Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center
Spring/Summer 2002

The Front-lines of Public Health.

The anthrax bioterrorist attacks this past fall clearly demonstrated that any laboratory in the state could be called upon to provide Public Health related services to citizens and health care providers. From our perspective, the private or clinical laboratories are truly on the front lines of public health and one of our responsibilities is to provide current information about emergencies such as those related to bioterrorism and emerging infectious diseases. The NPHL received questions, phone calls and in many cases, specimens from all areas of the state. We were especially fortunate in Nebraska to have received support from the CDC and Association of Public Health Laboratories to participate in an outreach program called the National Laboratory System. As a result, Mr. Tony Sambol was available to visit laboratories throughout the state following the bioterrorist incidents and deliver protocols or answer questions that various technologists had about safety and isolation procedures. Tony developed a CD-ROM containing protocols and other information that was so successful it was adopted by the CDC and eventually delivered to over 4,500 laboratories throughout the United States after 9/11..

We are now in the process of organizing a state-wide network to facilitate communication and assess laboratory capability in all regions of the state. Such a network will be important for handling new challenges in the future as well as for our daily efforts at improving public health. The ability to utilize state of the art information technology is one of the key components of a health monitoring program and we will work to provide information using the web, but we also recognize that many technologists do not have access to the internet at the workbench and therefore alternative approaches are also necessary. In recognition of the continuing need for information, this edition of our newsletter summarizes key topics ranging from a new strain of methicillin-resistant *Staphylococcus* to West Nile virus to irradiation of the US mail. We invite your comments and would be pleased to find an answer to any questions that might arise. *Steven Hinrichs, M.D., Director NPHL*

Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Nebraska

Paul Fey, Ph.D.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an ever-increasing hospital infection-control problem. Currently in the United States, approximately 50% of all *S. aureus* isolates from intensive care units are resistant to methicillin (or oxacillin). MRSA isolates are also typically resistant to multiple non-*B*-lactam anti-staphylococcal antibiotics. Therefore, vancomycin is typically administered to patients with MRSA infections. Even though the prevalence of MRSA in hospitals is high, staphylococcal infections that are acquired in the community setting are typically methicillin-susceptible. Recently, however, community-acquired MRSA (CA-MRSA)

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Banking of Isolates for Epidemiological Purposes

Peter C. Iwen, Ph.D.

Large scale banking of clinical isolates at the NPHL began in the fall of 1997, shortly following the move of the public health laboratory from Lincoln to UNMC. The original goal of the banking program was to obtain *Salmonella* isolates from throughout the state for serotyping. Since that time, other isolates of epidemiological importance have been added to the submission list. In 2001, over 650 isolates were forwarded to the NPHL which represented 11 different genera of bacteria **(Table 1).**

The main goal for the banking program today is to provide typing information for outbreak investigations and to evaluate antibiotic resistance trends as they

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NPHL Investigates Effects of Sterilizing Irradiation on Newborn Screening Tests

Douglas F. Stickle, Ph.D.

Contamination of U.S. Senate offices by anthrax spores delivered through the mail prompted the United States Postal Service (USPS) to institute a program of sterilization of mail for the Washington, D.C. area. This program was initiated in October 2001. Sterilization is being performed by irradiation. The level of irradiation needed to kill spore-forming bacteria such as *Bacillus anthracis* is known to affect the integrity of diverse items that may be sent though the mail, including electronics, film, food, and paper as well as medical diagnostic test kits and biological samples.

Among approximately 25,000 bloodspot specimens per year tested by the Nebraska Newborn Screening Program, as many as 10% of these samples are transported to the testing laboratories via the USPS. The potential effects of irradiation on bloodspot newborn screening tests were unknown. Although there are currently no plans by the USPS to irradiate mail in Nebraska, irradiation effects on newborn screening bloodspots were investigated by the University of Nebraska Medical Center in collaboration with Quest Diagnostics in Lincoln.

