## Week 6: Neonatal Infectious Diseases

### Neonatal Infectious Diseases I

**Tuesday, Aug. 4  2:30-4:00pm EDT**

**Moderators**
Pascal Lavoie  
Maria Carrillo-Marquez

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<thead>
<tr>
<th>EDT</th>
<th>Abstract</th>
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<tr>
<td>2:30 pm</td>
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<td>Introduction &amp; General Information</td>
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<tr>
<td>2:35 pm</td>
<td>3379766</td>
<td>The impact of Group B Streptococcus Intrapartum Antibiotic Prophylaxis on the Neonatal Microbiome at 1-month age</td>
<td>Suzie Hoops</td>
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<td>2:45 pm</td>
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<td>Detection of Congenital Cytomegalovirus by testing Neonatal Saliva in Minutes Using a Disposable Cartridge in a Near-Patient Platform</td>
<td>Vamsee Pamula</td>
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<tr>
<td>2:55 pm</td>
<td>3383211</td>
<td>Routine MRSA surveillance does not change the incidence of MRSA blood or CSF infections in the NICU</td>
<td>Justin Josephsen</td>
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<td>Antenatal Inflammation Uniquely Alters Postnatal Immune Response Patterns to Primary Group B Streptococcus Infection and Secondary Challenge in a Murine Model</td>
<td>Andrea Alletto</td>
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<td>John Vanchiere</td>
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<td>Ellen Kim</td>
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<tr>
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<td>Wrap Up</td>
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Note: Schedule subject to change based on presenter availability.
CONTROL ID: 3379766

TITLE: The impact of Group B Streptococcus Intrapartum Antibiotic Prophylaxis on the Neonatal Microbiome at 1-month age

PRESENTER: Suzie Hoops

AUTHORS (LAST NAME, FIRST NAME): Mukhopadhyay, Sagori; Hoops, Suzie; Frager, Nicole; Dhudasia, Miren B.; Hartman, Erica; Gerber, Jeffrey S.; Knights, Dan; Puopolo, Karen M.


CURRENT CATEGORY: Neonatology

CURRENT SUBCATEGORY: Neonatal Infectious Diseases/Immunology

KEYWORDS: Neonatal microbiome, GBS prophylaxis, Bifidobacterium.

SESSION TITLE: Neonatal Infectious Diseases/Immunology: S. Aureus, CMV, and GBS | Neonatal Infectious Diseases/Immunology: S. Aureus, CMV, and GBS

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

Background: Intrapartum antibiotic prophylaxis (IAP) is administered to laboring women colonized with Group B Streptococcus (GBS) to prevent mother-to-child transmission of the bacteria. Effects of this practice on the neonatal gut microbiome beyond the immediate neonatal period remains uncertain.

Objective: To determine species-level changes in gut microbiome composition of infants at one month of age born to women administered GBS IAP compared to those born to women without intrapartum antibiotic exposure.

Design/Methods: Prospective observational study of healthy, vaginally delivered, term neonates. GBS IAP was defined as administration of penicillin, ampicillin, vancomycin, clindamycin or cefazolin≥4 hours prior to delivery. Shotgun metagenomic sequencing was performed on fecal samples collected from infants at regular intervals. Differences in microbiome composition and proportion of contributing species were assessed using non-parametric tests and false-discovery-rate (FDR) correction for multiple hypothesis testing where appropriate. Models were adjusted for breastfeeding status at 1 month of age and for exposure to neonatal antibiotics (initiated within 72 hours of birth).

Results: Of 55 eligible infants, 13 (24%) were exposed to maternal GBS IAP: 12 to penicillin and 1 each to cefazolin and ampicillin. Demographic characteristics are shown in Table 1. No significant differences in alpha diversity or beta diversity were seen between the 2 groups (Figure 1). The phylum Actinobacteria were significantly lower in relative abundance among infants exposed to GBS IAP (FDR-corrected p-value 0.009) (Figure 2). Four species of genus Bifidobacterium within Actinobacteria, including B. adolescentis, were significantly decreased in infants exposed to GBS IAP (FDR-corrected p-values < 0.2).

