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Clinical and Microbiologic Characteristics of Early-Onset Sepsis Among VLBW Infants: Opportunities for Antibiotic Stewardship

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Abstract

Background—Most very-low birth weight (birth weight <1500 grams, VLBW) infants receive empiric antibiotics for risk of early-onset sepsis (EOS). The objective of this study was to determine the characteristics of VLBW infants with culture-confirmed EOS at a single center during 25 years, to identify opportunities for antibiotic stewardship.

Methods—Retrospective cohort study including VLBW infants admitted from 1990–2015. EOS was defined as isolation of a pathogen in blood or cerebrospinal fluid culture obtained < 72 hours of age. Clinical and microbiologic characteristics of EOS case infants were obtained by review of medical, laboratory and administrative records. Blood culture, antibiotic initiation, and maternal discharge code data was available for all VLBW infants born 1999–2013.

Result—109 EOS cases (20.5/1000 VLBW births) occurred during the study period. Preterm labor, preterm rupture of membranes and/or the obstetrical diagnosis of chorioamnionitis were present in 106/109 cases (97%). Obligate anaerobic organisms accounted for 16% of cases. Time to culture positivity was 36 hours for 88% and 48 hours for 98% of cases. From 1999–2013, 97% of VLBW infants were evaluated for EOS and 90% administered empiric antibiotics; 22% of these infants were born by Cesarean section to mothers with pre-eclampsia and without preterm labor or chorioamnionitis and had a 12-fold lower incidence of EOS compared to the remaining infants.

Conclusion—Decisions to initiate and discontinue empiric antibiotics among VLBW infants can be informed by the delivery characteristics of infected infants, and by local microbiologic data.

Keywords

very-low birth weight; preterm birth; early-onset sepsis; antibiotic stewardship

BACKGROUND

The incidence of early-onset sepsis (EOS) increases with decreasing gestation age and birth weight and has remained relatively unchanged over two decades among extremely preterm

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infants (1). Among very-low birth weight (VLBW, birth weight < 1500 grams) infants, the incidence has ranged from ~11–20 cases/1000 VLBW births over the past 20 years (2–4). Morbidity and mortality associated with EOS increases with decreasing gestational age at birth; 30–50% of infants born 22–28 weeks gestation with EOS die of their infection (4). Identified risk factors for EOS and newborn clinical condition are useful in assessing risk of EOS among term and late preterm infants (5, 6). However, obstetric infection and/or inflammation is a major etiology of spontaneous preterm labor and subsequent birth (7), and nearly all premature infants have some degree of respiratory or systemic instability, making it difficult to use similar approaches to risk stratification among these infants. Unsurprisingly, the most premature infants are universally provided empiric antibiotic therapy from birth, and in ~50% of these infants, antibiotics are continued for ≥5 days despite sterile blood cultures (8). Recently, prolonged early antibiotic exposures have been associated with a subsequent increased risk of necrotizing enterocolitis and death, even when adjusted for gestational age and initial severity of illness (8–10). Faced with a patient population for whom both the administration of and withholding of antibiotics may each have life-threatening consequences, neonatal clinicians are challenged to identify those premature infants at low enough risk of EOS to minimize early antibiotic exposures. We hypothesized that VLBW infants born solely for maternal indications such as pre-eclampsia (PET), by cesarean section, and without exposure to the infectious risks associated with preterm labor and premature membrane rupture, may constitute a subgroup of VLBW infants at markedly decreased risk of EOS. To explore this hypothesis, we determined the delivery and microbiologic characteristics of all VLBW infants with culture-confirmed EOS born at a single birth center over a 25-year period. In addition, we quantified the frequency of blood culture and antibiotics for suspected EOS within a subgroup of these infants.