The study found that enzyme activities (biotinidase and galactose-1-phosphate uridyl transferase), and concentrations of the hormones thyroxine and thyrotropin, were reduced by irradiation, but that the reductions due to irradiation would be unlikely to cause false negative screening results for those tests. However, it was found that degradation of hemoglobins by irradiation would likely cause the interpretation of hemoglobinopathy screening to indicate the possible presence of hemoglobin variants, a result that would

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"CA-MRSA"

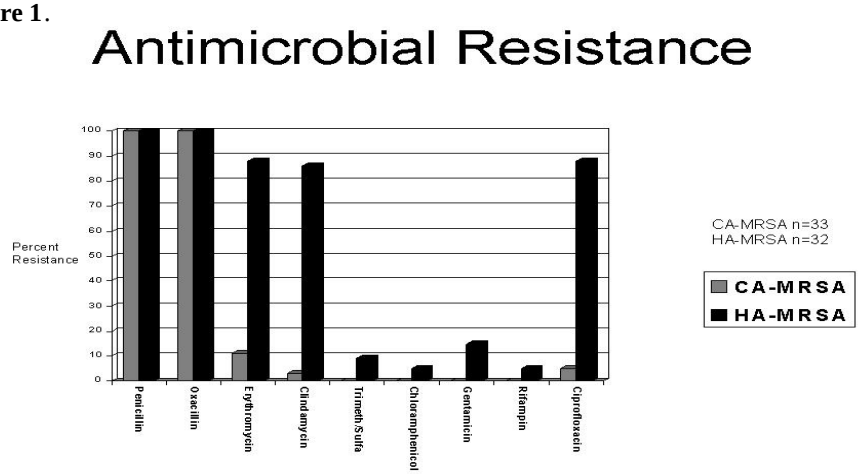
have been associated with several pediatric fatalities in the northern great plains region of the United States (1). Two recent epidemiological studies have demonstrated that the majority of CA-MRSA are generally susceptible to non-*B*-lactam antibiotics, found typically in skin and soft tissue infections, and appear highly-related as assessed by pulsed-field gel electrophoresis (PFGE) (2,3).

One of the questions surrounding the evolution of CA-MRSA is whether they are actually "escapees" from the hospital environment or whether the gene (*mecA*) that mediates methicillin-resistance has been recently acquired by a previously methicillin-susceptible *S. aureus* strain that is endemic in certain communities. The NPHL has begun to address this question by studying CA-MRSA isolated from Thurston County, Nebraska in 1998-1999. CA-MRSA isolates (33) were compared with 32 hospital-associated MRSA (HA-MRSA) using antibiotic susceptibility testing, PFGE, superantigen production, and *spa* typing. As shown in **Figure 1** and by others, CA-MRSA are more susceptible to non-*B*-lactam antibiotics compared to HA-MRSA. Of note was the small percentage of resistance to erythromycin, clindamycin, and ciprofloxacin in CA-MRSA compared to HA-MRSA. Superantigen typing demonstrated that 32/33 CA-MRSA isolates produced either SEB or SEC toxin. Five isolates (15%) produced SEB while 26 (81%) produced SEC. No isolates produced Toxic Shock Syndrom Toxin (TSST-1). In contrast, no HA-MRSA isolates produced SEB, SEC, or TSST-1. Both SEB and SEC have been implicated as the causative agents of non-menstrual TSST (nmTSST). As a further distinction, both PFGE and *spa* typing suggested that all CA-MRSA strains were highly-related yet distinct from HA-MRSA. The CA-MRSA from Nebraska were also highly-related or identical to those strains from the northern great plains that caused pediatric fatalities. These data suggest that CA-MRSA strains are a unique strain of MRSA, distinct from HA-MRSA, that are circulating in certain communities capable of causing serious disease. Recent work by Ma, etal. demonstrated that the *mec* element itself is distinct in CA-MRSA giving further credence to the notion that CA-MRSA are actually previously methicillin-susceptible *S. aureus* strains that have recently acquired the *mecA* gene; and not "escapees" from the hospital environment (4).