Conclusion(s): GBS IAP is associated with phylum- and species-level differences at 1 month of age in exposed newborns.

Table 1: Infant Characteristics
Figure 1: Gut microbiome (1-month age) diversity indices of infants with and without exposure to GBS IAP

Figure 2: Differences in proportion of Actinobacteria in the gut microbiome (1-month age) of infants with and without exposure to GBS IAP

Table 1: Infant Characteristics

Figure 1: Gut microbiome (1-month age) diversity indices of infants with and without exposure to GBS IAP

Figure 2: Differences in proportion of Actinobacteria in the gut microbiome (1-month age) of infants with and without exposure to GBS IAP

**IMAGE CAPTION:**
Table 1: Infant Characteristics
**Background:** Congenital cytomegalovirus (cCMV) is the most common congenital infection and a leading cause of hearing loss and intellectual disability. In the U.S., 1 in 150 newborns are estimated to have cCMV, however, most infants have no clinically detectable symptoms and therefore, are not identified. Identification of infants with cCMV can facilitate early detection of CMV-associated hearing loss and provide interventions including antiviral therapy to improve outcomes. Although most newborn screening (NBS) is performed using dried blood spots (DBS), the sensitivity of CMV detection in DBS is a challenge, even when using DNA amplification whereas saliva and urine CMV detection have high sensitivity. Due to lack of infrastructure for transport of saliva to NBS labs, the test will likely need to be performed in hospitals similar to bilirubin, hearing screening, and pulse oximetry.

**Design/Methods:** CMV sequences published by Boppana and custom sequences for internal control lambda were used. Heaters and sensors were integrated into a digital microfluidic (DMF) cartridge to enable rapid amplification of target DNA sequences using fluorescent probe chemistries (R44DC016576). Reaction droplets (containing sample, master mix, primers, probes, and lambda DNA) were subjected to 50 cycles including denaturing (93°C) and extension (60°C) stages. Saliva samples from newborns are run on the cartridge.

**Results:** The entire 50 cycle reaction, including on-cartridge droplet dispensing, reagent mixing, and thermal cycling, was completed in 5 min. Cycle thresholds were measured from PCR curves for CMV at concentrations from 10-100,000 cp/µL with the results following expected dose response. Figure below shows PCR from 1,500 CMV cp/µL multiplexed with lambda. CMV patient samples showed amplification while the normal samples did not.

**Conclusion(s):** This study demonstrates performance of cCMV PCR assays in a disposable cartridge in a completely automated fashion to enable in-hospital screening. All reagents are fully integrated into the cartridge eliminating the need for reagent preparation or loading. Rapid PCR (in less than 5 mins) circumvents the necessity to setup infrastructure to transport saliva samples and can accelerate adoption of near-patient testing and rapid return of results. Further testing is required to establish the performance and clinical utility of this device in a clinical setting.

![CMV PCR assay on DMF cartridge in 5 minutes.](image-url)
KEYWORDS: MRSA, Contact isolation.

SESSION TITLE: Neonatal Infectious Diseases/Immunology: S. Aureus, CMV, and GBS | Neonatal Infectious Diseases/Immunology: S. Aureus, CMV, and GBS

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:
Background: Evidence for routine MRSA surveillance is limited, and it is not clear if routine MRSA nasal swabs affect the incidence of true MRSA infection. Contact isolation for neonates in the NICU is associated with delayed patient care, staff avoidance, and increased adverse events. At this time, there are no guidelines to recommend for or against surveillance at routine intervals. A preliminary analysis of over 5 years of weekly MRSA surveillance in our NICUs revealed no correlation between MRSA colonization and MRSA infections in the blood or CSF. Thus, we stopped routine MRSA surveillance 2 years ago and followed MRSA infection rates.