METHODS

Study design and population

This is a retrospective cohort study of all VLBW infants admitted to the Brigham and Women's Hospital neonatal intensive care unit (NICU), a 50-bed level III NICU located in Boston, Massachusetts. This study was approved by the Partners Health Care Human Subjects Review Board. VLBW infants admitted to the NICU with birth dates from 1/1/1990 to 5/31/2015 were included in the description of clinical and microbiologic characteristics of EOS cases; > 95% were born at the center. Antibiotic use data was available beginning in 1999. In 2013, a new local guideline for VLBW EOS evaluation was instituted. Because this represented a change from prior policies, antibiotic use among VLBW infants was assessed for the period 1/1/1999 – 12/31/2012.

Study definitions

EOS evaluation was defined as blood or cerebrospinal fluid (CSF) culture obtained at ≤ 72 hours of life. Center practice during the study period was to send two blood culture bottles for all EOS evaluations (one aerobic, one anaerobic; minimum of 1 milliliter (mL) of blood inoculated into each culture bottle). *EOS cases* were defined as one or more blood or CSF cultures obtained at ≤72 hours of life growing a recognized pathogenic species. We previously reported on the incidence of VLBW EOS from 1990–2007 as defined by NICHD

Neonatal Research Network reports (3, 4, 11); in contrast, in the present study cultures growing commensal organisms (coagulase negative staphylococci, diphtheroids, or *Bacillus* species) were considered cases *only* if isolated from both culture bottles and treated with an appropriate course of antibiotics. Cultures were incubated in the hospital microbiology laboratory on a continuous-reading system (BacT/Alert aerobic and anaerobic culture bottles, utilizing fastidious antimicrobial (FA) neutralizing media (Biomerieux, Inc)). *Time to positivity* (TTP) was defined as the difference between time when the culture was obtained from the infant (recorded by the clinician obtaining the culture) and time when microbial growth was first detected, communicated in real time to the NICU care team. For this analysis, one EOS case (GBS; TTP 69 hours) was excluded because the culture was incubated in the hospital blood culture system despite the fact that it was inoculated into a culture bottle (obtained at an outside hospital) that was not designed for use with that system, making the TPP unreliable. Empiric EOS therapy was defined as the initiation of parenteral antibiotic therapy at ≤ 72 hours of life, prior to knowledge of blood or CSF culture results. *Delivery characteristics*: preterm labor was defined as the spontaneous or induced onset of uterine contractions < 37 0/7 weeks gestation. Preterm rupture of membrane (PROM) was defined as spontaneous or induced rupture of membrane at <37 0/7 weeks gestation. Chorioamnionitis, and PET were recorded from obstetrician progress notes.

Data sources

Microbiology data was obtained from the hospital microbiology database. Neonatal and maternal clinical data was obtained by review of maternal and neonatal paper and electronic medical records. Data for infant demographic, microbiologic and antibiotic exposure variables along with maternal discharge ICD-9 codes was provided by a centralized data warehouse at Partners Healthcare (Research Patient Data Registry).

Analysis

Data analysis was performed by using SAS 9.3 (SAS Institute, Inc, Cary, NC). Discrete data are presented as frequencies and were compared using Fisher’s exact test or Chi-square test; continuous data is presented as a central tendency and compared using t-test or Wilcoxon rank test as appropriate.

RESULTS

Incidence of VLBW EOS cases and contaminants

During the study period 5313 VLBW infants were admitted to the NICU and 109 infants were diagnosed with culture-confirmed EOS (overall incidence, 20.5 cases/1000 VLBW live births). The incidence decreased over time (1990–1996, 24.8/1000; 1997–2007, 20.5/1000; 2008–2015, 14.3/1000; p=0.05 for comparison of 1990–1996 versus 2008–2015). An additional 19 infants had a commensal organism isolated from only one of two culture bottles (coagulase-negative staphylococci, 12; micrococcus species, 3; *Corynebacterium* species (diphtheroids), 3; *Bacillus* species, 1) with overall incidence 3.6 contaminant cultures/1000 VLBW live births.

