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# The Laboratorian SM

## PENICILLIN TOLERANCE IN GROUP B STREPTOCOCCUS

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Group B Streptococcus (*Streptococcus agalactiae*, GBS) is a gram-positive β-hemolytic bacterium that is the most common cause of neonatal blood infections and meningitis, and a frequent cause of pneumonia. Under the 2002 Centers for Disease Control (CDC) guidelines, pregnant women are screened for GBS at 35-37 weeks of gestation. Treatment recommendations for women who test positive for GBS are β-lactam antibiotics such as penicillin G, given at least 4 hours prior to delivery. Following the implementation of the CDC GBS screening guidelines, neonatal GBS disease declined from 1.7 per 1,000 live births in 1993 to 0.34 per 1,000 live births in 2005. Despite a dramatic drop in the incidence of infection in the United States, GBS remains a leading cause of newborn morbidity and mortality, resulting in an estimated 1,425 early onset cases and 63 deaths annually.

To date, no cases of penicillin resistance have been reported in GBS in the scientific literature. Our laboratory, however, has identified a phenotype of penicillin tolerance in which the microorganism is inhibited for growth in the presence of the drug, but remains viable for an extended period of time as compared to susceptible strains. These strains can then start propagating once the drug concentration decreases below the effective levels. The proposed bactericidal mechanism of penicillin on GBS and other gram-positive bacteria is initiated by the inhibition of penicillin binding proteins (PBPs). PBPs are enzymes that synthesize the bacterial peptidoglycan cell wall. The inhibition of the PBPs by penicillin triggers the up-regulation and secretion of large amounts of bacterial autolytic enzymes, resulting in cell wall degradation and cell lysis. Our hypothesis is that penicillin tolerant (PT) strains are more resistant to autolysin digestion due to differences in the construction of the cell wall or differences in the level of secreted autolysins. This phenomenon has been identified and reported in a number of other organisms, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus*

*pyogenes*, *Enterococcus faecalis* and *Mycobacterium tuberculosis*.

We examined clinical isolates of GBS, of which approximately 15.7% were found to be tolerant to penicillin and other β-lactam antibiotics, such as ampicillin and cefazolin. Whereas, non-β-lactams, such as erythromycin, clindamycin or vancomycin were still effective. We have identified two novel amino acid polymorphisms in a PBP found within approximately 50% of the penicillin tolerant GBS strains and are virtually absent in the penicillin susceptible GBS strains. These GBS tolerant strains, as well as a susceptible strain expressing the PBP tolerant gene, were found to be significantly more resistant to cell wall digestive enzymes and found to have evidence of structural changes in the peptidoglycan cell wall. This supports our hypothesis that penicillin tolerant strains are more resistant to autolysin digestion due to differences in the construction of the cell wall. These data have been submitted to a peer-reviewed journal for publication.

Currently, the mechanism(s) of the other 50% of the PT strains are under investigation. Here, tolerance may be due to differences in cell wall synthesis rate, cell wall thickness, autolysin secretion, or disruption in the penicillin-induced autolysin upregulation via a signal transduction mechanism. Additionally, investigating the incidence of PT GBS infections by collecting clinical isolates from mothers and their infected neonates is underway. Although increasing the duration of penicillin treatment will eventually kill PT GBS strains, this is not a practical option when dealing with labor and delivery. However, we hypothesize that by alerting physicians to the phenomena of penicillin tolerance we can directly affect patient care by prompting the prescription of alternative non-β-lactam antibiotics, such as erythromycin, clindamycin, or vancomycin, and further decrease the incidence of illnesses and deaths associated with newborns infected with GBS.

