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# Early antibiotic exposure in very-low birth weight infants and infection risk at 3–7 days after birth

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## Abstract

**Objective:** To determine rates of late-onset infection (LOI) during postnatal days 3–7 among preterm infants, based on antibiotic exposure during days 0–2.

**Study Design:** Retrospective cohort study of infants born <1500 grams and ≤30 weeks gestation, 2005–2018. We analyzed the incidence and microbiology of LOI at days 3–7 based on antibiotic exposure during postnatal days 0–2.

**Results:** The cohort included 88,574 infants, of whom 85% were antibiotic-exposed. Fewer antibiotic-exposed compared to unexposed infants developed LOI (1.5% vs 2.1%; adjusted hazard ratio, 0.28, 95% CI 0.24–0.33). Among antibiotic-exposed compared to unexposed infants, Gram-negative (38% versus 28%, p=0.002) and fungal (11% vs. 1%, p <0.001) species were more commonly isolated, and gram-positive organisms (49% vs 70%, p <0.001) were less commonly isolated.

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AUTHOR CONTRIBUTIONS

*Concept and design:* Coggins, Willis, Benjamin, Laughon, Mukhopadhyay, Greenberg, Puopolo *Acquisition, analysis, or interpretation of data*: All authors

*Drafting of the manuscript*: Coggins

*Critical revision of the manuscript for important intellectual content:* All authors

*Statistical analysis:* Willis, Benjamin

*Administrative, technical, or material support:* Benjamin, Clark

*Study supervision:* Benjamin, Laughon, Greenberg, Puopolo

**Conclusions:** We observed low overall rates of LOI at days 3–7 after birth, but antibiotic exposure from birth was associated with lower rates, and with differing microbiology, compared to no exposure.

### Keywords

antibiotics; preterm; neonate; sepsis; infection

## INTRODUCTION

Early-onset neonatal sepsis (EOS) remains an important cause of morbidity and mortality in the neonatal intensive care unit (NICU), and disproportionately affects preterm infants(1,2). Given the risk of early-onset infection and concerns for infection-related mortality among very preterm infants, empiric antibiotic administration at birth is common. In one study of over 50,000 preterm infants in the US, 79% of very low birth weight (VLBW, birth weight <1500 grams) and 87% of extremely low birth weight (ELBW, birth weight <1000 grams) infants received antibiotics within the first three days after birth(3). Further, variation in the proportion of infants administered prolonged early antibiotics was noted, ranging from 0–80% of VLBW and 0–92% of ELBW infants across 113 centers(3).

Empiric antibiotic administration is meant to protect infants at risk of EOS, yet multiple studies associate prolonged antibiotic exposures in the first days after birth with increased rates of subsequent morbidities, including late-onset infection (LOI), necrotizing enterocolitis (NEC), brain injury, bronchopulmonary dysplasia, and mortality(4–8). Early antibiotic exposure may alter the gut microbiome(9) and potentiate antimicrobial resistance(10,11). Antibiotic stewardship efforts among very preterm infants focus on the identification of infants at lowest risk of EOS, for whom early antibiotic exposure may be deferred(12,13). Low EOS risk infants include those born for maternal indications (e.g., preeclampsia), via Cesarean delivery without labor, and rupture of membranes at delivery(13–15). Given the immaturity of the preterm immune system and the frequency of invasive procedures required by VLBW infants in the first days after birth (and associated risk of infection from such procedures), this use of risk stratification could result in increased risk of infection during this period. In addition, early antibiotic administration might protect very preterm infants from early LOI, regardless of risk for EOS.

In 2018, the American Academy of Pediatrics (AAP) published updated recommendations for management of preterm infants at risk of EOS, which endorsed deferral of empiric antibiotic administration among infants deemed low-risk based on delivery characteristics(13). Multiple retrospective observational studies identify extremely low EOS rates in these infants(14–16), including a single-center reports with no increase in infectious outcomes following implementation of low-risk delivery criteria to optimize antibiotic utilization(12). However, among infants born prior to these 2018 recommendations, there was minimal guidance for preterm EOS risk stratification, and little data to characterize the prevalence of infection in the first week after birth relative to empiric antibiotic exposure or non-exposure among preterm infants.

The objective of this study was to estimate the rates of LOI identified on days 3–7 after birth among VLBW infants, conditional on exposure to antibiotics during days 0–2. Secondary objectives were to assess associations of early antibiotic exposure on the microbiology of first-week LOI, and with short-term morbidities and mortality during this period.

