of Delaware in Newark, DE. She worked previously in the Department of Biochemistry and Molecular Biology at Penn State College of Medicine in Hershey, PA; she recently joined the **Pennsylvania Department of Health** PFGE laboratory.
- **Kimbley Lloyd** earned her bachelor's degree in Biology from Alcorn State University, Lorman, MS, and a bachelor's degree in Clinical Laboratory Science from University of Mississippi Medical Center, Jackson, MS. She joined the Special Microbiology team at the **Mississippi Public Health Laboratory** in November 2001, and is now taking over the PFGE responsibilities.

Farewells

- **Melanie Pace,** from the Mississippi Department of Health laboratory, has retired.
- **Sharon Abbott,** from the California Department of Health Services, has retired; however, the laboratory is fortunate to have her continue to work part-time on special projects.
- **Jennifer Mark**, from the California Department of Health Services, has left MDL to take a full-time position in the Viral and Rickettsial Diseases Laboratory. In the near future she plans to pursue a master's degree.

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Publications and Abstracts

- Hunter SB, Vauterin P, Gerner-Smidt P, Kubota K, Kincaid J, Hise KB and Swaminathan B. **Development of an Automated Provisional Pattern Naming System for PulseNet**. International Conference for Emerging Infectious Diseases (ICEID), presented in Atlanta, Georgia, March 2004.
- Lockett JL, Van Duyne MS, Flores SP, Smelser C, Head M, Jones J, Pupedis K, Bresler F. **PulseNet's Role in an Outbreak of *Salmonella Kiambu* Associated with Contaminated Beef Jerky.** International Conference for Emerging Infectious Diseases (ICEID) presented in Atlanta, Georgia, March 2004.
- Blanton EM, Sulka AC, Griffin PM, **Outbreaks from Refrigerated Ready-to-Eat Foods Prepared Outside the Home.** International Conference for Emerging Infectious Diseases (ICEID), presented in Atlanta, GA March 2004.
- Kretsinger K, Johnson GS, Lockett JL, FDA Working Group, Woodruff RS, Schoonmaker-Bopp D, Fry AM, Moore MR, **Multi-state Outbreak of Salmonella Muenchen Associated with Precut Melons, Eastern and Central United States, May – July, 2003.** International Conference for Emerging Infectious Diseases (ICEID), presented in Atlanta, GA March 2004.
- Munro J, Ng L, PulseNet Canada Laboratories, **The Evolution of PulseNet Canada: The National Molecular Subtyping Network.** International Conference for Emerging Infectious Diseases (ICEID), presented in Atlanta, GA March 2004.
- Perez NE, Sulka AC, Braden C, **Camp-associated Foodborne Disease Outbreaks in the United States.** International Conference for Emerging Infectious Diseases (ICEID), presented in Atlanta, GA March 2004.

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