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### UPCOMING EVENTS ►►

- 10/1-3 | **ACOG: Annual District Meeting (District V)**  
Indianapolis, IN
- 10/1-4 | **ACOG: Annual District Meeting (Districts VIII and IX)**  
Napa, CA
- 10/16-18 | **ACOG: Annual District Meeting (Districts I & III)**  
Orlando, FL
- 10/16-18 | **ACOG: Annual District Meeting (District IV)**  
Asheville, NC

## LEGAL CORNER

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**Theresa K. Begley, Esq. Compliance Officer**

### Physician Signatures-Still Confusing

For some time, laboratories have been trying to make sense out of conflicting directives and communications from Medicare, Medicaid, and state licensing departments about whether or not the laboratory can process laboratory tests without a physician's signature on the test requisition.

Many labs, MDL included, have struggled with how to handle test requisitions that come to the lab unsigned. Should the lab hold the test results until it receives a signed requisition from the physician? Or should the lab release the test results and request that the physician send a signed requisition or test order after the fact?

Historically, the Centers for Medicare & Medicaid Services ("CMS") have stated that test requisitions do not need to be signed by the physician as long as the physician clearly documents in the patients' medical records his or her intent that the test be performed. In August 2008, CMS confirmed this position.

However, CMS recently requested comments on a proposed rule to purportedly "clarify" its policy on physician signatures. The proposed rulemaking restates CMS's position that test requisitions do not need to be signed by physicians. However, CMS muddies the water by attempting to distinguish between a test requisition and a test order and concludes that the requisition, which need not be signed, and the order, which must be signed, are two separate documents.

Because for most labs, the test requisition serves as the test order, we do not believe that the new rule will change CMS's historical position that requisitions sent to the lab do not need to be signed. However, this latest word from CMS seems to emphasize that the physician has an independent duty, regardless of whether he or she signs the requisition, to include his or her signature either in the text of the patient's medical record or by a signed order attached to the medical record.

Moreover, physicians should take note of the fact that their failure to sign test requisitions could lead to an audit of the physicians medical records by Medicare, Medicaid or commercial insurers.

Some experts say that just because CMS may not require a signature on the physician order for laboratory tests does not mean that laboratories should not obtain physician signatures on the test requisitions. For example, some state Medicaid plans, including New Jersey and other payors require physician signatures for patients covered under such plans or policies.

Therefore, MDL will continue to encourage its physician clients to sign all test requisitions, in addition to including signed orders in the patient's medical record that a test was ordered and the clinical justification for ordering the test.

1. Physician Signature Requirements for Diagnostic Tests, Pub 100-02 Medicare Benefit Policy, Transmittal 94, Centers for Medicare & Medicaid Services, (August 29, 2008)
2. M. Clinical Laboratory Fee Schedule: Signature on Requisition, 74 Fed. Reg. 33462 (July 13, 2009).
3. See letter April 1, 2008 Terrence L. Kay Hospital and Ambulatory Policy Group [www.clinical-labs.org/.../ACLAPhysicianSignatureLettertoTerryKay.pdf](http://www.clinical-labs.org/.../ACLAPhysicianSignatureLettertoTerryKay.pdf)

# Journal Watch

**Murayama SY, Seki C, Sakata H, Sunaoshi K, Nakayama E, Iwata S, Sunakawa K, Ubukata K.** 2009. Capsular type and antibiotic resistance in *Streptococcus agalactiae* isolates from patients, ranging from newborns to the elderly, with invasive infections. *Antimicrob Agents Chemother.* 53:2650-3.

The authors investigated 189 Group B Streptococcal clinical isolates from Japanese patients with invasive infections. Early (10.8%) and late (70.8%) onset GBS infections in children occur primarily through vertical transfer from mother to neonate. Whereas GBS infections in adults, which manifest as sepsis (75%), cellulites, arthritis, necrotizing fasciitis, meningitis, and bacterial endocarditis, occurred primarily in people greater than 50 years of age with underlying health issues (88.7%) such as diabetes, liver dysfunction, or immune compromise. These GBS strains were characterized based on capsular type by PCR, for antimicrobial susceptibility, and for the presence of resistance genes. They found that predominantly capsular type III (67.7%) were isolated from children, whereas types Ib (31.5%) and V (18.5%) were predominant among adults. 9.2% and 28.2% of the strains isolated from children and adults were resistant to the fluoroquinolone, levofloxacin, respectively. Interestingly, the levofloxacin resistant isolates were predominantly capsular type Ib in both the children (100%) and adults (91.4%). PFGE molecular typing data suggests that these type Ib, levofloxacin resistant strains are closely related and may represent a single clone that acquired fluoroquinolone resistance and has spread rapidly throughout Japan.

