Pathogens Distribution and Antimicrobial Resistance in Bloodstream Infections in Twenty-Five Neonatal Intensive Care Units in China, 2017-2019

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Abstract

Background

Overcrowding, abuse of antibiotics and increase of antimicrobial resistance negatively affect neonatal survival rates in developing countries. We aimed to define pathogens and their antimicrobial resistance (AMR) of early-onset sepsis (EOS), hospital-acquired late-onset sepsis (HALOS) and community-acquired late-onset sepsis (CALOS) in 25 neonatal intensive care units (NICUs) in China.

Study design

This retrospective descriptive study included pathogens and their AMR from all neonates with bloodstream infections (BSIs) admitted to 25 tertiary hospitals in China from January 1, 2017, and December 31, 2019. We defined EOS as the occurrence of BSI at or before 72 hours of life and late-onset sepsis (LOS) if BSI occurred after 72 hours of life. LOS were classified as CALOS if occurrence of BSI was ≤ 48 hours after admission, and HALOS, if occurrence was > 48 hours after admission.

Results

1092 pathogens of BSIs collected from 25 NICUs including 349 EOS isolates, 702 HALOS isolates and 41 CALOS isolates. Gram-negative bacteria (GNB) dominated in EOS (56.7%) and HALOS (62.2%). The most frequent pathogens causing EOS were *Escherichia coli* (27.2%) and group B streptococcus (GBS; 14.6%) whereas in CALOS they were GBS (46.3%) and *Staphylococcus aureus* (41.5%). *Klebsiella pneumonia* (27.9%), *Escherichia coli* (15.7%) and fungi (12.8%) were the top three isolates in HALOS. Third-generation cephalosporin resistance rates in GNB ranged from 9.7–55.6% in EOS and 26–63.3% in HALOS. Carbapenem resistance rates in GNB ranged from 2.7–31.3% in HALOS and only six isolates in EOS were carbapenem resistant. High rates of multidrug resistance were observed in *Klebsiella pneumonia* (60.7%) in HALOS and in *Escherichia coli* (44.4%) in EOS. All gram-positive bacteria were susceptible to vancomycin except for three *Enterococcus faecalis* in HALOS. The adverse outcome rates in HALOS were high, ranging from 4.6–16.2%.

Conclusions

Escherichia coli, *Klebsiella pneumonia* and GBS were the leading pathogens in EOS, HALOS and CALOS, respectively. The high proportion of pathogens, high degree of antimicrobial resistance and high proportion of adverse outcomes in HALOS underscore understanding of the pathogenesis and devise effective interventions in developing countries.

Background

Neonatal bloodstream infection (BSI) is the third most common cause of neonatal morbidity and mortality globally, and is an ongoing major global public health challenge (1, 2). Asia and Africa have the highest burden of BSIs in the world (2). Scarcity in resources, insufficient surveillance and infection

control, abuse of antibiotics and increase of antimicrobial resistance in low-income and middle-income countries (LIMICs) may contribute to this situation (3, 4). The risk of emergence and spread of antibiotic resistance in South East Asia is thought to be among the highest among all the WHO regions (5, 6). Monitoring resistance in disease causing pathogens is of particular importance for neonatal BSIs in LIMICs, where most treatments are empirically prescribed but should be based on reliable contemporaneous resistance data.

Neonatal sepsis was classified into early onset sepsis (EOS) and late onset sepsis (LOS) routinely (7, 8). EOS generally reflects vertical transmission from mothers while LOS cases were likely due to pathogens acquired after delivery and often from nosocomial infections (9). Currently, data on antimicrobial resistance (AMR) distinguishing between community-acquired LOS (CALOS) and hospital-acquired LOS (HALOS) in neonates are scarce (3, 10). To combat the threat of AMR, distinguishing emerging resistance in neonatal BSI is vital in order for HALOS to be at least partially preventable. We conducted a three years multi-center retrospective study to investigate the current data on common causative pathogens and AMR patterns in BSIs, distinguishing between EOS, HALOS and CALOS.