## METHODS

### Data Source and Study Population

This was a retrospective cohort study analyzing prospectively-collected data from all VLBW infants born ≤30 weeks' gestation between January 1, 2005 and December 31, 2018 and admitted to one of 292 NICUs managed by the Pediatrix Medical Group. Data are extracted from the group's shared electronic medical record, deidentified, and entered into the Pediatrix Clinical Data Warehouse to support quality improvement and research efforts (17). Data were collected from birth until hospital discharge, transfer, or death. Infants were excluded if they were outborn, transferred from the study site, or died on days 0–2 after birth (where day 0 is defined as the calendar day of birth). Infants with EOS (defined as culture-confirmed bloodstream infection [BSI] or meningitis during days 0–2 due to a bacterial or fungal organism), NEC, or spontaneous intestinal perforation (SIP) during days 0–2 were excluded. Infants with viral infections identified during days 0–7 were excluded. The primary study cohort included all eligible infants during the study period. The Duke University Institutional Review Board approved the study as exempt research with a waiver of informed consent.

### Study Definitions

**Exposure—**Antibiotic exposure was defined as any parenteral antibiotic administration during days 0–2 after birth. Antifungal agents were not included in the exposure definition, given the inability to differentiate antifungal treatment from antifungal prophylaxis.

**Outcomes—**The primary outcome was LOI during postnatal days 3–7. LOI was defined as isolation of a bacterial or fungal pathogen from blood or cerebrospinal fluid culture obtained ≥3 days after birth. LOI due to coagulase-negative *Staphylococcus* (CoNS) was included if CoNS was isolated from (a) 2 or more cultures obtained within 4 calendar days, or (b) 3 cultures within 7 days, or (c) 4 cultures within 10 days. Secondary outcomes included rates of NEC/SIP(18,19), death, and combined LOI or death during days 3–7. We additionally described the age at death and LOI, and organism distributions among infants with LOI, between antibiotic-exposed and unexposed infants.

### Clinical Data Collection

Infant demographic characteristics included gestational age (GA), birth weight, sex, race, and ethnicity. Small for gestational age (SGA) status was defined as birth weight $<10^{th}$ percentile on the Olsen growth chart(20). Maternal and delivery characteristics included: delivery mode, receipt of antenatal corticosteroids, prolonged premature rupture of membranes (PPROM, defined as >18 hours), chorioamnionitis, and maternal group B *Streptococcus* colonization. Characteristics of neonatal clinical management during days 0–2 included mechanical ventilation, administration of surfactant, vasopressors (defined

as dopamine, dobutamine, epinephrine, norepinephrine, or milrinone), and/or systemic corticosteroids (defined as hydrocortisone or dexamethasone).

### Statistical Analysis

Infant demographics, clinical characteristics, and outcomes were summarized using descriptive statistics. Among the primary cohort of all eligible preterm infants, we determined the prevalence of LOI, NEC/SIP, death, and combined LOI/death during days 3–7. We examined the distribution of timing of culture procurement, antibiotic exposure, LOI onset, and death during days 3–7 between these groups. The distribution of organisms isolated during LOI episodes was described among infants with and without early antibiotic exposure. To estimate the association between early antibiotic exposure and outcomes occurring on days 3–7 (LOI, NEC/SIP, death, and combined LOI/death), we performed Cox proportional hazards regression modeling. This modeling approach was chosen in order to account for infants who were transferred during days 3–7 as LOI, NEC/SIP, and death outcomes could not be definitively ascertained. Transfer may have been initiated for infectious conditions, and excluding these infants might introduce systematic selection bias. Infants were censored on the day of outcome occurrence or transfer, whichever came first. Covariates were prespecified based on their potential relationships with early antibiotic exposure and with outcomes of interest, and included GA, SGA status, sex, race (acknowledged as a social construct and included in the model due to potential disparities in antibiotic utilization and LOI incidence by race (21,22)), discharge year, delivery mode, presence of chorioamnionitis, presence of PPROM, and NICU care provision during days 0–2 (invasive ventilation, surfactant, and vasopressors). Two-sided p-values $<0.05$ were considered statistically significant. All analyses were performed using Stata (v17.0, Stata Corporation, College Station TX).

## RESULTS

### Characteristics of Study Participants

Of 88,574 eligible infants, 75,353 (85%) were exposed to antibiotics during postnatal days 0–2 and 13,221 (15%) were unexposed. All baseline characteristics differed significantly between antibiotic-exposed and unexposed infants ($p \leq 0.001$, Table 1). Compared to antibiotic-unexposed infants, antibiotic-exposed infants had lower median GA (27 vs 29 weeks) and were less frequently SGA (13% vs 22%) (Table 1). Among lowest-gestation infants (22–25 weeks), only 4% (619/16,447) were antibiotic-unexposed (Supplemental Table 1). Antibiotic-exposed infants were more frequently born in the setting of PPROM (20% vs 3%) and less frequently born via Cesarean section (71% vs 92%). Overall, 47,385 infants (53% of the cohort) were born via Cesarean section, without rupture of membranes prior to delivery or maternal chorioamnionitis, and without a major congenital anomaly. Of these, 36,637/47,385 (77%) were antibiotic-exposed in days 0–2; the remaining 10,748 infants comprised 81% of the antibiotic-unexposed group. Antibiotic-exposed infants more frequently received mechanical ventilation, surfactant, vasopressors, and systemic corticosteroids (Table 1). Approximately 8% of the cohort (7,313 infants) were transferred during the day 3–7 period.