**Castor ML, Whitney CG, Como-Sabetti K, Facklam RR, Ferrieri P, Bartkus JM, Juni BA, Cieslak PR, Farley MM, Dumas NB, Schrag SJ, Lynfield R.** 2008. Antibiotic Resistance Patterns in Invasive Group B Streptococcal Isolates. *Infect Dis Obstet Gynecol.* 2008:727505.

This study investigates the antimicrobial susceptibility of 3,813 Group B Streptococcus clinical isolates obtained during 1996-2003 from invasive GBS disease from four participating institutions in GA, MN, NY, and OR of the Centers for Disease Control and Prevention's Active Bacterial Core surveillance (ABCs) system of the Emerging Infections. All 3,813 were sensitive to penicillin, ampicillin, cefazolin, cefotaxime, and vancomycin. Clindamycin and erythromycin resistance was 12.7% and 25.6%, respectively, and associated with serotype V ( $P < .001$ ). Clindamycin resistance increased from 10.5% to 15.0% and erythromycin resistance increased from 15.8% to 32.8%. These data support the need for GBS erythromycin and clindamycin susceptibility testing and surveillance for those patients who are penicillin allergic and at high risk for anaphylaxis.

**Andrews WW, Schelonka R, Waites K, Stamm A, Cliver SP, Moser S.** 2008. Genital tract methicillin-resistant *Staphylococcus aureus*: risk of vertical transmission in pregnant women. *Obstet Gynecol.* 111(1): 113-8.

This study attempted to estimate the frequency of genital tract colonization by methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women and evaluate the association of such colonization with infant outcome. Between July 2003 and July 2006, anovaginal screening cultures for Group B Streptococcus (GBS) were prospectively obtained in the third trimester (35 to less than 37 weeks of gestation) and were also processed for identification of *Staphylococcus aureus* including methicillin-resistant strains. Maternal colonization by MRSA was linked to a computerized database of invasive neonatal infections that occurred at the study center during the study period. Among 5,732 mothers (who delivered 5,804 infants) with GBS screening cultures and infant infection data available, 22.9% were GBS-positive and 14.5% were positive for *Staphylococcus aureus*. A total of 24.3% of the *Staphylococcus aureus* isolates were MRSA. The overall MRSA colonization rate was 3.5%. Colonization by any *Staphylococcus aureus* (relative risk 1.6, 95% confidence interval 1.4-1.9) as well as MRSA (relative risk 2.2, 95% confidence interval 1.6-2.8) was significantly more common among GBS-positive than among GBS-negative women. No cases of

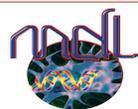
early-onset invasive neonatal infection by MRSA occurred among infants in the study. Genital tract colonization with MRSA affected 3.5% of pregnant women. Such MRSA colonization is associated with colonization by GBS but does not predispose to a high risk of early-onset neonatal MRSA infection.

**Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, Harrison LH, Lynfield R, Mohle-Boetani J, Zansky S, Albanese BA, Stefonek K, Zell ER, Jackson D, Thompson T, Schrag SJ.** 2009. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. *Clin Infect Dis.* 49: 85-92.