Dr. Tom Safranek, State Epidemiologist, has asked for your help in determining the prevalence of CA-MRSA in Nebraska through the submission of erythromycin-susceptible MRSA isolates to the NPHL for further study. Currently, approximately 20% of these erythromycin-susceptible MRSA isolates have the "CA-MRSA signature" as described. The NPHL appreciates your collaboration in this effort and would like to continue to receive erythromycin-susceptible MRSA isolates collected in your laboratory.

1. Centers for Disease Control and Prevention. 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*--- Minnesota and North Dakota, 1997-1999. Morb. Mortal. Wkly. Rep. 48:707-710.
2. Groom AV, Wolsey DH, Naimi, TS, et al. 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian Community. JAMA;286:1201-1205.
3. Naimi TS, LdDell KH, Boxrud DJ, etal. 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* Minnesota, 1996-1998. Clin. Infect. Dis.;33:990-996.
4. Ma, X.X., Ito, T., Tiensasitorn, C. etal. 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. 46:1147-1152.

Figure 1.



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"Effects of Sterilizing Irradiation"

require follow-up testing. Overall, the study concluded that alternative means of transport for newborn screening bloodspots would be advisable were irradiation of USPS mail be undertaken on a large scale. A report of this NPHL study will be presented at the 2002 National Meeting of the American Association for Clinical Chemistry, July 28-August 1, Orlando, FL.

Detailed information about the USPS irradiation program, and about the means and effects of sterilizing irradiation, can be found in the following sources:

Barnaby JF, Feder AC. A nation challenged: killing anthrax. New York Times 2001, October 25.

Day TG, Government postal service customer announcement, October, 2001 (*http://www.usda.gov/dalusdaoperations/USPS.htm*).

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New Screening Method for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Amy Armbrust, MT(ASCP)

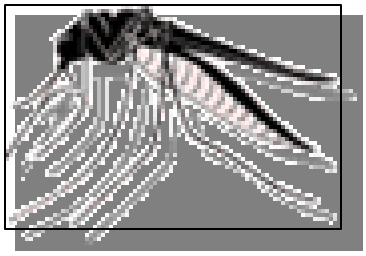
Beginning January 22, a new test for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was implemented. The test uses amplified DNA technology to improve sensitivity and allow detection of these organisms from urine as well as swab specimens. Because the collection of urine sample does not require special examination facilities it is expected that more screening can be performed in the population at highest risk, including sexually active males and females under the age of twenty. The establishment of a urine screening program at any clinic requires approval by Phil Medina, the STD Program Coordinator. Clinical studies have shown the value of using an amplified procedure to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae.* The performance characteristics of the Becton Dickinson Probetec assay were abstracted from published studies. (**Table 1**) The amplified test is more sensitive and specific than either culture or the previous GenProbe assay, but the positive predictive value of the test will vary depending on the prevalence of the organism in the patient population, whether the patient is male or female and the type of specimen collected. Large amounts of blood and mucus may interfere with the assay as well as medications that color the urine orange. Specimens can be submitted from either males or females using the swab collection kit or a voided urine sample in a sterile container.

Table 1. Chamydia/Gonorrhea Performance Values

Chlamydia	Sensitivity	Specificity
Male urine	93%	95%
Male swab	92.5%	95%
Female urine	80.5%	96%
Female swab	92.8%	96%
Gonorrhea	**Sensitivity**	**Specificity**
Male urine	97%	96%
Male swab	98.5%	96%
Female urine	84.9%	98%
Female swab	96.6%	98%

The Chlamydia/Gonorrhea Amplified Probe test will be performed Monday through Friday with results available the same day on specimens received by 6 AM. Specimen requirements are as follows: swabs must be collected with the Probetec, gender specific collection kit. The swab must be labeled with patient identification, date and time of collection. The eyes, throat and rectum are not approved sites and require culture methods for organism recovery. If a legal or criminal action is anticipated, culture is the appropriate method. Swabs can be maintained at room temperature for up to five days after collection. Urine must be refrigerated and not frozen and also received within five days by the laboratory.