Objective: To determine if weekly MRSA surveillance is associated with a decrease in MRSA infections

Design/Methods: Seven-year, retrospective cohort study of all infants admitted to 2 affiliated, urban NICUs (Levels 3 & 4) from 6/12 to 1/20. Prior to December 2017, infants received weekly nasopharyngeal (NP) MRSA testing and were placed in contact isolation if MRSA colonized (MRSA Surveillance Cohort). A review of all MRSA colonizations and infections in our NICUs was conducted; no correlation was found. Routine MRSA surveillance was stopped in 2018, and infants were only placed in contact isolation if a MRSA infection (blood, CSF, wound) was identified (No Surveillance Cohort). MRSA was identified with an electronic query of the EMR. The cost of MRSA screening was $102 per sample and $23 per day for isolation supplies. Analysis of GA, central line days, and other key factors is ongoing.

Results: There were 10,005 patients in the entire study period with birthweights from 400g to 5030g. During the MRSA Surveillance period, 30,340 MRSA NP swabs were performed on 7,536 patients, of which 12% had MRSA colonization. The rate of MRSA infection in the blood or CSF was not different in the two cohorts (1.1 vs. 2.4 infections per 1000 infants, p=0.22). Vancomycin exposure decreased from 10.1% to 5.9% of all infants (p<0.0001) when MRSA surveillance was stopped (Table). During the active surveillance program, the annual cost of MRSA surveillance/isolation was $590,000, representing $68,000 per positive infection.

Conclusion(s): The rates of MRSA infection did not change when a weekly MRSA surveillance program was discontinued, and its discontinuation was associated with a decrease in vancomycin use. Given the high costs of routine MRSA screening, the cost to isolate infants with MRSA, and the lack of a clear clinical benefit, NICUs should consider whether this practice is warranted.

<table>
<thead>
<tr>
<th>MRSA Surveillance</th>
<th>No Surveillance for MRSA</th>
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<tbody>
<tr>
<td>Total infants</td>
<td>7305</td>
</tr>
<tr>
<td>Infant deaths, any cause, any type</td>
<td>151.5 (1.5)</td>
</tr>
<tr>
<td>Infant deaths, after 35 weeks</td>
<td>282 (15.6)</td>
</tr>
<tr>
<td>Infant deaths, 4-6 weeks</td>
<td>5.6</td>
</tr>
<tr>
<td>Incidence of group B infections</td>
<td>18/1000 infants</td>
</tr>
<tr>
<td>Infectious deaths</td>
<td>0.2</td>
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</tbody>
</table>

Conclusion(s): The rates of MRSA infection did not change when a weekly MRSA surveillance program was discontinued, and its discontinuation was associated with a decrease in vancomycin use. Given the high costs of routine MRSA screening, the cost to isolate infants with MRSA, and the lack of a clear clinical benefit, NICUs should consider whether this practice is warranted.

IMAGE CAPTION:
Background: Chorioamnionitis is a common antenatal inflammation that precedes preterm delivery and increases infant susceptibility to infections such as group B Streptococcus (GBS, Streptococcus agalactiae). We previously reported that exposure to maternal inflammation can modulate postnatal immunity to viral infection (Pediatric Research 2014). Whether these modifications impact the postnatal immune response to GBS is an unanswered question.

Objective: Our goal was to determine if antenatal inflammation influences natural immunity in exposed offspring by evaluating immune responses to primary and secondary GBS infection.

Design/Methods: E17 timed-pregnant C57/Bl6 dams were treated with IP lipopolysaccharide (LPS) or saline control (Ctrl). GBS (COH1,10^5 cfu) or sham (saline)-infected mice were euthanized one week post infection. Spleens and livers were harvested for immune cell analysis by multiparameter flow cytometry. For primary GBS studies, pups were inoculated IP on PND 5. For re-challenge studies, surviving GBS or sham infected mice received IP GBS 4 weeks after the first inoculation.