Methods

Settings and Infection Control Methods

Twenty-five tertiary hospitals participated in the current study. Among the hospitals, twenty-three tertiary hospitals were located in Shandong province which involves 13 major cities, one tertiary hospital in Hebei province and the other one in the Inner Mongolia Autonomous Region. Nineteen hospitals were general hospitals and 6 were maternal and child health care hospitals. Because all 25 hospitals have their own maternity/obstetric ward, most neonates were born on site and only a few were transferred. The number of beds ranged from 20 to 60. The ratio of nurses to bed ranged from 0.4 to 1.2 and physician to nurse ranged from 0.3 to 0.5. The bed occupancy rate was maintained above 90%. All 25 hospitals have an infection control committee. Trained infection control nurses were available at all units at all times. All NICUs had a hand hygiene policy, but audits of staff compliance were not undertaken. Disinfectant dispensers filled with Betadine 7.5% were provided at hand-wash sinks, and clean disposable tissue papers for hand-drying were sufficiently available. None of 25NICUs have laminar flow devices. Windows are opened regularly to keep the air fresh under the premise of keeping warm, and all NICUs equipped with ultraviolet air sterilizers to sterilize the air repeatedly. Surveillance cultures were only used when an outbreak was suspected but were not routinely undertaken.

Identification and Susceptibility Testing

Blood cultures were performed for any infant presenting with clinical signs or symptoms of sepsis according to the local guidelines of each hospital. Blood samples were collected by trained nurses or physicians. Venipuncture sites were prepared with 75% isopropyl alcohol, followed by iodine tincture, and then wiped with alcohol. Skin site was allowed to dry for 1 min prior to venipuncture. A general policy of using one culture bottle exclusively for newborns with at least 1 ml of blood sample was adopted by all

hospitals. Sample was delivered to the microbiology laboratory within 2 hours of collection by staff members. Species identification was performed at recruited hospital laboratories on automated detection systems using Bactec FX system (Becton Dickinson, USA) in 15 hospitals and BacT/ALERT 3D system (bioMérieux, France) in 10 hospitals. Antimicrobial susceptibility testing of pathogens was undertaken by disk diffusion and Etest MIC, according to Clinical and Laboratory Standards Institute guidelines (11).

Definitions

Our definition of neonatal sepsis was formulated with consideration to Chinese consensus of diagnosis and treatment. Neonatal sepsis was defined as the growth of a single pathogen (bacterium or fungus) from the blood of an infant who fulfilled all three of the following criteria: (1) One or more of the following infection-related clinical manifestations: respiratory distress, apnea; tachycardia or bradycardia; systemic hypotension or hypoperfusion; hypothermia or fever ($T > 38.5^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$); convulsions, hypotonia, irritability or lethargy; feeding intolerance or intestinal obstruction. (2) One or more abnormal hematologic index: white blood cell count ($< 5 \times 10^9/\text{L}$ or $> 30 \times 10^9/\text{L}$ for age $\leq 3\text{d}$ or $> 20 \times 10^9/\text{L}$ for age $> 3\text{d}$), increase of immature/total neutrophil (≥ 0.16 for age $\leq 3\text{d}$ or $\geq 0.12 \times 10^9/\text{L}$ for age $\geq 3\text{d}$), C-reactive protein level ($\geq 10\text{mg/L}$) or abnormal procalcitonin level ($\geq 0.5\text{ mg/L}$). (3) Antibiotics used for at least 5 days (12, 13).

EOS was defined as occurrence of sepsis at or before the first 72h of life while LOS was defined as occurrence of sepsis after the first 72 h of life. Among LOS, infants with sepsis onset ≤ 48 hours after admission were considered as having CALOS, and those with onset > 48 hours after admission were considered as having HALOS (10).

Antimicrobial susceptibilities were reported as susceptible or resistant (intermediate or resistant) based on microbiology reports. Resistance proportions were reported as number of resistant pathogens/number of pathogens tested. MDR gram negative bacteria were defined by resistance to at least three of the following antimicrobial categories: carbapenems (imipenem and meropenem), penicillins (piperacillin, Ampicillin, and piperacillin/tazobactam), broad-spectrum cephalosporins (ceftazidime and cefepime), monobactams (aztreonam), aminoglycosides, and fluoroquinolones (14).