The company that provides the Probetec assay is planning on introducing a liquid transport media similar to that used by the GenProbe assay. This change is expected to occur in September and more information will be available in the future.



NPHL Arbovirus Surveillance Activities

Tony Sambol, M.S.

In 2001, the Nebraska Public Health Laboratory (NPHL) finished the second year of diagnostic laboratory testing for arbovirus detection in Nebraska. The Nebraska Health and Human Services System (NHHSS), in cooperation with the Centers for Disease Control and Prevention (CDC), had contracted with the NPHL to provide this service. This activity is part of the CDC Cooperative Agreement for West Nile Virus Surveillance and Epidemiological Project that was started in July 2000 by the Nebraska State Medical Entomologist, Dr. Wayne Kramer.

The arbovirus family is a group of over 500 viruses that are transmitted by arthropod insects, most commonly the mosquito, with approximately 150 associated with human illness. Symptoms of arbovirus infection range from subclinical, to a mild febrile illness with headache, to encephalitis. Severe infections can cause lingering neurological sequlae, which can rarely result in death of the infected individual.

WNV, which had been restricted to Africa, west Asia and southern Europe, was detected in the summer of 1999 for the first time in North America in the New York City area it has been associated with crows, house sparrows and other birds which are susceptible to WNV and can act as carriers of the virus once infected. WNV has now been detected in approximately twenty-four states, ranging from New York to Florida, and has traveled as far west as the Mississippi River. WNV was detected in a diseased crow in Davenport, IA last Fall. (See Figure 1) Epidemiologists will continue to watch the further spread in of WNV into the western United States.

Arboviruses that are indigenous to Nebraska include the St. Louis encephalitis (SLE) and Western equine encephalitis (WEE) viruses. According to the CDC, there have been 27 laboratory-confirmed human cases of WEE and 14 cases of SLE in Nebraska since 1964. Dr. Kramer, using funds provided by the CDC, detected SLE in two mosquito specimen pools out of 1,359 pools collected in Scotts Bluff County in 1994 and 1995. In 1995, 36 out of 2,788 mosquito specimen pools tested were positive for WEE. Thirty of the 36 positive mosquito pools were collected in Scotts Bluff County, with four from the City of Norfolk and two from the City of Grand Island.

Arbovirus surveillance for SLE, WEE, and WNV will continue to be included in the testing done in Nebraska. During the past two years, Dr. Kramer directed the statewide collection of surveillance samples for testing. Mosquito trapping and "sentinel" chicken sera specimens were conducted in seven cities throughout Nebraska including Omaha, Bellevue, Lincoln, Norfolk, Grand Island, North Platte and Scottsbluff.

During the 2001 season, the NPHL Special Pathogens Laboratory tested 706 sentinel" chicken sera for arbovirus antibodies. Approximately 40,000 mosquitoes were trapped and identified for testing, with approximately 36,000 of these

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"NPHL Arbovirus Surveillance"