### Prevalence of LOI, NEC, and Death days 3–7

The primary outcome was LOI during days 3–7, which occurred among 1,437 infants (1.6% of the overall cohort); LOI occurred among 1.5% of antibiotic-exposed infants and 2.1% of antibiotic-unexposed infants. Among antibiotic-exposed and unexposed infants, we identified rates of NEC/SIP (1.7% and 0.8%, respectively), death (2.1% and 1.2%), and combined LOI or death (3.5% and 3.2%) in the first 7 days after birth (Table 2). Infants born 22–25 weeks' gestation constituted the majority of infants who died (67%, 1158/1726), but only 6% (65/1158) of these infants were antibiotic-unexposed (Supplemental Table 1). Beyond day 7, mortality was more common among antibiotic-exposed than unexposed infants (7% vs 3%) with median 21 days to death in both groups (Table 2). When the comparison of antibiotic-exposed and antibiotic-unexposed infants excluded those infants treated with antibiotics on each day 0–7, the relationships between early antibiotic exposure and LOI, death, and LOI or death at days 3–7 remained significant, and the relationship with NEC/SIP at days 3–7 remained non-significant (data not shown).

There were 108 infants who both developed LOI *and* died between days 3–7, accounting for 0.1% of the overall cohort of eligible infants, and 6% of all 1,726 infants who died during days 3–7. Of these 108 infants with LOI who died, 87 (81%) were antibiotic-exposed during days 0–2, and 21 (19%) were antibiotic-unexposed. The majority (72 of 108, 67%) were born 22–25 weeks' gestation (Supplemental Table 1).

In adjusted Cox proportional hazards models, antibiotic exposure during days 0–2 was associated with significantly lower hazards of LOI during days 3–7, compared to infants without early antibiotic exposure (aHR 0.28; 95% CI 0.24, 0.33; $p<0.001$, Table 3). Despite higher proportions of antibiotic-exposed infants dying during days 3–7, the adjusted hazards of death among this group were lower than antibiotic-unexposed infants (aHR 0.61; 95% CI 0.51, 0.74; $p<0.001$).

### Timing of Antibiotic Utilization, LOI Onset, and Death

A blood or cerebrospinal (CSF) culture was sent during postnatal days 0–2 among 97% of antibiotic-exposed infants and 63% of antibiotic-unexposed infants (Table 4). Initial antibiotic administration occurred on the day of birth (day 0) among 95% of antibiotic-exposed infants, and the duration of antibiotic exposure was ≤3 calendar days in 44% of these infants. Among infants with early antibiotic exposure, 23,625/75,353 (31%) had antibiotic duration of at least 7 days from birth (Table 4). Among infants unexposed to antibiotics during days 0–2, 13% (1,759/13,221) were exposed to antibiotics during days 3–7; within this group, antibiotic initiation most frequently occurred on days 3 (589 infants, 33%) and 4 (380 infants, 22%) (Table 4). Among antibiotic-unexposed infants during days 0–2 who received antibiotics during days 3–7, 28% (493/1,759) received antibiotic durations of at least 4 calendar days.

The proportion of LOI identified on each day increased with each successive day during the day 3–7 period, regardless of antibiotic exposure status during days 0–2. LOI occurred least frequently on day 3 (6% of antibiotic-exposed and 14% of antibiotic-unexposed infants with LOI) and most frequently on day 7 (34% and 27%, respectively) (Table 4). In contrast, death

during days 3–7 most commonly occurred on day 3 (28% of antibiotic-exposed and 29% of antibiotic-unexposed infants who died) and progressively decreased in frequency each day through day 7 (Supplemental Table 2).

### LOI Microbiology

Among all eligible infants, LOI during days 3–7 was most frequently attributed to CoNS (29% of LOI episodes), *Escherichia coli* (16%), and *Staphylococcus aureus* (12%). Among antibiotic-exposed infants, LOI was most frequently due to CoNS (32%) and *E. coli* (16%). Nearly all fungal LOI (123 of 124 episodes, all *Candida*) occurred among antibiotic-exposed infants, comprising 11% of all LOI in that group. Among antibiotic-unexposed infants, *S. aureus* occurred most frequently (36% of LOI episodes), followed by CoNS (21%) (Table 5).