We defined our adverse outcomes (15) as any of the following: death (all cause after neonatal unit admission until discharge or transfer); any of grade ≥ 3 peri-intraventricular hemorrhage (IVH) or Periventricular leukomalacia (PVL) (16); moderate or severe bronchopulmonary dysplasia (BPD) defined as babies needing mechanical ventilation or oxygen dependency at 36 weeks of postmenstrual age or discharge (17); retinopathy of prematurity (ROP) requiring treatment by laser or cryotherapy (18); or necrotizing enterocolitis (NEC) $>$ stage 2, according to Bell's criteria (19).

Data Collection and Statistical Analyses

The medical record of each infant with positive blood culture was reviewed by a local neonatologist and data were documented onto a unified standardized worksheet from all 25 NICUs. Data of the worksheet included the medical institution, number of cots, staffing ratios, medical record number, gestational age,

birth weight, gender, date of birth, date of blood cultures obtained, isolates identified, clinical significance of isolates and antimicrobial susceptibility. Other clinical data were also collected including body temperature, heart rate, white blood cell count, procalcitonin and C-reactive protein in the 72h before and after blood cultures were collected. The ethics committees of all 25 participating hospitals approved the study and allowed data sharing. Procedures were in accordance with the Helsinki Declaration of the World Medical Association.

Descriptive analysis was performed to characterize the study population. Frequencies, proportions and summary statistics were used to describe relevant variables.

Results

Overall

Of the 66516 specimens collected, 2718 (4.1%) were culture positive, yielding a total of 2751 isolates from January 1, 2017, to December 31, 2019 in 25 Chinese NICUs. However, only 1092 (39.7%) isolates were classified as disease causing pathogens that met inclusion criteria, excluding 1644 (59.8%) contaminants and 16 (0.5%) isolates within the identical BSI episodes of a previous pathogen (Figure I). Of these 1092 pathogens, 349 (32%) pathogens were responsible for EOS, 702 (64.3%) for HALOS and 41 (3.7%) for CALOS. No neonate experienced both EOS and HALOS. Four infants had HALOS caused by polymicrobial pathogens, namely one with *Escherichia coli* and *Klebsiella pneumonia*, one with *Klebsiella pneumonia* and *Enterococcus* species, and two infants with *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The characteristics of the study population are presented in Table I. Pathogens responsible for HALOS were more common in neonates with lower birth weight and lower gestational age. Data from HALOS patients noted 287 (41.4%) very low birth weight (<1500 g) neonates and 380 (54.4%) neonates with gestational age less than 34w. In EOS, 178 (51%) were term infants and 203 (58.2%) were neonates with normal birth weight. There were strong gestational age and birth weight predominance in CALOS, with 40 (97.6%) occurring in neonates with normal birth weight and 37 infants (90.2%) being of term. Pathogens were detected more frequently in males than females (624 vs. 464). The adverse outcome rates in EOS and HALOS range from 1.7% to 7.4%, and from 4.6% to 16.2%, respectively.

Pathogen Distribution

Table II shows the pathogen distribution that caused neonatal EOS, HALOS and CALOS. GNB was dominant in both EOS and HALOS, with the proportion of 56.7% (198/349) and 62.2% (437/702), respectively. In contrast, 87.8% (36/41) of pathogens that caused CALOS were GPB. *Escherichia coli* and GBS were the most common pathogenic bacteria of EOS, accounting for 27.2% (95/349) and 14.6% (51/349). 84.3% (43/51) of GBS was identified from term infants and 15.7% (8/51) in preterm infants with EOS. In contrast, *Escherichia coli* was responsible for 61.1% (58/95) of pathogens in preterm infants, and 38.9% (37/95) in term infants with EOS. *Klebsiella pneumonia*, the leading pathogen of HALOS, was responsible for 27.9% (196/702) of the cases, followed by *Escherichia coli* (15.7%, 110/702) and Fungi (12.8%, 90/702). *Klebsiella pneumonia* was primarily identified among preterm infants (75.5%; 148/196).

97.8% (90/92) of fungi cases were identified in HALOS, and all of them were of candida spp. GBS and *Staphylococcus aureus* were responsible for 46.3% (19/41) and 41.5 % (17/41) of CALOS cases.

