Nebraska Public Health Laboratory Newsletter

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NPHL Updates

By Peter C. Iwen, PhD, D(ABMM), Associate Director, NPHL

Spring time for the laboratory brings a transition from the "respiratory season" to the "enteric and arboviral season". As is true with every season, the laboratory also remains prepared for the "unexpected" issues that can occur. Tony Sambol, Assistant Director of the Nebraska Public Health Laboratory (NPHL) provides an overview of some "unexpected" infectious diseases that have recently been encountered in Nebraska and the vigilance of the NPHL personnel to keep current in dealing with these diseases. Dr. Gerald Capraro, Clinical Microbiology Fellow, addresses the emergence of cholera in Haiti. This article provides guidance to the laboratorians on methods to identify *Vibrio cholerae* in the laboratory. Additionally, NPHL has recently validated a method to subtype the toxigenic strain of *V. cholerae* that is circulating in Haiti.

Healthcare reform is another topic that will certainly impact the laboratory of the future. An article by Scott Campbell, IT Consultant, gives a basic discussion of terminology used to help standardize laboratory reporting for electronic health records. This overview will continue in an upcoming edition of the newsletter as a method to help the laboratorian be more informed on the future of electronic accessioning and reporting.

To highlight our collaboration with our research partners at UNMC, Roxanne Alter, Research Coordinator in the Center for Staphylococcal Research, provides an overview on biofilm formation. Research is ongoing to better understand the genetics of biofilm formation for the management of patients with infections caused by biofilm-producing bacteria.

Finally, we remind you to reserve the dates of June 5-8th for the national Association of Public Health Laboratories (APHL) Annual Meeting & 5th State Environmental Laboratory Conference in Omaha at the Qwest Center. NPHL is host for this event and encourages our laboratory partners to participate in this activity. For program and registration information, consult the APHL website (http://www.aphl.org/profdev/conferences).

INSIDE THIS ISSUE:

Emerging and Reemerging Infectious Diseases in Nebraska

By Tony Sambol, MA SM(NRM), Assistant Director, NPHL

Before the events of 911, the Centers for Disease Control and Prevention (CDC) worked with public health laboratories (PHLs) to develop procedures to test for and confirm agents that could be used in a terrorist event. Although no bioterrorist event has happened since the anthrax attacks in October 2001, PHLs across the U.S. have tested environmental samples (all hoaxes to date) as well as animal, human, and environmental isolates of concern. In collaboration with our partners in the food, water and animal laboratories, isolates detected from human and animal specimens have been confirmed as *Brucella* spp., *Francisella tularensis*, and *Coxiella burnetti*. Additionally, many suspicious isolates for *Bacillus anthracis* and *Yersinia pestis* have been tested – all negative. While available to test for these special pathogens, personnel in the Nebraska Public Health Laboratory (NPHL) continue to work with the CDC and the state epidemiologist to prepare for the newly emerging or reemerging diseases that may affect our region. Reemerging vaccine preventable diseases such as measles, mumps, whooping cough, and chickenpox have become more common. Additionally, antimicrobial resistant organisms such as MRSA, VRE and MDR-MTB continues to be recognized on a routine basis.

As expected, Nebraska is no stranger to the possibility of encountering an emerging infectious disease. NPHL has tested for avian influenza A/H5 from a traveler to Thailand who returned ill. In another instance, an individual returning from Africa was suspected to have Rift Valley Fever. Although both cases were negative, these situations show the necessity of having the tests available to evaluate for these agents.

In 2009, Nebraska experienced an influenza epidemic caused by a new strain of virus called A/H1N1v. Within two weeks, the CDC had the reagents, equipment and supplies to PHLs to test for this emerging pathogen. As pertaining to arbovirus infections, a case of encephalitis caused by the eastern equine encephalitis (EEE) virus was detected in a horse where travel history was involved. Additionally, while the mosquito borne West Nile virus is now endemic in our state, occasional human cases of St. Louis encephalitis (SLE) virus, another mosquito borne virus, have been detected. Dengue fever has also been identified in a Nebraska resident who traveled to an endemic area. Finally, the lab continues to watch for cases of cholera from people travelling to Haiti for humanitarian relief work and malaria (*Plasmodium* spp.) which are known to occur in Nebraskans

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that have travelled outside the U.S.