## DISCUSSION

In this large cohort of preterm VLBW infants, we identified infants who were antibiotic-exposed and unexposed during days 0–2 after birth, and described their outcomes during days 3–7. It is critical to recognize that our study cohort was born in 2005–2018, prior to the publication of the AAP EOS guidance that strictly defined preterm infants who may be considered at lowest risk of EOS and could be spared initiation of empiric antibiotics after birth(13). Therefore, our retrospective study reflects practice decisions made by clinicians for reasons that we could not determine. Nonetheless, practice variation did exist, and our study describes the associated outcomes. There are 3 key findings in this study. First, early antibiotic exposure was associated with lower hazards of both LOI and death during days 3–7, though LOI and death occurred in <3% of all study infants regardless of earlier antibiotic administration. Second, infants exposed to antibiotics at days 0–2 differed fundamentally from unexposed infants, with distinct demographic and perinatal characteristics and higher illness severity among antibiotic-exposed infants in the first two days after birth. Third, among those developing LOI during days 3–7, the microbiology of infection differed depending on antibiotic exposure at days 0–2.

Although 11% of our study cohort suffered LOI before NICU discharge, we found that only 1.6% suffered this infection during days 3–7 – and those infants administered antibiotics on days 0–2 after birth had a lower incidence (1.2%). Our regression models, which were adjusted for numerous perinatal and neonatal characteristics, identified significantly lower hazards of both LOI and death in days 3–7 among antibiotic-exposed infants. Numerous factors may contribute to lower hazards of LOI in the setting of early antibiotic exposure. Among infants receiving antibiotics limited to days 0–2 (a common “rule-out sepsis” duration), post-discontinuation antibiotic effect may provide persistent circulating antibacterial effect (for ampicillin, has been estimated at up to 30 hours for *E. coli* and 80 hours for GBS)(23). Nearly one-quarter of our antibiotic-exposed study cohort received antibiotics for the entire study period of interest (days 0–7); antibiotic pretreatment in LOI evaluation is known to delay time to blood culture positivity(24) or may sterilize cultures entirely(25). In either case, empiric antibiotic exposure may delay nosocomial bacterial colonization contributing to later LOI, or alter the developing preterm microbiota(9,26). Any “protective” effect of early antibiotic exposure on prevention of LOI did not extend beyond

this period: 11% of early antibiotic-exposed, and 7% of early antibiotic non-exposed infants ultimately suffered LOI before discharge.

Although proportionally more antibiotic-exposed than unexposed infants died during days 3–7, antibiotic exposure was associated with significantly lower adjusted hazards of death during this time period. This finding is challenging to interpret, as we were unable to ascertain the precise cause of death among these infants. However, death appeared to be largely unrelated to infection: only 6% of all deaths during days 3–7 occurred in infants who also had LOI. Inverse patterns of LOI and death timing further suggest that these outcomes may be unrelated, as death occurred most frequently on day 3 and decreased over time, while LOI onset peaked on day 7. Given the fundamental differences in baseline characteristics among antibiotic-exposed and unexposed infants, and despite adjustment for numerous factors, it is possible that residual confounding persists. The impact of adjustment upon a low mortality event rate may have also contributed. If more antibiotic-unexposed infants died than would be expected based on observed patient characteristics, this could be reflected in the inverted relationship we identified in adjusted analyses. While not a primary focus of this study, our findings during days 3–7 did not persist beyond day 7: death was more frequent among antibiotic-exposed infants (who were more preterm with higher early illness severity), with similar median time to mortality between groups. We did attempt to understand the relationship between early death and early antibiotic administration among infants born ≤25 weeks' gestation, infants at statistically highest risk for mortality. The majority of death (67%) and LOI (54%) occurring in the overall cohort occurred among these extremely preterm infants, and the vast majority of these infants (96%) were antibiotic-exposed. It is unclear why the remaining 4% were antibiotic-unexposed, given that practice trends during the study period were overwhelmingly inclined towards antibiotic exposure in this population. It is possible that some of these periviable infants were antibiotic-unexposed due to intention for a palliative approach to care, but we could not determine such intentions from our data.