Antimicrobial Resistance

Table III presents antimicrobial resistance patterns in common GNB and GPB in EOS and HALOS respectively. Most GNB in HALOS showed a high degree of antimicrobial resistance, not only to commonly used ampicillins (87.5%–100%) and third-generation cephalosporins (26%–63.3%) but also to reserved antibiotics such as carbapenems (2.7%–31.3%). A high proportion of *Klebsiella pneumoniae* (60.7%, 119), *Escherichia coli* (37.3%, 41), *Pseudomonas aeruginosa* (37.3%, 6) and *Acinetobacter baumannii* (35.7%, 10) in HALOS were multidrug resistant. Carbapenem resistance was uncommon in EOS: 44.4% (4/9) of *A. baumannii* isolates and 2.1% (2/95) of *Escherichia coli* were resistant. The proportion of resistant isolates was highest for *Escherichia coli* in EOS: 84.9% (79/93) were ampicillin resistant, 49.5% (47/95) were third-generation cephalosporins resistant and 44.4% (42/95) were multidrug resistant. None of the five GNB caused CALOS were carbapenem and multidrug resistant.

Among GPB in HALOS, methicillin resistance was detected in 77.8% (63/81) of coagulase-negative staphylococci and 80% (28/35) of *S. aureus*. Significant methicillin resistance rate was identified in *Staphylococcus aureus* which was 88.2% (15/17). High rates of resistance were observed in GBS in CALOS to erythromycin (89.5%, 17/19) and clindamycin (80%, 15/19). All the isolates of coagulase-negative staphylococci, *Staphylococcus aureus* and GBS were susceptible to vancomycin, but three enterococci isolates (16.7%, 3/18) in HALOS were resistant.

Discussion

Due to different empirical uses of antibiotics and implementation of preventive measures, the bacterial spectrum may vary geographically and temporally (9). In this multicenter retrospective study, we highlight *Klebsiella pneumoniae*, *Escherichia coli* and GBS as the leading causes of EOS, HALOS and CALOS, respectively. This study was conducted in China, a lower-middle income country with paucity of high-quality data. HALOS caused by *K pneumoniae* predominated in our study and was associated with a high degree of AMR and adverse outcomes. The result would be important for clinicians in Chinese NICUs to guide optimal clinical prevention strategies and for consideration of empirical antibiotic treatment during clinical management.

Nosocomial infection is a major health problem particularly in NICUs in developing countries (20). In current study, about two thirds of pathogens identified were responsible for HALOS. Approximately 60% of HALOS were due to GNB which was similar to the results from previous Chinese studies (21, 22). A review of 11,471 bloodstream samples indicated that GNB was detected from no less than 60% of positive blood cultures in all the developing settings of the world (23). Similar to that reported from South Asia (3) and Egypt (7), *Klebsiella pneumoniae* was the most common GNB causing LOS. By contrast, Coagulase negative staphylococcus (CoNS) was significantly predominant in western countries for LOS, such as 40% in Switzerland (10). The preponderance of CoNS might indicate the developed regions'

adoption of neonates with lower gestational age and lower birth weight and prolonged use of central catheters which are risk factors for CoNS infection (24). The predominance of GNB in our developing counties may largely be attributed to the lack of standard infection-control practices. Insufficient hand hygiene, lack of essential equipment and supplies including sinks, running water and disposables, overcrowding and understaffing are described to be key contributions to nosocomial infection caused by GNPs (25). We find that HALOS frequently correlated with low gestational age, low birth weight and comorbidities which were also mentioned in another recent prospective population-based cohort study (10). Therefore, implementation of these basic hygiene practices should be emphasized more in Chinese NICUs to minimize the hazards of the high incidence of HALOS caused by GNB. Also, empiric antibiotics selected to treat suspected HALOS in Chinese NICUs need to effectively treat GNP, especially *Klebsiella pneumoniae*.

Another remarkable finding in the Chinese NICUs was the relatively high percentage of fungi in HALOS. Nearly all the identified fungi infections were responsible for HALOS, which was paralleled with other Chinese studies (21, 22). Similarly, recent studies have also reported outbreaks of fungal nosocomial infection in Chinese NICUs which alerted clinicians (26, 27). Prolonged antibiotic therapy, broad spectrum antibiotic exposure may be the connection to high prevalence of fungi nosocomial infections (28). This highlights the need to develop new and more effective approaches to prevent HALOS.