Besides these diseases, the CDC's *Epi-X* and a private electronic publication called *ProMed Digest* daily list diseases or agents occurring throughout the world. Some notable pathogens include: avian influenza virus (A/H5), poliomyelitis virus, ceftriaxone and ciprofloxacin resistant *Shigella flexneri* 2a, hantavirus, Venezuelan equine encephalitis (VEE) virus, Rift Valley fever virus, Chikungunya virus, and yellow fever virus. Laboratorians should keep guard and continue to be vigilant in testing. The increase loss of animal habitat or frequency and ease of travel throughout the world, provide the means that any place in Nebraska could become the epicenter of a disease outbreak.

A Case Study in *Vibrio cholerae*

By Gerald A. Capraro, PhD Clinical Microbiology Fellow

Case Study

While in Haiti on a recent mission trip, a 30 year old female developed profuse watery diarrhea and severe dehydration. On physical exam the patient was alert but her pulse was non-palpable and her blood pressure was not measurable. Her intestinal sounds were prominent and she was afebrile. She went to a local hospital where limited selection of microbiological testing was done. No specialized media was used in the culture (only the basic blood agar, chocolate agar and MacConkey agar). The concerned was that the patient had cholera. How would you advise the microbiologists to test for *Vibrio cholerae*? (Hint: What easy biochemical test can be helpful in differentiating this organism from other enteric organisms?) What is the typical antibiogram of the *V. cholera* strain found in Haiti? If you suspected this organism and were at a hospital with more resources, what special media would you request be added to the routine stool culture?

Discussion

The outbreak strain of *Vibrio cholera* currently circulating in Haiti is toxigenic *V. cholerae* Type O1, serotype Ogawa, biotype El Tor. The first reported case of cholera in Haiti was on 21 October 2010 following the devastating earthquake that left the country with few medical resources. Aid workers from Nebraska and other parts of the U.S., that have been in Haiti providing medical and other assistance, are also at risk of acquiring this infection. Although *V. cholerae* is rarely identified in the clinical laboratory, this case is provided to assist microbiologists in the identification of *V. cholera* since a large number of individuals are travelling to Haiti from our region.

Specimen Collection and Transport

When *V. cholerae* is suspected, collect stool specimens during the acute stage of disease. Rectal swabs are acceptable specimens if stool is unavailable. Since *V. cholerae* is particularly susceptible to desiccation, inoculate the specimen to appropriate media within 2 to 4 hours; otherwise, place the stool in a commercially available transport media, such as Cary-Blair, to maintain viability of the organism. Specimens are transported at room temperature and are NOT refrigerated or frozen. Additionally, since large amounts of glycerol can be toxic to *Vibrio* spp., buffered glycerol in saline is unacceptable for transport.

Isolation and Identification

V. cholerae is a halophilic gram negative rod that can be curved, straight or comma-shaped on Gram stain. The organism is catalase and oxidase positive. *V. cholerae* will grow on MacConkey (MAC) agar as colorless colonies and on sheep blood agar (SBA) as small to medium (1 to 3 mm in diameter) nonhemolytic colonies that are smooth and opaque with a greenish hue. *V. cholerae* ferment sucrose and as a result, cannot be differentiated from other normal enteric flora on Hektoen-Enteric (HE) and xylose-lysine-desoxycholate (XLD) media. On *Vibrio*-selective thiosulfate-citrate-bile salt-sucrose (TCBS) agar, *V. cholerae* will ferment sucrose and produce yellow colonies. This property allows differentiation of *V. cholerae* from other non-sucrose fermenters such as *V. parahaemolyticus*, *V. mimicus* and most strains of *V. vulnificus* that will produce green colonies. Two words of caution regarding the use of TCBS media: 1) yellow colonies may become green after refrigeration and 2) oxidase testing is unreliable when performed directly on colonies growing on TCBS.