Results: Primary GBS (vs. sham) infection of LPS-exposed offspring was associated with marked myeloid enhancement (Gr-1+CD11b+ neutrophils: spleens P<0.05, livers P<0.001; Ly6G-CD11b+ monocytes: spleens P<0.05, livers P<0.01) but suppression of CD3+ lymphocytes (livers, P<0.01; spleens, P<0.05). In spleens, CD4+ cells of LPS-exposed GBS (vs. sham) infected pups showed increased Th2 (P<0.05), Th17 (P<0.01) and IL-10 (P<0.001) responses; in contrast only IFNγ+ (Th1) frequencies were increased in CD8+ cells (P<0.01). After GBS re-challenge, liver but not splenic monocytes were prominent in the LPS-GBS group (P<0.001 vs. Ctrl-GBS), while neutrophil and lymphocyte frequencies in livers or spleens were not different.

Conclusion(s): Our present studies suggest that exposure to antenatal inflammation alters postnatal innate and adaptive immune response patterns to primary and secondary GBS infection. Prominence of these findings in the liver are consistent with mounting evidence of its critical role in modulating inflammation and immunity. Our observations may have relevance to the development of natural protective immunity in preterm infants born after chorioamnionitis, a topic of ongoing investigation. This work was supported in part by grants (to JMK) from the NIH (AI140206), the Glennon and Fleur de Lis Foundations, and Saint Louis University.

(No Image Selected)
isolates by validating the association of azithromycin exposure during pregnancy with the GBS antibiotic susceptibility profile and the presence of known resistance genes mef, ermB, and ermTR.

**Design/Methods:** This retrospective study utilized chart reviews, microbiological data, and molecular data of 333 archived GBS isolates from pregnant patients who were screened for GBS between January 1, 2013 and April 1, 2018 to identify an association between antibiotic exposure and the presence of resistance genes in GBS. Serotyping was performed to identify potential circulation of clonal GBS strains in the community.

**Results:** Among all isolates included in this study (n=333), nearly 60% (198/333) were resistant to erythromycin and clindamycin, and 3.6% exhibited inducible clindamycin resistance. Significant relationships were found between azithromycin exposure, chlamydia infection, antibiotic susceptibility phenotype, and molecular haplotype. Positive correlation was found between the antibiotic susceptibility phenotype and the molecular haplotype (p < 0.01). The serotype of circulating GBS strains was comparable to reference populations in the U.S. and Canada.

**Conclusion(s):** These data support the hypothesis that azithromycin exposure may be an important driver of antibiotic resistance among maternal colonizing GBS isolates in this population. Continued surveillance of regional GBS colonizing and invasive strains will be important to understanding the population structure of maternal GBS, to monitor evolving resistance mechanisms, and to inform vaccine policies. Prospective studies are necessary to evaluate the impact of antibiotic exposures on the maternal microbiome and pregnancy outcomes.

(No Image Selected)
multidisciplinary neonatal antibiotic stewardship team was convened and multiple periodic interventions were implemented. As a balancing measure, the rates of coagulase negative staphylococcal (CONS) infection were analyzed and the morbidity was reviewed. Statistical analysis included chi-square tests as well as student’s t-test.

**Results:** A historic review of data analyzing approximately 700 patients (2015-2016) revealed low MRSA colonization prevalence (3.7%) and high VUR (37.7/1000 patient days). There were no statistically significant differences in MRSA colonization or colonization acquisition after screening practices changed from weekly to screening at admission and discharge only (Table 1). Less than 2% of patients acquired MRSA colonization during hospitalization and none of these patients developed a blood-stream MRSA infection. After implementation of Antibiotic Stewardship, the VUR gradually decreased from 44.0/1000 patient days in 2016 to 22.7 in 2019 (Figure 1) with no significant increase in MRSA colonization, acquisition or blood-stream infection (Table 2). In addition, there were no statistically significant differences in CONS infection rates and no mortality related to this pathogen.

**Conclusion(s):** Antibiotic stewardship and a multidisciplinary approach significantly reduced vancomycin utilization with no significant increase in MRSA colonization, MRSA infection or CONS infection rates. Further studies are required to evaluate the long-term effects of vancomycin restriction in the NICU.