A strength of this study was that all 25 hospitals have their own maternity/obstetric ward and almost all neonates were born onsite with only a few transferred. This makes the pathogens in EOS more precise and representable. In the current study, *Escherichia coli* was the most common pathogen in EOS, followed by GBS. Similarly, the latest surveillance from a national neonatal research network in US demonstrated the shift from GBS to *E. coli* as the predominant pathogen and the increase in *Escherichia coli* infections among very low-birth-weight infants (29). *Escherichia coli* predominated among preterm infants in current study which was similar to those reported in most developed countries (6, 10, 29). Previously, GBS was reported to be a rare cause of EOS and was documented in only few reports from China and other Asian countries (7, 30). Reason for the low proportion of GBS in EOS may be partially due to the overuse of antenatal antibiotics in China. However, we did not have the data on antenatal antibiotic use. *Escherichia coli* was the most common pathogen in EOS and the second common in HALOS. The extraordinary similarity of this spectrum supports the assumption that the cause of EOS may not only be due to vertical transmission from mothers but also can be caused by unsanitary practices in the labour rooms and NICUs. Further investigations are of urgent need to identify the causes of GNP in EOS in China and subsequently develop targeted prevention strategies.

Most CALOS occurred in term or near-term newborns, accounting for a small percentage of BSI. GBS and *Staphylococcus aureus* were the leading responsible pathogens. Similar to reports in developed counties, infants with CALOS had a higher birth weight and gestational age and fewer adverse outcomes compared to those who developed HALOS (10). The relatively low incidence rate may be related to the lack of strict management of antibiotics in China and relatively easy access. Antibiotics may have been applied before admission resulting in false negative results in blood cultures.

Ampicillin/piperacillin and third-generation cephalosporin are the first choices for empirical treatment to neonatal sepsis in China (31) however ampicillin and gentamicin are commonly recommended in some areas (32). Unfortunately, in our study, GNB from HALOS exhibited a high degree of resistance to first and second line drugs recommended by the World Health Organization, ampicillin (87.5%-100%) and third generation cephalosporins (26%-63.3%), whereas only 6%-20.9% exhibit resistance to gentamicin. Furthermore, a relatively high proportion of GNB was resistant to the WHO classified “watch group” antibiotics, such as meropenem (2.7%-31.3%). Nowadays, multidrug-resistance GNB is of increasing concern in neonates because few therapeutic options are available (33). *Klebsiella pneumonia* and *Escherichia coli* received much attention because of their high rates of MDR in HALOS (60.7%) and EOS (40.4%), respectively. Research found that the most frequent mechanism of MDR in GNB was ESBL production (34). Paralleled to the rates of MDR, approximately two thirds of *Klebsiella pneumonia* in HALOS and half of *Escherichia coli* in EOS were resistant to third-generation cephalosporins. The increasing resistance to second and third line antibiotic medication is of great concern. When suspecting sepsis in a neonate that might be caused by a GNB, empiric use of the available antibiotic should be more and more tailored to the local antibiogram.

Another issue of equal concern is the high proportion of methicillin-resistant *Staphylococcus aureus* in LIMCs, especially in south Asia where 56% of *S. aureus* infections were reported to be methicillin-resistant (23). In our study, more than two thirds of *Staphylococcus aureus* were methicillin resistant. Fortunately, all of the cases remained susceptible to watch group antibiotics such as vancomycin, which was in agreement with most other studies (7, 16).

Antimicrobial resistance is a rapidly emerging, potentially disastrous problem. Resistance to alternative antibiotics is seriously limiting our antibiotic options. In general, empirical antibiotic treatment should be guided by the antimicrobial resistance patterns of bacterial pathogens commonly detected from local infections. We need more reliable and accurate diagnostic methods to rule out sepsis, thereby preventing abuse of antibiotics in NICUs.

Strengths of this study include its enrollment of pathogens from all neonates admitted to the NICU, distinguishing pathogens and AMR between EOS, HALOS and CALOS, and large sample size. The following limitations should be considered: 1) the choice of a definition on neonatal sepsis is a limitation inherent to many studies. The diagnosis of neonatal sepsis is mainly based on Chinese Consensus formulated by Chinese Pediatric Society which may not have been widely accepted (24). The diagnosis of infection of CONS usually relies on confirmation with second blood culture, but this practice is not routinely followed in LMICs. Therefore we are not certain whether CONS represented true pathogen infections or potential contaminants; 2) antibiotics are usually used in offsite born neonates prior to admission that limit potential pathogens identified for CALOS. 3), we merely collected the data on the proportion of causative pathogens between EOS, HALOS and CALOS, but we were unable to calculate the incidence rates.