LOI microbiology differed strikingly by early antibiotic exposure status, with gram-negative and fungal infections occurring more commonly among antibiotic-exposed infants, and *S. aureus* predominating among antibiotic-unexposed infants. Vertical transmission of differing maternal flora might have partly influenced LOI pathogen distribution(27), particularly among antibiotic-exposed infants (who experienced 85% of all gram-negative LOI). Meanwhile, the predominance of gram-positive organisms (*S. aureus,* CoNS) in unexposed infants is suggestive of nosocomial pathogen acquisition, which may result from colonization of mucosa or indwelling devices. Substantial proportions of CoNS LOI in both groups highlight that healthcare-acquired infections are not solely late phenomena and can occur soon after NICU hospitalization. However, these findings also suggest that early antibiotic exposures (in addition to the underlying preterm birth mechanism) might impact the developing infant microbiome and could alter the tropism of LOI as early as the first postnatal week. Particularly striking was the near-universal clustering of fungal LOI among infants who were antibiotic-exposed at birth; while extreme prematurity is a known risk factor for invasive candidiasis, antimicrobial exposure (particularly third-generation cephalosporins and carbapenems) reduces commensal bacterial burden and is also a significant risk factor for fungal LOI in preterm infants(28–30). Compared to term

infants, preterm microbiota have lower microbial diversity with higher relative proportions of pathogenic bacteria(31,32), which is exacerbated among preterm infants receiving early antibiotics(33,34). Additional research in larger cohorts is required to inform the relationship between early antibiotic exposure, microbiome composition, and later infectious complications in preterm infants.

In 2018, the AAP published updated clinical guidance proposing criteria to identify preterm infants at low EOS risk(13). These criteria were derived from retrospective studies of large preterm cohorts that identified very low EOS rates among certain preterm infants, ranging from 0–0.6%(7,14,15). As noted, our current study is not an evaluation of implementation of such criteria; rather, we performed this study to support potential implementation by addressing the short-term affects that early antibiotic administration might have on early hospital-acquired infection. There was substantial overlap between low-risk criteria (birth by Cesarean section, for non-infectious maternal criteria, without labor or rupture of membranes prior to delivery) and the perinatal characteristics of antibiotic-unexposed infants in our study (81% of whom met partial low-risk criteria). Nonetheless, a significant limitation of our study is that we could not determine why clinicians made decisions regarding early antibiotic prescription. A 2012 AAP consensus statement recommended antibiotic discontinuation at 48 hours among infants evaluated for EOS with sterile cultures(35). Yet we found that 56% of early antibiotic-exposed infants received >3 days of antibiotics, and almost one-quarter received >7 days of antibiotics within the first week after birth, despite 1–2% incidence of culture-confirmed early-onset sepsis(2). Other studies in contemporary cohorts have reported similarly high rates (20–70%) of prolonged empiric antibiotic administration, with substantial center variation(3,4,6,36). In contrast, among infants not receiving early empiric antibiotics in our study, only 13% were subsequently administered antibiotics during days 3–7, suggesting that clinicians were firm in their initial determination.

Strengths of this study included analysis of a large cohort of preterm VLBW infants encompassing both academic and community NICUs, enhancing its generalizability. However, we also acknowledge limitations. This study focused specifically on early antibiotic exposure and associated outcomes of LOI restricted to days 3–7 after birth; we did not primarily focus on other outcomes, nor on outcomes occurring after the first postnatal week. While we could determine the precise timing of death within this cohort, we could not ascertain the cause of death for infants who died, nor whether the mechanism of death differed between antibiotic-exposed and unexposed infants. As an observational study of practice variation, we could not determine the indications for antibiotic prescription. Further research (both randomized controlled trials and prospective cohort studies) in the current era of AAP preterm EOS management guidance is needed to better characterize decision-making in preterm empiric antibiotic prescribing and to describe its associated short- and long-term outcomes. Increased standardization of current data-driven preterm empiric antibiotic prescribing guidelines may further inform future studies of outcomes among infants based on early antibiotic exposure status.

## CONCLUSIONS

LOI occurred infrequently among very preterm infants between days 3–7 after birth, regardless of antibiotic exposure status during days 0–2. Nevertheless, antibiotic exposure was associated with lower hazards of LOI and death during days 3–7 compared to unexposed infants, after adjustment for multiple potential confounders. Empiric antibiotic administration at birth is intended to protect infants at risk for EOS, but any benefit of this exposure with respect to infection at days 4–7 is small. It is important to emphasize that the reduced prevalence of LOI associated with early antibiotic exposure was not sustained beyond the limited day 3–7 period. Early antibiotic exposure did not appear to affect the overall prevalence of LOI and mortality after day 7, which was significantly higher among early antibiotic-exposed infants, and likely reflects risk related to underlying patient factors. Distinct LOI microbiology between antibiotic-exposed and unexposed infants during days 3–7 potentially represents maternal, nosocomial, or antibiotic-mediated influences on developing infant microbiota. Strategies to better assess patient-level infection risk in days 0–7 are needed to inform early antibiotic prescribing practices in preterm infants.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Conflicts of Interest/Funding Sources:

Dr. Coggins reports receiving research funding from the National Heart, Lung and Blood Institute of the National Institutes of Health (T32HL007891). Dr. Puopolo reports receiving research funding from the National Institutes of Health, from two contracts with the Centers for Disease Control and Prevention, and from the Children's Hospital of Philadelphia. Dr. Laughon reports receiving support from the National Heart, Lung and Blood Institute of the National Institutes of Health (K24HL143283). Dr. Greenberg has received support from industry for research services (https://dcri.org/about-us/conflict-of-interest/). None of the authors have conflicts of interest to declare relevant to this study.