**Table 1:** MRSA Screening

<table>
<thead>
<tr>
<th></th>
<th>2015-2017 (Weekly Screening) n=906</th>
<th>2016-2019 (Screening at admission and discharge) n=1081</th>
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<tbody>
<tr>
<td>Positive</td>
<td>34 (3.7%)</td>
<td>31 (2.9%)</td>
</tr>
<tr>
<td>Negative</td>
<td>902 (96.3%)</td>
<td>1049 (97.1%)</td>
</tr>
<tr>
<td>Patients</td>
<td>903 (99.9%)</td>
<td>1081 (100%)</td>
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</table>

**Figure 1:** Vancomycin Utilization Rate

**Table 2:** MRSA Colonization and Vancomycin Utilization

<table>
<thead>
<tr>
<th>Year</th>
<th>2013</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>764</td>
<td>367</td>
<td>367</td>
<td>298</td>
<td>326</td>
</tr>
<tr>
<td>MRSA positive patients</td>
<td>133.8</td>
<td>131</td>
<td>131</td>
<td>20.5</td>
<td>20.5</td>
</tr>
<tr>
<td>MRSA colonization acquisition rate</td>
<td>1.5%</td>
<td>0.9%</td>
<td>0.9%</td>
<td>0.8%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Vancomycin Utilization Rate (Days/1,000 patient days)</td>
<td>764</td>
<td>367</td>
<td>367</td>
<td>298</td>
<td>326</td>
</tr>
</tbody>
</table>

**IMAGE CAPTION:**
Table 1: MRSA Screening

Figure 1: Vancomycin Utilization Rate

Table 2: MRSA Colonization and Vancomycin Utilization
Background: Feeding human milk (HM) is most beneficial in premature infants, especially in very low birth weight (VLBW) infants. However, VLBW infants are at risk of HM-acquired cytomegalovirus (CMV) infection.

Objective: To investigate rate of virolactia of HM obtained from mothers who delivered VLBW infants, rate of HM-acquired CMV infection, to assess changes in CMV DNA viral load in time and effects of freezing and heating in CMV DNA viral load.

Design/Methods: A prospective randomized controlled study was performed to compare effectiveness of CMV inactivation in HM of three different preparation methods; Freezing-thawing (FT), FT + Low temperature Holder pasteurization (LP), and FT + high temperature short-term pasteurization (HP). The study population included gestational age < 32 weeks or birth weight < 1,500 gram admitted at Asan Medical Center and Haeundae Paik Hospital NICU. Urine CMV culture and PCR were obtained at birth, 4, 8, 12 weeks, and HM CMV culture and PCR were obtained at birth, 3, 6, 9, 12 weeks.

Results: During the study period, a total of 629 infants were admitted. Of them, 250 (41.9%) mothers produced CMV PCR positive HM. After exclusion, 158 infants were enrolled of which 48, 61, and 69 infants were randomized into FT, FT + LP, and FT + HP group, respectively. Out of 158 infants, 7 (4.4%) infants acquired CMV infection via HM after birth, and 2 (1.3%) infants showed CMV-associated symptoms. CMV infection occurred in 2 (4.9%), 4 (8.3%), and 1 (1.4%) infant in FT, FT + LP, and FT + HP group, respectively. All 7 infants survived except 1 who died due to reasons not associated with CMV. The CMV DNA load in HM showed a peak at 3 to 6 weeks and gradually decreased for 2-3 months. Four subtypes of HM CMV PCR shedding were found. CMV DNA load significantly decreased after either freezing or pasteurization, however, CMV DNA load was not associated with CMV infection. We could not find an evidence of Holder pasteurization is more effective than freezing only in reducing HM-acquired CMV infection. Two infants fed with FT+LP HM developed symptoms at <30 days and < 32 weeks.

Conclusion(s): HM-acquired CMV infection is 4.4% in VLBW infants, and its short term impact was minimal. Based on our findings, we suggest feeding either frozen HM or pasturizing HM at high temperature up to 4–6 weeks after birth or until reaching 32 gestational age. Meanwhile, all VLBW infants should be encouraged to feed their own mother’s milk in neonatal intensive care unit.