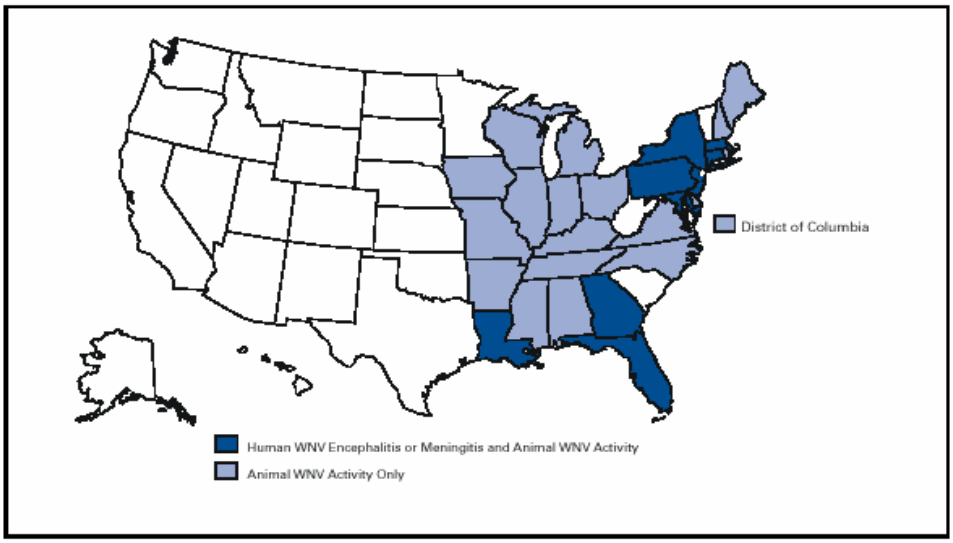
being *Culex tarsalis,* the mosquito species capable of transmitting WNV.

Following the CDC's recommendations, arbovirus surveillance activities for the detection of SLE, WEE, and WNV in Nebraska will include the following:

- active bird surveillance monitoring of chickens "sentinel camps" located throughout the state,
- active surveillance sampling of mosquito populations located near these "sentinel camps",
- enhanced veterinary surveillance by alerting veterinarians to monitor the presence of neurological diseases in birds, horses, and other animals,
- enhanced human surveillance by alerting health care providers to monitor for cases of encephalitis and aseptic meningitis
- dead bird surveillance and testing of appropriate specimens.

One goal of Dr. Kramer is to enhance statewide educational efforts concerning arbovirus detection and surveillance. These efforts will be directed towards the public, physicians, veterinarians, local health department personnel, and the agencies involved in mosquito and animal control. It is important that clinical laboratory personal have a general understanding of the WNV surveillance program, as they may be contacted by the public. In this regard, the public should be told that large birds found dead are usually not appropriate for testing because fresh tissue is needed. Specific questions can be directed to Dr. Kramer. The combined efforts of all laboratories will assist various programs within the NHHSS network to build upon the existing infrastructure of arbovirus surveillance. Through the continuing work that is being done, the enhanced planning and development of an arbovirus surveillance and response program will aid in the prevention and/or control of arboviral outbreaks within Nebraska.

FIGURE 1. Areas reporting West Nile virus (WNV) activity — United States, 2001*



* As of November 20, 2001.

Histoplasmosis in Nebraska

Peter C. Iwen, Ph.D.

Although histoplasmosis is not a reportable disease in Nebraska, laboratory observations suggest that the incidence of this disease has increased over the past few years. (Iwen, PC and SH Hinrichs. 2000. Expanding endemic area for *Histoplasma capsulatum*? ASM News, 66: 588.)

The dimorphic fungus causing this disease, *Histoplasma capsulatum* is considered geographically restricted to the soil in an area of the United States that is an approximate 600 miles radius from the point of merger of the Ohio and Mississippi Rivers. The southeastern corner of Nebraska as far north as Omaha is located on the extreme northwestern edge of this endemic zone. However, numerous cases of histoplasmosis have been diagnosed through laboratory testing of samples collected from patients residing in the central and western parts of Nebraska, suggesting that the endemic area may be larger than previously expected.

To evaluate this observation further, the NPHL has developed a molecular assay for the typing of *H. capsulatum* into strains. The ability to recognize strains of this organism will help in an epidemiological evaluation for this disease.

To make this typing program successful, we are requesting that all laboratories throughout the state submit to the NPHL any *H. capsulatum* detected. These isolates can be submitted through the Regional Laboratory courier to the "Attention of Dr. Peter Iwen". Submit the isolate on Sabouraud dextrose agar contained within a screw-capped tube. When sending, seal the tube with paraffin paper, double wrap the tube in plastic and seal tightly in a leak-proof container so breakage does not occur upon transport. The *H. capsulatum* may be submitted along with the other isolates that are sent for banking.