Microbiology of EOS cases

The microbiology of the VLBW EOS cases is shown in Table 1. Consistent with previous reports from our center and from national registries, the most common organism isolated was *E. coli*, followed by GBS (4, 11). Obligate anaerobes were isolated in 17 cases (16%): 15 cases of *Bacteroides* species. and one each of *Fusobacterium nucleatum* and *Clostridium perfringens*. Compared to the infants with growth of aerobic or facultative anaerobic species, infants with obligate anaerobic infection were significantly smaller, born at earlier gestation, and more likely to be born to a mother who received intrapartum treatment with ampicillin (see Table, Supplemental Digital Content 1). Detailed microbiologic data was available for the 46 cases that occurred from 1999–2012. During this period, 14/46 (30%) of cases were identified by growth of obligate or facultative anaerobic species in the anaerobic bottle only. In an additional 25/46 (54%) of those cases, facultative anaerobic species were isolated from both aerobic and anaerobic culture.

Time to recognition of infection

TPP was only recorded for growth from aerobic cultures. The majority of organisms grew within 36–48 hours: 61/84 (72.6%) were positive by 24 hours; 74/84 (88.1%) by 36 hours; 82/84 (97.6%) by 48 hours. One case of *S. mitis* and one case of *S. sanguinis* had TTP 55 and 49 hours, respectively. Median TTP for 35 gram-positive cases was 22 hours (IQR 15–35), significantly longer compared to 45 gram- negative cases at 13 hours (IQR 12–17), $p < 0.001$. Median TPP for *E. coli* was 13 hours (IQR 11.25–15.75) and for GBS was 20.5 hours (IQR 15–25.3). All gram-negatives were reported within 37 hours. No significant difference was observed in median TTP of 55 infants whose mothers had received antibiotics prior to delivery (median TPP 15 hours, IQR 12–26) compared to the 29 infants who were not exposed to any intrapartum antibiotics (median TPP 18 hours, IQR 13–26), p 0.26.

Delivery characteristics of EOS cases

The demographics and delivery characteristics of the 109 EOS cases are shown in Table 2. Preterm labor, PROM and/or chorioamnionitis was noted in the maternal obstetrical records in 106/109 (97.2%) of the cases. Three EOS cases were delivered in the absence of these three risk factors. In two cases, the mothers presented with unexplained decreased fetal movement, and both were subsequently delivered by emergent Cesarean section due to non-reassuring fetal testing. Both infants grew *Listeria monocytogenes* from blood culture. The final case was an infant delivered via cesarean section for maternal PET in the absence of PROM or labor. *Streptococcus mitis* was isolated from 1 of 2 blood culture bottles; repeat blood cultures were sterile despite infant treatment with antibiotics to which the *S. mitis* isolate was resistant.

EOS evaluation and empiric antibiotics among VLBW infants

During the period 1/1/1999–12/31/2012, 2851 VLBW infants were admitted to the NICU. We excluded 55 infants who died within 24 hours of life and did not have microbiology,

SUPPLEMENTAL DIGITAL CONTENT
Supplemental Digital Content 1. Table

pharmacy or linked maternal data available; and 48 infants for whom maternal ICD-9 discharge codes were unavailable. Data was therefore analyzed for 2748/2851 (96.4%) of infants (Table 3). We identified 605 infants (22%) who were delivered to mothers with a diagnosis of PET, by Cesarean section and without diagnoses of PROM or chorioamnionitis. As seen in Table 3, the frequency of blood culture among these infants was not different from the remaining infants in the cohort. Empiric antibiotics were administered to 85% of these infants, albeit at a significantly lower rate compared to the entire cohort. Ampicillin and gentamicin were administered to 97% of the infants who received antibiotics; cephalosporins were administered alone or in addition to 138 infants. The EOS rate was 12-fold lower among infants in the defined subgroup compared to the other infants in the cohort. The one case that did occur was the aforementioned case of *S. mitis*.