Clinical microbiology laboratories in non-endemic areas do not routinely include TCBS agar in a stool culture; however, some labs may maintain this media for cases of suspected *V. cholerae*. **Figure 1** provides a flow chart for the isolation and identification of *V. cholerae* for laboratories with and without TCBS. Laboratories that do not use TCBS should screen growth on SBA for oxidase positive colonies but should be mindful of other oxidase positive organisms (e.g., *Aeromonas* spp. and *Plesiomonas shigelloides*) that can cause gastrointestinal disease. The deoxycholate string test can be used to separate possible isolates of *Vibrio* spp. from *Aeromonas* spp. and *Plesiomonas shigelloides*. Susceptibility to O/129 can also be used to identify *Vibrio*. Regardless of whether TCBS is used, physicians must communicate the suspicion *V. cholerae* with the laboratory.

The scientific literature is replete with studies documenting that commercially available identification systems demonstrate variability to identify *Vibrio* spp. Currently, no commercial identification system has all 12 clinically relevant *Vibrio* spp. in their database. Thus, the flow chart in **Figure 1** is designed to help microbiologists presumptively identify *V. cholerae* without reliance on these systems (1). In 2003, O'Hara *et al*. reported on the accuracy of six commercial identification systems for *Vibrio* spp. (2). Systems included in this study were API 20E, Crystal E/NF, MicroScan Neg ID2 and Rapid Neg ID3, and Vitek GNI+ and ID-GNB. The authors stated that "extreme care must be taken in the interpretation of answers from these six commercially available systems for the identification of *Vibrio* species."

Finally, submit suspect *Vibrio* spp. to the public health laboratory for typing and toxin testing. Cholera is monitored under the CDC emerging infection program and *Vibrio* Surveillance System.

(Vibrio, continued on page 3)

(Vibrio, continued from page 2)

Susceptibility Testing

If *V. cholerae* is isolated and susceptibility testing is deemed to be necessary, please refer to CLSI document M100-S20 (relocated to CLSI M45-A2 in 2011) for guidance and interpretive criteria (3). The susceptibility profile of the *V. cholerae* outbreak strain from Haiti has been characterized and is available in **Table 1** and on the CDC website (4).