Group B Streptococcus (GBS), traditionally considered to be a neonatal pathogen, is an important cause of morbidity and mortality among older adults and among those with underlying medical conditions. This study used population-based surveillance to examine trends in adult GBS disease during the period 1990-2007 and to describe the epidemiology of adult GBS disease to guide prevention efforts. Active Bacterial Core surveillance was conducted in selected counties in ten US states. A case was defined as isolation of GBS from a normally sterile site in a nonpregnant resident of a surveillance area who was 18 years of age. Rates were calculated using US Census data. Demographic and clinical information was abstracted from medical records. Serotyping and susceptibility testing were performed on isolates collected from a subset of case patients. A total of 19,512 GBS cases were identified in nonpregnant adults during 1990-2007 (median patient age, 63 years); the incidence of adult GBS disease doubled from 3.6 cases per 100,000 persons during 1990 to 7.3 cases per 100,000 persons during 2007 ( $P < .001$ ). The mean difference in incidence between black and white persons was 4.6 cases per 100,000 persons (range, 3.1 cases per 100,000 persons during 1991 to 5.8 cases per 100,000 persons during 1999). Common clinical syndromes in 2007 included bacteremia without focus (39.3%), skin and/or soft-tissue infection (25.6%), and pneumonia (12.6%). Most (88.0%) GBS cases in adults had 1 underlying condition; diabetes was present in 44.4% of cases. Serotypes V, Ia, II, and III accounted for 80.8% of infections during 1998-1999 and 78.5% of infections during 2005-2006. Invasive GBS disease in nonpregnant adults represents a substantial and increasing burden, particularly among older persons, black persons, and adults with diabetes. Prevention strategies are needed.

**Enomoto M, Morioka I, Morisawa T, Yokoyama N, Matsuo M.** 2009. A Novel Diagnostic Tool for Detecting Neonatal Infections Using Multiplex Polymerase Chain Reaction. *Neonatology.* 96(2):102-108.

In newborns with infections, it is necessary to detect various pathogens rapidly and accurately, because the infections are often fatal when diagnosis is delayed. However, no diagnostic tools that rapidly detect pathogens causing neonatal infectious diseases are available. The objective of this study was to establish a rapid diagnostic tool using multiplex polymerase chain reaction (PCR) to detect 8 major pathogens that often cause neonatal infections, including Group B Streptococcus, *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, *Ureaplasma urealyticum*, herpes simplex virus, Cytomegalovirus, and *Candida albicans*, and to validate this tool in the neonatal intensive care unit (NICU). One hundred and thirty clinical samples were obtained from newborns with any infectious signs or histories. DNA was extracted from these samples and multiplex PCR was performed with a mixture of 8 primer pairs, all designed to amplify pathogenic DNA and produce different sizes of amplicons. Seventy-seven samples with suspicion of bacterial infections were also examined by bacterial culture to evaluate the accuracy of the multiplex PCR results. Six of the eight pathogens could be rapidly detected by their multiplex PCR method, within 3.5-4.5 h. These positive results led them to immediately diagnose and select proper drugs against each pathogen. In comparison with culture results, their test characteristics were as follows: specificity: 93%, negative predictive value: 96%, and concordance rate: 90%. They established and validated a rapid diagnostic tool for detecting pathogens using multiplex PCR, which may be useful for the confirmed diagnosis of neonatal infections in the NICU.



Medical Diagnostic Laboratories, L.L.C.

## New Test Announcements

**Test 1220:** Factor V Leiden and Factor II Prothrombin by Real-Time PCR

**Test 1125:** 2009 H1N1 influenza virus (Swine Flu) with tamiflu resistance by Pyrosequencing.

## Test Replacement

As of October 1, 2001 the following test replacement will take effect:

**Discontinued: Test 1121**

*Neisseria meningitidis* by Real-Time PCR

**Replacement: Test 1121**

*Neisseria meningitidis* by Real-Time PCR

(Reflex to penicillin tolerance by Pyrosequencing)

## Medical Diagnostic Laboratories, L.L.C.

### Abstracts

1. **Chadwick SG, Mordechai E, Adelson ME, Gyga SE.** Susceptibility profiles of Community-Associated MRSA Isolates from Cervicovaginal Swab Samples. 49th Interscience Conference on Antimicrobial Agents & Chemotherapy, San Francisco, CA. September 12-15, 2009.