DISCUSSION

In this single-center, 25-year experience with over 5000 VLBW admissions, we found that 97% of VLBW infants with culture-confirmed EOS were born in association with three risk factors: preterm rupture of membranes, preterm labor and/or the obstetrical diagnosis of chorioamnionitis. Additionally, we found that nearly a quarter of our 1999–2012 study cohort was born without these risk factors, due to maternal PET, by Cesarean section - and that this subgroup had a 12-fold lower incidence of EOS compared to the remaining infants in the cohort. Despite this differential risk, blood cultures were universally obtained and empiric antibiotics were administered to >80% of the lower-risk subgroup - suggesting that neonatal clinicians are unaware of the magnitude of this differential risk. The frequency of antibiotic initiation among preterm infants and persistence of administration despite sterile cultures has been documented by others (8, 12). The clinical association of prolonged antibiotic use with subsequent poor outcomes has strong biologic plausibility with emerging evidence of host immune-microbiome interactions that are disrupted with antibiotic exposure (8, 13–18). Yet antibiotic decisions are challenging for neonatal clinicians when most VLBW infants exhibit some degree of respiratory or systemic instability. Our findings present several opportunities for antibiotic stewardship among VLBW infants.

One opportunity for antibiotic stewardship may be for clinicians to consider birth indication as well as birth weight and gestation. Preterm deliveries for maternal health indications account for ~30% of preterm births and the absence of labor has been reported in ~25% of the extremely low-birth weight population (7, 19). In this study, <1% of VLBW EOS cases occurred among infants born for such reasons, suggesting that clinicians could safely consider not initiating empiric antibiotics among such infants, or at the very least, discontinuing antibiotics with sterile cultures. Preterm labor and/or PROM each provides opportunity for the fundamental pathogenesis of ascending colonization of the uterine/fetal compartment with maternal gastrointestinal and genitourinary flora and subsequent transition to invasive infection. In many cases it can be difficult to distinguish cause and effect. It should be noted, however, that we found that induction of labor and assisted ROM – even if prompted by maternal conditions such as PET - also placed VLBW infants at risk of infection, exposing them to the pathogenesis of EOS. In contrast, an infant born by Cesarean section elected for maternal health indications, in the absence of any attempt to induce labor or ROM, has not been exposed to the conditions associated with development

of EOS. Our findings should not be interpreted as advocacy for this delivery scenario, only that such delivery characteristics could be considered when making the decision to begin empiric antibiotics as well as when making the decision to discontinue antibiotics with sterile cultures.

When considering delivery indication and antibiotic decisions, the three cases that occurred without a history of preterm labor or PROM deserve comment. The two cases of early-onset *Listeria* infection presented in a nearly-identical manner: the mothers presented with decreased fetal movement, in the absence of preterm labor, PROM or any sign of chorioamnionitis, with non-reassuring fetal testing. The pathogenesis of *Listeria* infection differs from most other types of EOS, with maternal infection preceding fetal infection; maternal infection may or may not be symptomatic. *Listeria* readily invades the placenta and can infect the fetus either by ascending infection, direct tissue invasion or hematogenous spread (20). Although uncommon, this type of clinical presentation should be specifically noted by neonatal clinicians when developing approaches to antibiotic stewardship. The final and only case that occurred in a mother delivered for severe PET by Cesarean section, with ROM at delivery in the absence of labor and or any attempts to induce labor – occurred in an infant with birth weight 510 grams. The organism (*S. mitis*) was isolated after 25 hours of incubation in only 1 of 2 culture bottles, and was resistant to ampicillin and gentamicin; however, repeat cultures were sterile after treatment with these antibiotics, raising the possibility that this was a contaminant culture.