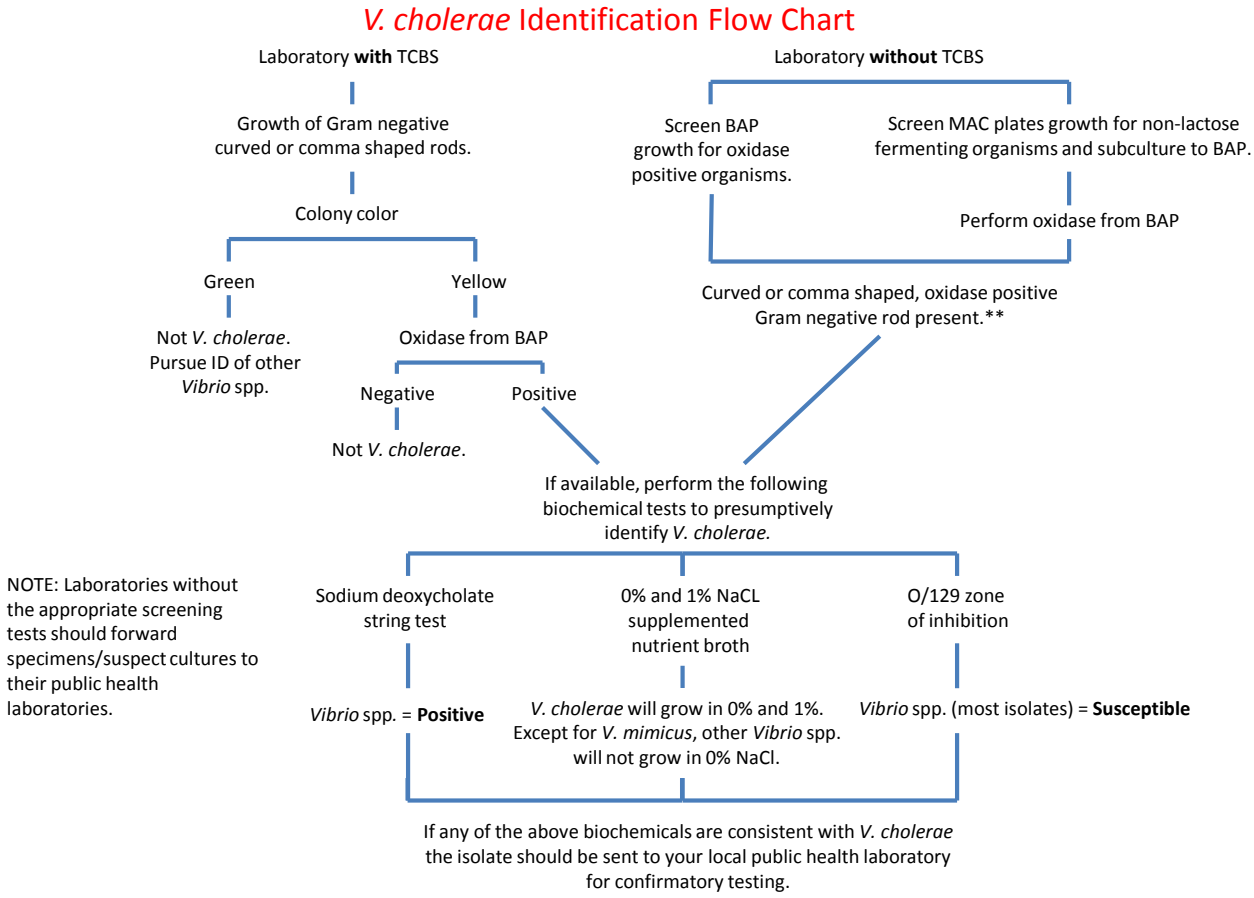
Reporting

Isolation of *V. cholerae* must be reported immediately to the attending physician and to hospital epidemiology/ infection control personnel, as well as to public health authorities.

References

1. American Society for Microbiology. 2011. A practical guidance document for the identification of *Vibrio cholerae*. Clinical Microbiology Issues Update. http://www.asm.org/index.php/policy/cmiu-1-11.html.
2. O'Hara, CM. *et al*. 2003. Accuracy of six commercially available systems for identification of members of the family *Vibrionaceae*. J Clin Micro 41: 5654 – 5659.
3. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. Wayne, PA: CLSI Document M100-S20
4. http://www.cdc.gov/cholera/index.html

Figure 1: Flow chart for the presumptive identification of *V. cholerae* from clinical specimens.



**In rare cases *Campylobacter* spp. may be recovered from non-microaerobic incubation. When curved, oxidase positive GNRs are recovered from BAP, the selective Campylobacter medium should also be checked to confirm that no *Campylobacter* species have been isolated.

Table 1: Antibiotic susceptibility profile of *V. cholerae*, biotype El Tor isolates circulating in the current cholera epidemic in Haiti.

Antibiotic	Interpretation
Azithromycin	Susceptible
Tetracycline	Susceptible
Doxycycline	Susceptible
Ciprofloxacin	Reduced Susceptibility
Sulfisoxazole	Resistant
Furazolidone	Resistant
Nalidixic Acid	Resistant

Brief Review of Terminology in Public Health, Part I

By W Scott Campbell, MBA, BS, IT Consultant

"A rose by any other name would smell so sweet"
W. Shakespeare

Shakespeare's famous line makes us wonder what is so important about a single word. In the public health laboratory, we may ask ourselves the same question. A specimen is received, a test performed, and a result issued, right? Surprisingly, it is not so easy.

Consider the following common phrase, "My child stayed home with a bug today." Most parents would understand that the child is home sick…or would they? Does the child have the cold bug, the stomach bug, or the flu bug? Or perhaps, the child has a pet beetle or a pet arachnid (not technically a bug)? Hmm, it's not so clear anymore. Now, imagine a real life lab experience. A specimen is collected from a patient with symptoms of fever, sore throat and a cough. The specimen is sent to the public health lab and tests positive for influenza. The result is sent to the patient's provider and to the state health department. We're done, right? Not so fast…what type of specimen was tested – sputum, blood, fecal? What was the source of the specimen – throat, nasopharyngeal swab? What type of influenza was detected – seasonal, H1N1 or H1N1 pandemic? How did you test the sample to reach that conclusion – rapid flu test, PCR, culture…? Imagine getting 100's or 1000's of results like this and then try to make sense of them. Here enters the world of medical terminology.