## DATA AVAILABILITY

The dataset analyzed during the current study derives from the Pediatrix Medical Group database and is not publicly available.

## ABBREVIATIONS

| | |
|---|---|
| **AAP** | American Academy of Pediatrics |
| **BSI** | bloodstream infection |
| **CoNS** | coagulase-negative staphylococci |
| **EOS** | early-onset sepsis |
| **GA** | gestational age |
| **LOI** | late-onset infection |
| **NEC** | necrotizing enterocolitis |

| | |
|---|---|
| **NICU** | neonatal intensive care unit |
| **PPROM** | prolonged preterm rupture of membranes |
| **SGA** | small for gestational age |
| **SIP** | spontaneous intestinal perforation |
| **VLBW** | very low birth weight |

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**Table 1:**

Infant demographic, delivery, and clinical characteristics

| Variable | Median [IQV] or n (%) | | |
|---|---|---|---|
| | **Overall cohort (n=88,574)** | **Antibiotic exposure days 0–2 (n= 75,353)[1]** | **No antibiotic exposure days 0–2 (n= 13,221)[1]** |
| **Demographics** | | | |
| Gestational Age (weeks) | 28 [26 – 29] | 27 [26 – 29] | 29 [28 – 30] |
| Birth weight (g) | 1,004 [775 – 1,225] | 990 [760 – 1,220] | 1,070 [870 – 1,260] |
| Small for gestational age | 12,837 (15%) | 9,891 (13%) | 2,946 (22%) |
| Male sex | 45,408 (51%) | 39,283 (52%) | 6,125 (46%) |
| Race/Ethnicity[2] | | | |
| White, non-Hispanic | 38,192 (45%) | 31,889 (44%) | 6,303 (50%) |
| Black, non-Hispanic | 25,771 (30%) | 22,080 (30%) | 3,691 (29%) |
| Hispanic | 16,228 (19%) | 14,376 (20%) | 1,852 (15%) |
| Other[3] | 5,074 (6%) | 4,200 (6%) | 874 (7%) |
| **Perinatal characteristics** | | | |
| Multiple gestation | 22,838 (26%) | 19,655 (26%) | 3,183 (24%) |
| Delivery by Cesarean section | 65,163 (74%) | 53,201 (71%) | 11,962 (90%) |
| Antenatal corticosteroids | 75,879 (86%) | 63,796 (85%) | 12,083 (91%) |
| Prolonged ROM >18 hours | 15,301 (17%) | 14,927 (20%) | 374 (3%) |
| Chorioamnionitis | 298 (<1%) | 298 (<1%) | 0 (0%) |
| Maternal GBS status | | | |
| Positive | 7,547 (9%) | 6,686 (9%) | 861 (7%) |
| Negative | 16,082 (18%) | 13,781 (18%) | 2,301 (17%) |
| Unknown | 64,945 (73%) | 54,886 (73%) | 10,059 (76%) |
| **Initial management, DOL 0–2** | | | |
| Mechanical ventilation | 56,900 (64%) | 51,794 (69%) | 5,106 (39%) |
| Surfactant administration | 61,521 (69%) | 54,387 (72%) | 7,134 (54%) |
| Vasopressor administration[4] | 14,894 (17%) | 14,415 (19%) | 479 (4%) |
| Systemic corticosteroid exposure[5] | 3,712 (4%) | 3,576 (5%) | 136 (1%) |

1. All listed characteristics differ significantly among antibiotic-exposed versus unexposed infants (p≤0.001) by Pearson's chi-square or Wilcoxon's rank sum test

2. Due to missing data, total number of infants with available race/ethnicity data equals 85,256 (72,545 antibiotic-exposed infants during days 0–2 and 12,720 antibiotic unexposed infants)