To facilitate an epidemiological investigation, we are asking that the laboratories maintain a patient source record of the isolates detected. Any questions concerning this can be directed to Dr. Iwen at 559-7774 or by e-mail at piwen@unmc.edu.

The Nebraska Public Health Laboratory Newsletter is a publication of the Department of Pathology and Microbiology, Samuel M. Cohen, M.D., Ph.D., Professor and Chairman, at the University of Nebraska Medical Center. The views expressed here do not necessarily reflect the opinions of the Nebraska State Department of Health and Human Services.

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Please direct suggestions, questions, or comments to: Editor, NPHL Newsletter, Department of Pathology and Microbiology, 981180 Nebraska Medical Center, Omaha, Ne., 68198-1180

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"Banking of Isolates"

appear and become more prevalent. The availability of these isolates also enables the NPHL to participate in the National Antimicrobial Resistance Monitoring System (NARMS) in collaboration with the CDC, the FDA, USDA, the Food Safety and Inspection Service, the Agricultural Research Service, and other state health departments. This program was developed as a mechanism to monitor antimicrobial resistance in enteric bacteria isolated from humans and animals. The information is used to define trends, essential for the initiation of prevention programs to limit the development and spread of antibiotic resistant microorganisms.

Over the past year, the NPHL has utilized isolates from this collection in a variety of studies. Pulse field gel electrophoresis (PFGE) typing is currently performed on all *E. coli* 0157:H7 isolates submitted to the laboratory. The PFGE patterns are posted electronically (PulseNet System) for a national tracking of the clonality concerning the spread of this pathogen. Local outbreak events, such as that which occurred with meningococcal disease in students from Crete and DeWitt, Nebraska (*Neisseria meningitidis* serogroup B) and the increase in numbers of *Shigella flexnerii* in the Omaha area, were also evaluated using PFGE typing. Susceptibility testing is currently performed on *Campylobacter* species and *Salmonella* serogroups to determine antimicrobial resistance treads within our state. The preliminary results of this testing showed the emergence of increased resistance within specific *Salmonella* serotypes, including resistance to cephalosporins Our results also showed that resistance to fluoroquinolones in both *Salmonella* and *Campylobacter* species does not appear to be a problem within our state at the present time. Finally, the serotyping of *Salmonella* has been useful as an epidemiological tool to evaluate antibiotic resistance within specific serotypes.

In addition to these ongoing evaluations, the NPHL will be studying molecular methods for the species identification of non*jejuni Campylobacter* and for the serotyping of *Salmonella*. The NPHL is also participating in a program with the CDC to study the serotypes of *Haemophilus influenzae* isolates that were associated with invasive disease in children aged <5 years.

To make the banking program a success, we continue to depend on and appreciate the support of laboratorians throughout the state. Listed in **Table 2** is an updated list of the organisms we would like submitted to the NPHL for banking. Questions concerning this program can be addressed to Dr. Peter Iwen at 559-7774 or

Table 1. Isolates submitted to the NPHL for banking, 2001.

Campylobacter spp.		334
Salmonella serogroups		153
Shigella sonnei		86
Escherichia coli 0157:H7		53
Staphylococcus aureus	(methicillin-resistant)	18
Neisseria meningitidis	(from sterile body site)	16
Shigella flexnerii		11
Haemophilus influenzae		8
Aeromonas hydrophilia/caviae		2
Group B *Streptococcus*		2
Listeria monocytogenes		2
Yersinia enterocolitica		2

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Florig HK. Is safe mail worth the price? Science 2002;295:1467-1468.

Hanson DJ. Zapping the mail. Chem Eng News 2002;80:30-32.

