Neonate Bloodstream Infections (BSI) in China: Emerging Threat of Multidrug-resistant (MDR) pathogens

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Abstract

Background: Bloodstream Infections (BSIs) continue to be associated with significant morbidity and mortality worldwide, which multidrug-resistant (MDR) pathogens present an ever-growing burden in the hospital and community settings. Data on antimicrobial resistance and prevalence of BSIs pathogens among hospitalized neonates in China are few and not clearly defined.

Methods: we retrospectively collected clinical and bacteria data about patients with BSI from 2013 to 2017 in Chongqing Health Center for Women and Children. The patients were divided into the early- and late-onset BSI groups according to if BSI occurred within or beyond 72 hours after admission.

Results: 210 BSIs occurred from January 1st, 2014 to December 31th, 2019. Of all included 210 patients, there were 99(47.14%), 105 (50.00%),

and 6 (2.86%) infected with gram-positive bacteria, gram-negative bacteria, and fungi, respectively. For early-onset BSI, the predominated pathogen was *E. coli*, accounting for 59.6% with 29.72% producing of extended-spectrum beta-lactamases (ESBLs). CNS (40.3%), *K. pneumoniae* (23.8%), and *E. coli* (20.9%) constitute the main causes of late-onset BSIs. Notably, 75.0% of *K. pneumoniae* produced ESBLs and 81.3% were MDR, which were higher than early-onset BSIs. Late onset BSIs and antibiotic exposure were significantly associated with MDR infection.

Conclusion: Gram-negative bacteria gradually became the main pathogenic bacteria, among which *E. coli* and *K. pneumoniae* accounted for the largest proportion. The phenomenon of multi-drug resistance of bacteria is serious, and the first-line drug can't meet the practical needs. Late onset sepsis and antibiotic exposure were significantly associated with MDR infection.

Background

Premature birth, severe infection and suffocation are the leading causes of neonatal death and the most frequently observed infection in neonatal wards is bloodstream infections (BSIs)¹. In 2017, an estimated 48.9 million incident cases of BSIs were recorded worldwide and 11.0 million BSIs-related deaths were reported, representing 19.7% of all global deaths². It is important that progress in neonatal survival has not kept pace with global reductions in child mortality and neonatal deaths now account for 47% of deaths in children under the age of five years³.

Appropriate empirical treatment of BSIs is crucially important in reducing mortality. To make matters worse, the emergence of antimicrobial resistance (AMR) pathogens makes it more difficult to treat bacteremia.

AMR pathogens exhibit a high degree of resistance to first line drugs recommended by the World Health Organization—namely, ampicillin, gentamicin, and third generation cephalosporins such as cefotaxime, and even multidrug-resistant (MDR)⁴. A 2020 study of neonatal BSIs in Pune, India reported a high

prevalence of AMR with high mortality³. Effective interventions are urgently needed to reduce burden of BSIs and death due to AMR pathogens in hospital neonates⁵. However, there are scant data on the epidemiology of neonatal sepsis and associated prevalence of AMR in LMIC, especially in China. It is critical to better understand the regional epidemiology of neonatal sepsis and AMR among hospitalized neonates in order to guide future prevention efforts and policies

Methods

Study Design and Patients Collection

We conducted a retrospectively cohort study from January 2014 to December 2019 in Chongqing Health Center for Women and Children. Blood cultures were performed on suspected BSIs patients with at least 2 blood samples from different sites at the same time and the data of neonates with positive blood culture results were collected.

Bacterial Strains

Non-repetitive nosocomial bacterial strains were collected between 2014 and 2019 in the Chongqing Health Center for Women and Children. All the isolates were identified at the species level by the VITEK MS (bioMérieux, Hazelwood, MO, United States) system, and routine antimicrobial susceptibility testing was performed by the VITEK2 compact (bioMérieux, Inc., Durham, NC) system.