### Peer-Reviewed Papers

1. **Ingvarsdottir K, Blaho J.** 2009. The role of chromatin in the regulation of HSV-1 viral gene expression and replication. *Future Microbiol.* 4(6):703-12.
2. **Hilbert D, Paulish T, Mordechai E, Adelson ME, Gyga SE, Trama J.** 2009. Antimicrobial non-susceptibility of cervico-vaginal and rectal *Escherichia coli* isolates is associated with phylogeny and plasmid carriage. *Eur J Clin Microbiol Infect Dis.* In press.

## HUMIGEN, L.L.C.

### Abstracts

1. **Do T, Davis C, Ucisik-Akkaya, E, Morrison B, Dorak MT.** Molecular Mechanism of Sex-specific Association of Interferon Regulatory Factor 4 with Childhood Acute Lymphoblastic Leukemia (ALL). 35th Annual American Society for Histocompatibility and Immunogenetics. San Francisco, CA, November 2-6, 2009.
2. **Ucisik-Akkaya E, Davis C, Do T, Dorak MT.** Immunoregulatory Gene Polymorphisms and Childhood Acute Lymphoblastic Leukemia (ALL) Susceptibility. 35th Annual American Society for Histocompatibility and Immunogenetics. San Francisco, CA, November 2-6, 2009.
3. **Davis C, Ucisik-Akkaya E, Do T, Dorak MT.** Polymorphisms of Iron Regulatory Genes with Immune Functions are Associated with Childhood Acute Lymphoblastic Leukemia (ALL). 35th Annual American Society for Histocompatibility and Immunogenetics San Francisco, CA, November 2-6, 2009.

### Peer-Reviewed Papers

1. **Dai J, Megjugorac N, Gallagher GE, and Gallagher G.** 2009. IFN $\lambda$ 1 (II-29) inhibits GATA3 expression and suppresses Th2 responses in human naïve and memory T cells. *Blood*, 113(23):5829-38.
2. **Do T, Ucisik-Akkaya E, Davis C, Morrison B, Dorak MT.** 2009. TP53 R72P and MDM2 SNP309 Polymorphisms in Modification of Childhood Acute Lymphoblastic Leukemia Susceptibility, *Cancer Genet Cytogenet.* In press.

**Q:** I received a faxed specimen discrepancy notification from your specimen resolution center (SRC) stating that our office failed to indicate what tests were ordered for a specific specimen. Our office always orders the same tests on all patients whose specimens we send to your laboratory. Can't you just automatically run those same tests all the time?

**A:** We do not accept standing orders. In an effort to ensure that tests ordered on a given date of service are medically necessary for the diagnosis and treatment of that specific patient, we require requested tests to be clearly marked on each test requisition form submitted. If the tests are not marked on the requisition form or are marked in such a way that we feel some degree of ambiguity in your request, we will contact your office for clarification of the orders to ensure that we are only performing the tests the physician has deemed appropriate for that patient on that date of service.

If you have a question you would like addressed in future issues, please email your question(s) to [QAQ&A@mdlab.com](mailto:QAQ&A@mdlab.com)



**For results to the electronic Epidemiology Quiz, please visit [www.mdlab.com](http://www.mdlab.com) and click on the e-Quiz link.**

1. Group B Streptococcus (GBS) infections in the newborn can manifest as...  
a. Sepsis   b. Meningitis   c. Pneumonia   d. All of the above
2. **True or false.** If a newborn does not develop symptoms of GBS infection within one week of birth, they will not develop it at all.
3. **True or false.** The incidence of group B streptococcal disease in babies less than a week old declined by over 70% in the 1990s, coinciding with increased use of intrapartum antibiotic prophylaxis  
a. HLA-A   b. HLA-DR   c. HLA-C   d. HLA-G
4. The Centers for Disease Control and Prevention (CDC) recommends universal prenatal screening for vaginal and rectal group B strep colonization of all pregnant women at \_\_\_\_\_ weeks' gestation.
5. In the United States, GBS remains a leading cause of newborn morbidity and mortality, resulting in an estimated 1,425 early onset cases and \_\_\_\_\_ deaths annually.  
a. 7   b. 27   c. 63   d. 93