3. Includes American/Alaska Native, Asian, Pacific Islander, and other

4. Includes infusions of dopamine, dobutamine, epinephrine, norepinephrine, and/or milrinone

5. Includes hydrocortisone or dexamethasone

**Table 2:**

Outcomes by Early Antibiotic Exposure Status

| Outcome | Overall cohort (n=88,574) | Antibiotic exposure days 0–2 (n= 75,353) | No antibiotic exposure days 0–2 (n= 13,221) | p-value |
|---|---|---|---|---|
| **Outcomes during days 3–7** | | | | |
| LOI | 1,437 (1.6%) | 1,154 (1.5%) | 283 (2.1%) | <0.001 |
| NEC/SIP | 1,398 (1.6%) | 1,290 (1.7%) | 108 (0.8%) | <0.001 |
| Death | 1,726 (1.9%) | 1,566 (2.1%) | 160 (1.2%) | <0.001 |
| Composite: LOI + death | 3,055 (3.4%) | 2,633 (3.5%) | 422 (3.2%) | 0.08 |
| Age at death for those dying on days 3–7, (median [IQR]) | 5 [3 – 6] | 5 [3 – 6] | 4 [3 – 6] | 0.12 |
| **Outcomes after day 7** | | | | |
| LOI after day 7 | 9,834 (11%) | 8,916 (12%) | 918 (7%) | <0.001 |
| NEC after day 7 | 5,680 (6%) | 5,099 (7%) | 581 (4%) | <0.001 |
| Death after day 7, prior to NICU discharge | 4,840 (6%) | 4,518 (7%) | 322 (3%) | <0.001 |
| Age at death (days) for those dying after 7 days, (median [IQR]) | 21 [13 – 39] | 21 [13 – 39] | 21 [12 – 35] | 0.23 |

**Table 3:**

Early Antibiotic Exposure and Hazards of LOI, NEC, and Death during Days 3–7

| Outcome | Any antibiotic exposure days 0–2 (n, %) | No antibiotic exposure days 0–2 (n, %) | Hazard ratio [95% CI] | p-value |
|---|---|---|---|---|
| LOI | 1,154 (1.5%) | 283 (2.1%) | 0.28 [0.24, 0.33] | <0.001 |
| NEC/SIP | 1,290 (1.7%) | 108 (0.8%) | 1.09 [0.87, 1.36] | 0.453 |
| Death | 1,566 (2.1%) | 160 (1.2%) | 0.61 [0.51, 0.74] | <0.001 |
| Composite: LOI + death | 2,633 (3.5%) | 422 (3.2%) | 0.42 [0.37, 0.47] | <0.001 |

Model adjusted for gestational age at birth, SGA status, sex, race, delivery mode, discharge year, presence of chorioamnionitis, presence of prolonged rupture of membranes, need for invasive ventilation days 0–2, need for surfactant days 0–2, receipt of vasopressors day 0–2

**Table 4:**

Timing of Antibiotic Utilization, Duration, and LOI Onset

| | Median [IQR] or n (%) | | |
|---|---|---|---|
| | **Overall cohort (n=88,574)** | **Antibiotic exposure days 0–2 (n= 75,353)** | **No antibiotics exposure days 0–2 (n= 13,221)** |
| **Antibiotics initiated between days 3–7 (%)** | 1,759 (2%) | --- | 1,759 (13%) |
| **Days after birth at which antibiotics initiated** | | | |
| 0 | 71,529 (81%) | 71,529 (95%) | -- |
| 1 | 2,742 (3%) | 2,742 (4%) | -- |
| 2 | 1,082 (1%) | 1,082 (1%) | -- |
| 3 | 589 (1%) | -- | 589 (5%) |
| 4 | 380 (< 1%) | -- | 380 (3%) |
| 5 | 276 (< 1%) | -- | 276 (2%) |
| 6 | 246 (< 1%) | -- | 246 (2%) |
| 7 | 268 (< 1%) | -- | 268 (2%) |
| **Total calendar days of antibiotic exposure during days 0–7 (%)** | | | |
| 0 days | 11,462 (13%) | 0 (0%) | 11,462 (87%) |
| 1 days | 1,472 (2%) | 1,059 (1%) | 413 (3%) |
| 2 days | 5,415 (6%) | 5,099 (7%) | 316 (2%) |
| 3 days | 27,783 (31%) | 27,246 (36%) | 537 (4%) |
| 4 days | 10,880 (12%) | 10,599 (14%) | 281 (2%) |
| 5 days | 4,263 (5%) | 4,051 (5%) | 212 (2%) |
| 6 days | 3,637 (4%) | 3,637 (5%) | 0 (0%) |
| 7 days | 6,566 (7%) | 6,566 (9%) | 0 (0%) |
| 8 days | 17,096 (19%) | 17,096 (23%) | 0 (0%) |
| **Patients with blood or CSF culture sent days 0–2** | 81,550 (92%) | 73,257 (97%) | 8,293 (63%) |
| Patients with blood culture sent days 0–2 | 81,550 (92%) | 73,257 (97%) | 8,293 (63%) |
| Patients with CSF culture sent days 0–2 | 916 (1%) | 912 (1%) | 4 (< 1%) |
| **Patients with blood or CSF culture sent days 3–7** | 15,528 (18%) | 13,482 (18%) | 2,046 (16%) |
| Patients with blood culture sent days 3–7 | 14,580 (17%) | 12,538 (17%) | 2,042 (16%) |
| Patients with CSF culture sent days 3–7 | 1,644 (2%) | 1,541 (2%) | 103 (1%) |
| **Patients with at least one blood or CSF culture sent** | | | |
| Day 0 | 79,294 (90%) | 71,278 (95%) | 8,016 (61%) |
| Day 1 | 2,854 (3%) | 2,633 (4%) | 221 (2%) |
| Day 2 | 2,618 (3%) | 2,452 (3%) | 166 (1%) |
| Day 3 | 2,959 (3%) | 2,318 (3%) | 641 (5%) |
| Day 4 | 3,269 (4%) | 2,787 (4%) | 482 (4%) |