Definitions

Cases of BSI were defined as a blood culture positive for a known neonatal pathogen. Patients who were positive for well-described contaminants and without any infection symptoms were not considered as BSI. Further, patients were subclassified into 2 groups according to the period: (1) Early onset BSIs was defined as BSIs occurring day of life (DOL) 0 through DOL 3 day. (2) Late onset BSIs was defined as BSIs DOL after 3d. AMR and MDR were according to the criteria of the Clinical and Laboratory Standards Institute 2019 (CLSI 2019). For gram-positive bacteria, we defined methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) as MDR pathogens. For gram-negative bacteria, MDR were defined as bacteria were resistant to at least three antimicrobial classes. Extended-spectrum- β -lactamase (ESBLs) producing organisms were tested by the phenotypic tests of ESBL production.

Statistical Analysis

All analyses were performed using SPSS v.25.0 software (SPSS Inc., Chicago, IL, USA). Univariate analyses were performed separately for each of the variables. The odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the strength of any association. For all calculations, statistical significance was defined at $P < 0.05$ for 2-tailed tests.

Results

210 BSIs occurred from January 1st, 2014 to December 31th, 2019. Clinical data were collected from 179 neonates. As shown in Table 1, 59.8% of the patients were male, the mean birthweight of enrolled neonates was 2517.8 g (SD = 934.8), and mean gestation was 35.68 weeks (SD = 4.2), Nearly half of the patients were premature infants and low weight infants, accounting for 46.36% and 48.04% respectively. More than half of enrolled neonates (51.4%) were born by caesarean section. In addition, 14.5% of neonates were positive for embryonic membrane culture. Although 13.4% mothers received antibiotics within 7 days before delivery, less than half (5.6%) received antibiotics longer than 48 hours. Among all inflammatory indicators, the sensitivity of WBC was the worst, with only 13.41% newborns greater than $20 \times 10^9/L$. While the sensitivity of PCT was much higher, about three-quarters of patients (77.7%) had increased after the occurrence of BSIs.

Table 1
Demographic Details of Enrolled Neonates

	Number of neonates
Neonatal variables	
Sex	
Boys	107 (59.8%)
Girls	72 (41.2%)
Age, days	9.2 ± 7.6
Birthweight, g	2517.8 ± 934.8
Gestation, weeks	35.7 ± 4.2
Positive Culture of Embryonic membrane	26 (14.5%)
Maternal and perinatal variables	
Antenatal corticosteroids	31 (17.3%)
Maternal fever within 7 days before delivery	20 (11.2%)
Maternal antibiotics within 7 days before delivery	24 (13.4%)
Received for 48 h or more	10 (5.6%)
Caesarean delivery	92 (51.4%)
Pathogens	
Gram positive bacteria	99 (47.1%)
Gram-negative bacterium	105 (50.0%)
Fungus	6 (2.9%)
Inflammatory biomarkers	
WBC ($\geq 20 \times 10^9/L$)	24 (13.4%)
CRP (neonates > 0.6 mg/L, Three days after birth > 1.6 mg/L)	102 (57.0%)
PCT (neonates > 0.55 ng/mL, 12 ~ 24h > 4.7 ng/mL, 36 ~ 48h > 1.7 ng/mL, Three days after birth ≥ 0.05 ng/ml.)	139 (77.7%)

Pathogens Isolated From Neonates With Bsis

Of all included 210 patients, there were 99(47.1%), 105 (50.0%),

and 6 (2.9%) infected with gram-positive bacteria, gram-negative bacteria, and fungi, respectively. Interestingly, before 2018, gram-positive bacteria (50.9%) were the main pathogen, but the isolation rate decreased year by year. After 2018, gram-negative bacteria became dominated pathogen, even, nearly three-quarters of the pathogen were gram-negative bacteria (72.7%) in 2019. Fungus (2.9%) is the rarest pathogen of neonatal BSIs in our hospital, and there has been no fungus bacteremia for three consecutive years (Fig. 1). The distribution of bacteria was different in newborns with different weights (Fig. 2). *Escherichia coli* (*E. coli*) is the main cause of extremely low birth weight and macrosomia infants, accounting for 60.0% and 50.0% respectively. *Klebsiella pneumoniae* (*K. pneumoniae*) accounted for 33.3% of pathogenic isolates, followed by *E. coli* at 29.2% in very low birth weight infants. For low birth weight infants BSI from 2014 to 2019, the most common causes were *E. coli* (38.5%) and *K. pneumoniae* (28.8%). However, in normal weight infants, pathogens were predominantly other staphylococcus (45.8%) such as coagulase-negative Staphylococcus (CNS) and *E. coli* (28.1%).