A second stewardship opportunity may lie in the technical approach to blood culture. The use of blood culture as the gold standard for diagnosing neonatal EOS has been questioned (21), despite advances in blood culture techniques that include use of optimized enriched broths that can detect bacteremia at <10 CFU/mL, and media with antimicrobial neutralization properties that efficiently neutralize beta-lactam antibiotics and gentamicin (22–26). If antibiotic decisions are to be guided by blood culture results, such cultures must be reliable. Practice in this study was to inoculate at least 1 mL blood in each of two blood culture bottles to optimize detection of pathogens as is recommended (21), although at least one recent study of neonatal blood cultures suggests that smaller volumes may be adequate (27). The use of two separate bottles provided the opportunity to determine if commensal species are true infections by comparing growth (or not) in each blood culture bottles. Prior studies provide conflicting evidence for this approach to distinguishing contaminants, with observational studies of practice supporting the use of two cultures (28) while another study of rigorous blood culture technique found one culture adequate (29). In this study, the use of two cultures allowed for the determination of contaminant species in 15% of cultures with microbial growth. Finally, we found utility in the use of anaerobic blood culture which identified obligate anaerobic infection in 16% of cases; in 15/17 cases the infection was gram-negative bacteremia due to *Bacteroides* species. *Bacteroides* species are the most common anaerobic constituent of the adult gastrointestinal flora and *B. fragilis* is a well-described pathogen after adult gastrointestinal surgery and in obstetrical infection, including peripartum bacteremia (30–32). The relatively few prior reports of neonatal anaerobic infection are summarized in a 2008 review by Brooks (33) which notes 34% mortality associated with neonatal *Bacteroides* infection. Although the ability of laboratories to identify anaerobic organisms and report susceptibility varies (34), recognition of these

infections can optimize care. Most *Bacteroides fragilis* are resistant to ampicillin and gentamicin and require treatment with antibiotics with anaerobic activity (clindamycin, metronidazole) or with activity against beta-lactamase producers (ampicillin-sulbactam or carbapenems) (35–36). One report of 128 adult patients with *Bacteroides* bacteremia noted a significantly higher mortality among patients receiving antimicrobial therapy to which the isolate was resistant compared to those treated with appropriate antibiotics (35). By improving capture of EOS organisms, anaerobic culture may provide greater confidence in sterile blood culture results.

A third opportunity for antibiotic stewardship may be provided in our findings for culture TTP. This study is the first report of TTP limited to VLBW EOS blood cultures. Our findings are consistent with prior reports addressing TTP for EOS culture drawn primarily from term infants, each of which found significant differences between isolation of gram-positive and gram-negative organisms (37–39). Median TTP was reported as 12.6 hours for one center that reported using 0.5–1.0 mL per standard blood culture bottle (37); and 13.7 hours for a center that reported using 0.5–2.0 mL per pediatric blood culture bottle (38); and 22 hours at a center that reported using inoculation of 1 mL of blood each into pediatric blood culture bottles (39). Neonatal clinicians express concern that intrapartum antibiotics interfere with neonatal blood culture, resulting in falsely negative blood cultures among infected infants. Over 30% of women laboring at term now receive intrapartum antibiotics (40), and in our study, 68% of EOS case infants were born to women who received antibiotics prior to delivery. While we cannot make any conclusions regarding “false negative” blood culture, we did not find any difference in TTP between infants born to women who did or did receive intrapartum antibiotics. Similar results have been noted in other reports that primarily include term infants (37, 41). The findings of this study suggest neonatal clinicians can begin to consider discontinuing empiric antibiotics among VLBW infants with sterile cultures at 36–48 hours of incubation.

Our study is limited by its retrospective design. To strengthen the validity of our findings, delivery characteristics of EOS cases were obtained by both electronic medical record data collection and manual chart review, but prospective data collection would provide the most accurate information. Retrospective prevalence information for potentially-lower EOS risk deliveries was limited by the use of ICD-9 codes which cannot entirely rule out some attempt to induce labor or rupture membranes prior to Cesarean section delivery, resulting in an overestimation of prevalence. This approach may have also underestimated the prevalence of lower-risk deliveries by excluding other preterm Cesarean deliveries done for maternal non-infectious medical illness. While we therefore could not obtain an exact proportion of deliveries that occur in the absence of EOS risk factors, our estimates do suggest that if antibiotic decisions were based on delivery characteristics, a significant reduction in antibiotic use could be achieved. Our results are from a tertiary care center with a primarily inborn preterm population, and therefore are generalizable only to similar settings. Finally, no blood culture or maternal data was available for 55 infants who died at <24 hours of life. Of these infants 32/55 had a birth weight of <=500 grams and 52/55 were <=25 weeks gestation. We do not have the exact time of death for these infants, but the absence of any medication and blood culture data suggests that these infants either could not be resuscitated or died soon after birth.