Conclusion

In this study, EOS and HALOS were most commonly caused by GNB, with *Escherichia coli* and *Klebsiella pneumoniae* being the major infectants, respectively. Only a small proportion of pathogens were identified for CALOS, most commonly GBS. HALOS have a high proportion of pathogens, prevalence of AMR and adverse outcomes. Effective interventions are urgently needed to reduce HALOS in LMICs. Continued surveillance is warranted to identify pathogen distribution and AMR, and to distinguish between EOS, HALOS and CALOS causing agents.

Abbreviations

BSI: Neonatal bloodstream infection; AMR: Antimicrobial resistance; GBS Group B streptococcus; EOS: early-onset sepsis; HALOS: hospital-acquired late-onset sepsis; CALOS: community-acquired late-onset sepsis; GNB: Gram-negative bacteria; LMICs: low-income and middle-income countries; CONS: Coagulase negative staphylococcus.

Declarations

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Authors' contributions

YHY, the corresponding author, doctorate, and professor of medicine, designed the study, trained and supervised the data collectors, interpreted the results and revised the manuscript. The first author, namely, JL, played a role in the analysis and interpretation of the data and in the preparation and drafting of the manuscript. The co-first authors, participated in the design of the study, the collection and interpretation of the data and writing of the manuscript. All authors listed on the manuscript approved the submission of this version of the manuscript and would accept full responsibility for the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate

The Institutional Review Board of Shandong Provincial Hospital Affiliated with Shandong University approved this project. All authors have signed written informed consent and approved the submission of this version of the manuscript and take full responsibility for the manuscript. The legal guardian of all participants signed an informed consent form that their data could be used for various clinical studies.

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Tables

Table 1 Demographic and Clinical Characteristics of the Study Population

	All patients (n= 1088 infants)	EOS (n= 349 infants)	HALOS (n= 698 infants)	CALOS (n=41 infants)
Birth weight (grams), n (%)				
≤1500	352 (32.4)	65 (18.6)	287 (41.1)	0
1501- 2500	230 (21.1)	81 (23.2)	148 (21.2)	1 (2.4)
≥2500	506 (46.5)	203 (58.2)	263 (37.7)	40 (97.6)
Gestational age (weeks), n (%)				
≤28	104 (9.6)	23 (6.6)	81 (11.6)	0
28-34	386 (35.5)	87 (24.9)	299 (42.8)	0
34-37	130 (11.9)	61 (17.5)	65 (9.3)	4 (9.8)
≥37	468 (43)	178 (51)	253 (36.2)	37 (90.2)
Male sex, n (%)	624 (57.4)	184 (52.7)	441 (63.2)	29 (70.7)
Age during blood sampling (days) (median, IQR)	10 (2–22)	1 (0–2)	17 (10–27)	13 (9–23)
Length of hospital stay (days) (median, IQR)	36 (15–56)	17 (10–36)	39 (19–63)	16 (11–24)
Adverse outcomes, n (%)				
Grade ≥3 IVH or PVL	34 (3.1)	6 (1.7)	26 (3.7)	2 (4.9)
Moderate or severe BPD	106 (56)	17 (4.9)	89 (12.8)	0
ROP requiring treatment	54 (5.0)	7 (2.0)	47 (6.7)	0
NEC > stage 2	132 (12.1)	18 (5.2)	113 (16.2)	1 (2.4)
Deaths	58 (5.3)	26 (7.4)	32 (4.6)	0

Abbreviations: IQR, interquartile range; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia; BPD, bronchopulmonary dysplasia.