Resistance Patterns for Early- and Late-Onset Pathogen

Pathogen showed different resistant to ampicillin, gentamicin, and third generation cephalosporins such as cefotaxime which were first line drugs recommended by the World Health Organization (Fig. 3). Gram-negative bacterial (90.6%) and Staphylococcus spp. (89.28%) had the highest resistance rates to ampicillin, however, Streptococcus spp (13.3%). remained sensitive to ampicillin. About a third of gram-positive bacteria and a quarter of gram-negative bacteria are resistant to aminoglycosides each year. In addition, 50% of bacteria are resistant to cephalosporins.

Table 2 showed distribution of pathogens and resistance patterns between the early- and late-onset BSIs groups. For early-onset BSIs, the predominated pathogen was *E. coli*, accounting for 59.6% with 29.72% producing of extended-spectrum beta-lactamases (ESBLs), followed by Streptococcus spp. (22.6%). About two-thirds of early-onset BSIs were MDR. CNS (40.3%), *K. pneumoniae* (23.8%), and *E. coli* (20.9%) constitute the main causes of late-onset BSIs. Notably, 75.0% of *K. pneumoniae* produced ESBLs and 81.3% were MDR, which were higher than early-onset BSIs.

Table 2
Pathogen and Drug Susceptibility of Early Onset BSIs and Late Onset BSIs

Variable	Total	Early onset BSIs	Late onset BSIs
Gram negative			
Escherichia coli			
ES cephalosporins	29.2% (19/65)	29.7% (11/37)	28.6% (8/28)
Carbapenems	0 (0/65)	0 (0/37)	0 (0/28)
MDR	69.2 (45/65)	64.9% (24/37)	75.0% (21/28)
Klebsiella spp			
ES cephalosporins	75.0% (24/32)	0	75.0% (24/32)
Carbapenems	15.6% (5/32)	0	15.6% (5/32)
MDR	81.3%(26/32)	0	81.3%(26/32)
Gram positive			
Coagulase-negative staphylococci			
Meticillin	71.5% (45/63)	66.7% (6/9)	72.2% (39/54)
Vancomycin	0 (0/63)	0 (0/9)	0 (0/54)
Staphylococcus aureus			
Meticillin	21.4% (3/14)	0 (0/2)	25.0% (3/12)
Vancomycin	7.1% (1/14)	0 (0/2)	8.3% (1/12)
Enterococcus spp			
Meticillin	100.0% (6/6)	0	100.0% (6/6)
Vancomycin	0 (6/6)	0	0
Streptococcus spp			
penicillin	0 (0/16)	0 (0/14)	0 (0/2)
erythrocine	87.5% (14/16)	92.8% (13/14)	50.0% (1/2)

Risk Factors For Mdr Infection

We assessed the risk factors of BSIs with MDR and non-MDR. As shown in the Table 3, Late onset sepsis and antibiotic exposure were significantly associated with MDR infection (P < 0.05).