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Table 1

Microbiology of EOS cases

Organism	N (% total)
Gram-positive	**43 (39.4)**
Group B *Streptococcus*	22 (20.2)
Streptococcus mitis	4 (3.7)
Streptococcus sanguis	4 (3.7)
Streptococcus pneumoniae	3 (2.8)
Other Streptococcal species	3 (2.8)
Staphylococcus aureus	3 (2.8)
Coagulase-negative staphylococci	1 (0.9)
Clostridium perfringens	1 (0.9)
Listeria monocytogenes	2 (1.8)
Gram-negative	**64 (58.7)**
Escherichia coli[†]	40 (36.7)
Bacteroides fragilis	12 (11)
Other Bacteroides species[*]	3 (2.8)
Citrobacter species[#]	3 (2.8)
Haemophilus influenzae	2 (1.8)
Fusobacterium nucleatum	1 (0.9)
Klebsiella pneumoniae	1 (0.9)
Morganella morganii	1 (0.9)
Proteus mirabilis	1 (0.9)
Fungus	**2 (1.8)**
Candida albicans	1 (0.9)
Candida glabrata	1 (0.9)

[*] Other Bacteroides species were *Bacteroides bivius* (1), *Bacteroides melaninogenicus* (1), *Bacteroides vulgatus* (1);

[#] other Citrobacter species were *Citrobacter freundii* (2) and *Citrobacter koseri* (1)

[†] Two *E. coli* cases were polymicrobial, with additional growth of GBS and *Proteus mirabilis* respectively.

Table 2

Demographics and delivery criteria of EOS cases (N=109)

Infant Characteristics	
Birth weight, mean (SD) • BW < 1000 grams, N (%)	989 (288) 59 (54)
Gestational Age, mean (SD) • GA < 28 weeks, N (%)	26.8 (2.3) 83 (76)
Death before NICU discharge, N (%)	33 (30.3)
Delivery characteristics, N (%)	
Vaginal delivery	40 (36.7)
Preterm labor	80 (73.4)
PROM • PROM > 12 hours	63 (57.8) 55 (50.5)
Maternal fever >38.0°C	25 (22.9)
GBS positive • GBS unknown	19 (17.4) 49 (45.0)
Maternal antibiotics, any • Antibiotics ≥4 hours prior to delivery	74 (67.9) 55 (50.5)
Chorioamnionitis	54 (49.5)
Preeclampsia	3 (2.8)
Presence of either preterm labor *or* preterm ROM *or* chorioamnionitis	106 (97.3)

Table 3

Blood Culture and Antibiotic Use by Maternal Diagnosis (N=2748)

	PET/no RF N = 605	Others N= 2143	p - value
Blood culture, N (%)	591 (99.8)	2019 (99.7)	0.52
Antibiotics, N (%)	506 (85.3)	1990 (94.5)	<0.001
EOS, N (cases/1000 VLBW infants)	1 (1.7)	45 (21.0)	0.001

Includes data for all VLBW infants born 1999–2012 with ICD-9 codes. Pre-eclampsia/no perinatal risk factors for EOS (PET/RF) denotes mothers with an ICD-9 code for pre-eclampsia delivered by Cesarean section, without a code for chorioamnionitis or preterm rupture of membranes. Blood culture data missing for 58 infants (12 (2.0%) in PET/no RF group, 45 (2.1%) in the Others group). Antibiotic data missing for 50 infants (12 (2.0%) in the PET/no RF group, 38 (1.8%) in the Others group.) Comparisons and percentages exclude all missing data.