Table 2 Pathogen Distributions in EOS, CALOS and HALOS at 25 NICUs, January 2017–December 2019

Pathogens	EOS <i>n</i> =349 <i>n</i> (%)	HALOS <i>n</i> =702 <i>n</i> (%)	CALOS <i>n</i> =41 <i>n</i> (%)	Total <i>n</i> =1092 <i>n</i> (%)
Gram-positive bacteria	149 (42.7)	175 (25.0)	36 (87.8)	360 (33.0)
CoNS	28 (8.0)	81 (11.5)	0	109 (10.0)
GBS	51 (14.6)	15 (2.1)	19 (46.3)	85 (7.8)
Staphylococcus aureus	19 (5.4)	35 (5.0)	17 (41.5)	71 (6.6)
Enterococcus spp.	11 (3.2)	18 (2.6)	0	29 (2.7)
Listeria monocytogenes	22 (6.3)	1 (0.1)	0	23 (2.1)
Other Gram-positive bacteria	18 (5.2)	25(3.6)	0	43 (4.0)
Gram-negative bacteria	198 (56.7)	437 (62.2)	5 (12.2)	640 (58.6)
Escherichia coli	95 (27.2)	110(15.7)	4 (9.8)	209 (19.1)
Klebsiella pneumonia	31 (8.9)	196 (27.9)	0	227 (20.8)
Enterobacter spp.	19 (5.4)	50 (7.1)	1 (2.4)	70 (6.5)
Serratia marcescens	19 (5.4)	19 (2.7)	0	38 (3.5)
Acinetobacter baumannii	9 (2.6)	28 (4.0)	0	37 (3.4)
Pseudomonas aeruginosa	5 (1.4)	16 (2.3)	0	21 (2.0)
Other Gram-negative bacteria	20 (5.8)	18 (2.6)	0	38 (3.5)
Fungi	2 (0.6)	90 (12.8)	0	92 (8.4)

Table 3 Antimicrobial Resistance of Common EOS and HALOS Causing Pathogens at 25 NICUs, January 2017–December 2019

	EOS (No. isolates resistant/ no. tested (%))	HALOS (No. isolates resistant/ no. tested (%))
Klebsiella pneumonia		
Ampicillin	24/30 (80)	174/186 (93.5)
Third-generation cephalosporin	3/31 (9.7)	124/196 (63.3)
Gentamicin Piperacillin/Tazobactam	2/31 (6.5)	41/196 (20.9)
Carbapenem	3/30 (10)	38/194 (19.6)
Multidrug resistance	0	26/196 (13.3)
	3/31 (9.7)	119/196 (60.7)
Escherichia coli		
Ampicillin	79/93 (84.9)	95/106 (89.6)
Third-generation cephalosporin	47/95 (49.5)	48/110 (43.6)
Gentamicin Piperacillin/Tazobactam	31/95 (32.6)	20/110 (18.1)
Carbapenem	2/95 (2.1)	5/107 (4.7)
Multidrug resistance	2/95 (2.1)	3/110 (2.7)
	42/95 (44.4)	41/110 (37.3)
Enterobacter spp		
Ampicillin	14/19 (73.7)	42/48 (87.5)
Third-generation cephalosporin	3/19 (15.8)	13/50 (26)
Gentamicin Piperacillin/Tazobactam	1/19 (5.3)	3/50 (6)
Carbapenem	1/19 (5.3)	4/50 (8)
Multidrug resistance	0	4/50 (8)
	3/19 (15.8)	11/50 (22)
Acinetobacter baumannii		
Ampicillin	6/9 (66.7)	25/27 (92.6)
Third-generation cephalosporin	5/9 (55.6)	11/28 (39.3)
Gentamicin	3/9 (33.3)	4 /28 (14.3)
Piperacillin/Tazobactam Carbapenem	4/9 (44.4)	6 /28 (28.6)
Multidrug resistance	4/9 (44.4)	8/28 (28.6)

	4/9(44.4)	10/28 (35.7)
Pseudomonas aeruginosa		
Ampicillin	3/4 (75)	14/14 (100)
Third-generation cephalosporin	1/5 (20)	6/16 (37.5)
Gentamicin	0	1/16 (6)
Piperacillin/Tazobactam Carbapenem	0	5/16 (31.3)
Multidrug resistance	0	5/16 (31.3)
	1/5 (20)	6/16 (37.5)
CoNS		
Methicillin	18/28 (64.3)	63/81 (77.8)
Vancomycin	0	0
Staphylococcus aureus		
Methicillin	1/19 (5)	28/35 (80)
Vancomycin	0	0
GBS		
Clindamycin	38/50 (76)	11/15(73.3)
Erythromycin	42/51(82.4)	12/15(80)
Vancomycin	0	0
Enterococcus spp.		
Vancomycin	0	3/18 (16.7)

Figures

Figure 1

Study Flow Diagram