The Office of the National Coordinator, the U.S. Department of Health and Human Services Health IT Administration, the CDC and the Council of State and Territorial Epidemiologists (CSTE) have all acknowledged this issue and have come to agreement that some standard type of communicating lab tests and results is needed (i.e., a common terminology). In addition, this standard must be understood by computers to assist in managing the massive amount of information exchanged between provider and laboratory. The two standards selected by these governing bodies were SNOMED-CT (Systematized Nomenclature in Medicine – Clinical Terms) and LOINC (Logical Observation Identifiers Names and Codes). Both consist of alphanumeric codes that classify items into unique terms yet create links to other related items.

SNOMED-CT primarily addresses clinical findings associated with a patient or subject. In public health laboratories, SNOMED-CT addresses the ultimate finding or result of the test. For example, influenza virus (organism) is coded as 55014007, but influenza A virus subtype H1N1 is coded as 442352004.

LOINC, although similar to SNOMED-CT, deals with how specimens were tested and conclusions reached. The code for ordering a test to determine if influenza A H1 RNA is present using a probe and target amplification is 49520-0. In combination, LOINC and SNOWMED-CT make it possible for a computer to understand and present laboratory information to healthcare professionals without ambiguity.

By communicating lab tests and results in conjunction with SNOMED-CT and LOINC, important public health data can be quickly understood by state and national health officials with appropriate and timely responses as emerging events developed. The use of both SNOMED-CT and LOINC coding is being promoted by CMS (Centers for Medicare and Medicaid Services) by incenting healthcare providers and hospitals to employ electronic health records systems capable of communicating reportable lab findings electronically using these terminologies.

References:

1. Snomed-CT information: http://www.nlm.nih.gov/research/umls/Snomed/snomed_main.html
2. LOINC: www.loinc.org

Meet the Laboratorian – Jeanette Walter

Compiled by Karen Stiles MT(ASCP)SM, State Training Coordinator NPHL

This month, I had the pleasure to interview Jeanette, from McCook, Nebraska. Her supervisor, Joanne Allen, states she has been instrumental in the McCook microbiology laboratory.

What got you interested in pursuing a career in laboratory science? An English assignment in Junior High on careers. We had to write a paper on a career that was of interest. After researching a career as a medical technologist, I never considered a different career.

Where did you attend med tech school? Kearney State College for my college work and Lincoln General (before it merged with Bryan) for my training. Since the same pathology group was at both hospitals, I also rotationed at Bryan.

How long have you worked in your present location? I have been at McCook Community Hospital since June 1974. Prior to McCook, I worked 3 years at Good Samaritan in Kearney, when my husband was in school.

Are there any specific areas of the clinical laboratory that you have special interest or expertise? I would say a special interest would be in microbiology but nowhere close to expertise.

What do you think is the single biggest change in the laboratory since you started? Technology – going from labor intensive and time consuming testing for basic tests to automated and speedy testing for complex tests.

What do you like most about your job? Every day is something different with new challenges and I help people make their likes better.

What is unique about working at your facility? The ability to work in all areas of the laboratory. Also, we get to know the patients on a personal level because of the size of the community.

What do you see as future challenges for the field of medical technology? The field has become so complex with government regulations and new technology, which makes it impossible for a technologist to keep up with all subspecialities. We need to not lose track that we are there for the patients.

What advice would you give to a first year clinical laboratory scientist? Keep an open mind and learn to think outside the box when working in the laboratory and remember what you do affects the lives of your patients.

Biofilm: Impact on Clinical Laboratory Testing

By Roxanne Alter, MS, MT(ASCP), Research Coordinator; Center for Staphylococcal Research, UNMC

Clinical laboratory scientists are trained to think of a bacterium as a single-celled organism. However, as technology has become more sophisticated, our understanding of bacteria has progressed and we now realize that they are more complex organisms than once thought. In fact, bacteria are known to form highly structured communities called a biofilm, which attach to surfaces and are contained within a self-secreted, extracellular mesh of polysaccharides, protein and DNA. Bacteria growing in this biofilm are now recalcitrant to the action of antibiotics than cells growing in a planktonic state (1). Furthermore, biofilms are more resistant to the innate immune system of the host and may lead to chronic inflammation.