N'Gadi A. The effects on research specimens and museum collection items from electron beam irradiation of mail by the US Postal Service. Smithsonian Center for Materials Research and Education, November 2001 *(http://www.si.edu/scmre/mail_irrad*)

PLEASE NOTE:
For our long distance clients:

NPHL Client Services
1.800.334.0459

Table 2. Isolates to be routinely submitted to the NPHL for banking.[a,b]

Campylobacter spp.[c]	
Escherichia coli 0157:H7[d]	
Haemophilus influenzae[c]	(from sterile body site)
Listeria monocytogenes[d]	
Neisseria menigitidis[d]	(from sterile body site)
Salmonella serogroups[d]	
Shiga toxin positive stool culture filtrate[d]	
Shigella spp.	
Staphylococcus aureus[d]	(vancomycin nonsusceptible)
Streptococcus pneumoniae[c]	(from sterile body site)

[a]Isolates for submission to the CDC are first forwarded to the NPHL to facilitate shipping, handling, and result reporting.

[b]Banking may include any organism with an unusual susceptibility pattern or outbreak association per request of the Epidemiology Division of NHHSS.

[c]The viability of this organism rapidly decreases over time and it should be sub-cultured to fresh sheep blood agar (*Campylobacter* and *St. pnemoniea*) or chocolate agar (*N. meningitidis*) if >3 days prior to submission.

[d]This organism should be submitted as soon as possible after detection to facilitate epidemiological investigating.

[e]The NPHL will culture specimens that are shiga toxin positive to identify the toxin producing organisms. All E. coli 0157:H7 isolated are tested by PFGE.

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INSIDE THIS ISSUE:

Salmonella Serotyping in Nebraska, 2001.

Beth Knutson, MT(ASCP)

The CDC estimates that from 1 to 4 million cases of salonellosis occur each year in the U.S. with approximately 500 deaths. About 40,000 of these infections are confirmed by culture, with most isolates serotyped at state public health laboratories. The CDC tests about 1,000 problem isolates that are sent from the state public health labs each year for serotyping.

Currently, the CDC recognizes two species of *Salmonella*, *S. enterica* and *S. bongori*. *S. bongori* contains 18 serovars, and *S. enterica* contains the remaining 2300+ serovars, which are divided among 6 subspecies. The salmonellae are first classified by serogrouping based on their somatic (O) antigens, and then serotyped based on the flagellar (H) antigens present. To date, there are 2,435 serovars described by the CDC, with 20 to 35 new serotypes described each year by the World Health Organization (WHO). To serotype the salmonellae requires the availability of more than 250 O and H grouping, typing and single factor antisera. The CDC maintains an inventory of the O and H antisera needed to serotype the 100 most common isolates found in the U.S., and these reagents are available for the state public health laboratories to use.

Salmonella serotyping at the NPHL for 2001 included 16 different hospital and private laboratories throughout the state, that submitted 149 isolates (Table 1.) The top six serotypes identified in 2001 are listed in Table 2. Personnel from the NPHL have begun collaborating with the CDC to develop new molecular methods to serotype Salmonella which will increase accuracy and throughput in the future.

Table 1 NPHL *Salmonella* serotype tally, 2001.

Laboratory	Number	Location
Alegent Health	1	Omaha
Bergen Mercy Hospital	13	Omaha
Children's Hospital	7	Omaha
Fremont Area Medical Center	4	Fremont
Good Samaritan Hospital	4	Kearney
Great Plains Medical Center	9	North Platte
Methodist Hospital	11	Omaha
NHS	12	Omaha
Ehrling Berquist	1	Bellevue
Pathology Center	12	Omaha
Physicians Laboratory	16	Omaha
Quest	27	Lincoln
Regional West Medical Center	10	Scottsbluff
Shenandoah, Iowa	1	Shenandoah
St. Francis Medical Center	4	Grand Island
St Joseph's Hospital	7	Omaha

Table 2 Top six *Salmonella* serotypes isolated at NPHL.

Serotype	Serogroup	Number
Typhimurium var, Copenhagen	B	29
Enteriditis	D	25
Heidelburg	B	12
Typhimurium	B	12
Newport	C2	10
Braenderup	C1	7