| | Median [IQR] or n (%) | | |
|---|---|---|---|
| | **Overall cohort (n=88,574)** | **Antibiotic exposure days 0–2 (n= 75,353)** | **No antibiotics exposure days 0–2 (n= 13,221)** |
| Day 5 | 3,509 (4%) | 3,087 (4%) | 422 (3%) |
| Day 6 | 3,999 (5%) | 3,534 (5%) | 465 (4%) |
| Day 7 | 4,362 (5%) | 3,887 (5%) | 475 (4%) |
| **Day of positive blood or CSF culture** | | | |
| Day 3 | 111 (0.1%) | 72 (0.1%) | 39 (0.3%) |
| Day 4 | 197 (0.2%) | 147 (0.2%) | 50 (0.4%) |
| Day 5 | 266 (0.3%) | 207 (0.3%) | 59 (0.4%) |
| Day 6 | 396 (0.4%) | 338 (0.4%) | 58 (0.4%) |
| Day 7 | 467 (0.5%) | 390 (0.5%) | 77 (0.6%) |
| Total Day 3–7 | 1,437 (1.6%) | 1,154 (1.5%) | 283 (2.1%) |

**Table 5:**

Organism Distribution among Patients with LOI

| | n (%) | By Antibiotic Exposure Group, n (%) | | |
|---|---|---|---|---|
| **Organism** | **Overall Cohort (n = 1,450 isolates in LOI)** | **Antibiotic exposure days 0–2 (n = 1,164 isolates in LOI)** | **No antibiotic exposure days 0–2 (n = 286 isolates in LOI)** | **P-value** |
| **Gram-positive** | 768 (53%) | 569 (49%) | 199 (70%) | <0.001 |
| Coagulase-negative *Staphylococcus* | 427 (29%) | 367 (32%) | 60 (21%) | |
| *Staphylococcus aureus* | 174 (12%) | 71 (6%) | 103 (36%) | |
| *Enterococcus* spp. | 35 (2%) | 20 (2%) | 15 (5%) | |
| Gram-positive cocci, not speciated | 127 (9%) | 108 (9%) | 19 (7%) | |
| Group B *Streptococcus* | 4 (<1%) | 3 (<1%) | 1 (<1%) | |
| Clostridia | 1 (<1%) | 0 (0%) | 1 (<1%) | |
| **Gram-negative** | 524 (36%) | 443 (38%) | 81 (28%) | 0.002 |
| *E. coli* | 236 (16%) | 217 (19%) | 19 (7%) | |
| *Klebsiella* | 80 (6%) | 62 (5%) | 18 (6%) | |
| *Enterobacter* | 34 (2%) | 20 (2%) | 14 (5%) | |
| *Pseudomonas* | 33 (2%) | 29 (3%) | 4 (1%) | |
| *Citrobacter* | 15 (1%) | 11 (1%) | 4 (1%) | |
| *Acinetobacter* | 9 (1%) | 6 (1%) | 3 (1%) | |
| *Serratia* | 18 (1%) | 15 (1%) | 3 (1%) | |
| *Proteus* | 5 (<1%) | 3 (<1%) | 2 (1%) | |
| *Haemophilus influenzae* | 3 (<1%) | 3 (<1%) | 0 (0%) | |
| *Stenotrophomonas* | 3 (<1%) | 2 (<1%) | 1 (<1%) | |
| *Neisseria* | 1 (<1%) | 1 (<1%) | 0 (0%) | |
| Gram-negative rod, not speciated | 87 (6%) | 74 (6%) | 13 (5%) | |
| **Fungi (all *Candida*)** | 124 (9%) | 123 (11%) | 1 (<1%) | <0.001 |
| **Other** | 34 (2%) | 29 (3%) | 5 (2%) | 0.46 |

Footnote: The number of organisms isolated overall (1,450) is higher than the number of total LOI episodes (1,437), as a small number of infants had >1 organism isolated at the time of LOI diagnosis.