Donlan and Costerton (2) further define biofilm as a microbial community that exhibits an altered growth rate, which can withstand high shear environments and transcribe genes that help it evade the immune system with increasing virulence. Bacterial adaptation occurs through the highly developed orchestration of gene expression, as well as chemical and physical cues that rapidly set into motion an elaborate cascade of alterations in the cell's physiology and metabolism. Taken together, this biofilm armor protects the bacteria against many hazards.

In the laboratory, bacteria are typically evaluated in a "non-biofilm" population, such as colonies growing on an agar plate. Physiological and metabolic differences between cells growing in a planktonic state and those growing in a biofilm haven been reported (3). In many cases it is unclear how clinical microbiologist should identify and test bacteria isolated from a biofilm-mediated infection (4). Considerable diagnostic problems exist for the clinical laboratory when biofilm formation is taken into consideration. False-negative cultures that contain microscopically visible organisms; as well as difficult or inappropriate specimen collection techniques and recovery of biofilm-associated infections from swabs can occur. In addition, it is difficult to accurately predict antimicrobial susceptibility.

Due to the difficulty of identifying biofilm infections *in vivo*, an attempt has been made to outline criteria for diagnosis of biofilm infections from clinical specimens (2,5). The first step is the confirmation of an isolated infection along with a direct examination of tissue on the implanted device containing aggregates of bacteria in a matrix. Next, identification of failed antibiotic treatment with conflicting susceptibility of planktonic bacteria indicates the possible possible formation of biofilm. Finally, a failure of host clearance mechanisms is indicated when bacteria are found within host inflammatory cells.

Culturing of periprosthetic tissue is the standard method used for the microbiologic diagnosis of prosthetic-joint infection, but this method is neither sensitive nor specific. The Mayo Clinic studied 331 patients with total knee or hip prostheses, of which 252 had aseptic failure (6). In this study, cultures of samples obtained by sonication of prostheses was more sensitive than conventional prosthetic tissue culture for the microbiologic diagnosis of infection. Sonication of samples appeared to be advantageous in patients who had received antimicrobial therapy within 14 days before surgery.

Currently, under the direction of Dr. Paul Fey, comparative studies to examine failed explanted medical devices are being conducted using sonication and culture for bacterial biofilm formation. The results of testing are being compared to conventional culture methods to determine the best practice for studying bacterial biofilm.

References:

1. Steward, P.S., Costerton, J.W.: Antibiotic resistance of bacteria in biofilms. Lancet 2001, 358:135-8.
2. Donlon, R.M., Costerton, J.W.: Biofilms:survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev, 2002: 167-93.
3. Fey, P.D., Olson, M.E., Current concepts in biofilm formation of *Staphylococcus epidermidis*. Future Microbiol:2010,5:917-33.
4. Hall-Stoodley, L., Stoodley, P.: Evolving concepts in biofilm infections, Cell Microbiol 2009,11:1034-43.
5. Parsek, M.R., Singh, P.K.: Bacterial biofilms:an emerging link to disease pathogenesis. Ann Rev Microbiol 2003:57:677-701.
6. Patel, R., et. al. Sonication of removed hip and knee prostheses for the diagnosis of infection. New Engl J Med 2007:16:357-6.

2011 NPHL Upcoming events:

ASCLS Spring Meeting April 13-15

Nebraska Biological Challenge Set Exercise May & November

Packaging & Shipping Seminar Omaha, June 10 & Lincoln, June 13

Bioterrorism Preparedness Workshop TBA

Association of Public Health Laboratories (APHL) Upcoming Events:

APHL Annual Meeting and State Environmental Laboratory Conference

Omaha - June 5-8, 2011

Center for Preparedness Education Upcoming Events:

9th Annual Preparedness Symposia

April 19-20 Gering
May 10-11 Norfolk
June 13-14 Lincoln
July 26-27 Kearney

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IN THIS ISSUE

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