Table 3
Clinical Data Analysis of Neonatal Infection with MDR

Variable	Total	MDR (n = 58)	Non-MDR (n = 35)	P value
Male	56 (59.1%)	36 (62.1%)	20 (57.1%)	0.6
gestational age				
premature births	64 (68.8%)	40 (69.0%)	24 (68.6%)	0.9
term delivery	29 (31.2%)	18 (31.0%)	11 (31.4%)	
weight				
≤ 2500 g	62 (66.7%)	39 (67.2%)	23 (65.7%)	0.8
≥2500 g	31 (33.3%)	19 (32.8%)	12 (34.3%)	
delivery mode				
Caesarean section	58 (62.4%)	35 (60.3%)	23 (65.7%)	0.61
natural labour	35 (37.6%)	23 (39.7%)	12 (34.3%)	
pathogens				
E. coli	60 (64.5%)	38 (65.5%)	22 (62.9%)	0.79
K. pneumoniae	24 (25.8%)	15 (25.8%)	9 (25.7%)	0.98
Early onset sepsis	34 (36.5%)	16 (27.5%)	18 (51.4%)	0.02
Late onset sepsis	59 (63.4%)	42 (72.4%)	17 (48.5%)	
Inflammatory biomarkers				
WBC (*10^9)	11.9 ± 7.6	11.9 ± 7.6	11.1 ± 8.49	0.5
N%	63.8 ± 19.4	64.7 ± 19.0	62.38 ± 20.1	0.6
PLT (*10^9)	200.3 ± 108.7	197.9 ± 120.7	204.4 ± 88.4	0.1
PDW	13.7 ± 3.7	13.73 ± 3.9	13.7 ± 3.4	0.4
I/T	23.9 ± 12.0	22.5 ± 11.1	26.3 ± 13.1	0.1
CRP (mg/L)	34.2 ± 27.2	27.4 ± 27.6	25.2 ± 25.3	0.2
PCT (ng/ml)	35.1 ± 41.9	46.8 ± 61.9	49.3 ± 56.7	0.8
Maternal infection	20 (21.5%)	13 (22.4%)	7 (20.0%)	0.7
Culture of Embryonic membrane	21 (22.5%)	13 (22.4%)	8 (22.8%)	0.9
Antibiotic exspoure	18 (19.3%)	15 (25.8%)	3 (8.5%)	0.03

Discussion

In the present study, we found that the microbiota and distribution of pathogens causing BSIs have changed significantly in the last 2 years. Gram-negative bacteria emerged as the predominant causative organisms. In addition, about half of BSI patients were infected with MDROs.

This study deserves attention for some major reasons. First, we found about two-thirds of sepsis occurred after 72 h, and the average age was about 9 days. This pattern is in sharp contrast with that reported by Elsevier, who said most episodes of sepsis occurred at a quite early age, with nearly a quarter of culture-positive sepsis episodes occurring within 24 h of birth and two-thirds within 72h⁶. However, China^{7,8} and Korea⁹ have shown a predominance of late-onset sepsis. At the same time, we also found differences in the distribution of pathogens in different countries. A high prevalence of Gram-positive organisms was found before 2018 in our hospital, which contrasts with previous findings elsewhere where Gram-negative bacteria were the predominant organisms isolated^{4,10-12}. Actually, our study found CNS played a significant role in BSIs in neonates with normal weight infants. However, CNS in neonatal bloodstream infections are not clear, as isolation from a single blood culture may represent true bacteremia or contamination^{13,14}. Interestingly, Gram-negative bacteria especially Enterobacter have become the major pathogens in our hospital in the last two years^{7,9,15-17}. In our study, except normal weight newborns, the most prevalent pathogens were *E. coli* and *K. pneumoniae*. Elsevier had found that *Acinetobacter* spp. (22%) emerged as the most common isolates in their cohort, which was rare in our hospital.

Second, we found the pathogens associated with BSI and their individual resistance patterns may differ between early- and late-onset BSIs. Remarkably, only about one-third of *E. coli* produced ESBLs among early-onset BSIs, which was far less than *K. pneumoniae* did among late-onset BSIs. This result was quite contrast with a study, of 368 398 pediatric clinical isolates characterized in the United States from 1999 to 2011, that only 1.97% were identified as third-generation cephalosporin-resistant (G3CR), and 0.47% as ESBL producers and the majority of G3CR and ESBL isolates were *E. coli*¹⁸. On the contrary, ESBL-producing strains were not rare, especially in Indian. A retrospective analysis of neonatal gram-negative septicemia from 2002 to 2003 reported that 61% (46/75) of cases were due to ESBL-producing strains¹⁹. Data included 30 studies comprising 71326 children demonstrated that MDR was present in 30% in Asia and 75% in Africa²⁰. Clearly, not only is the level of antimicrobial resistance increasing, but it has now become a serious problem in China. This suggests that current national antibiotic guidelines for neonatal sepsis may not be appropriate for management of neonatal infections in our study setting.

Third, we found late-onset sepsis and antibiotic exposure were significantly associated with MDR infection. Doctors often use ampicillin or cephalosporin empirically to treat bacteremia, but these antibiotics could induce high expression of AmpC and ESBLs, leading to the resistance to β -lactam antibiotics²¹. Piperacillin-tazobactam (TZP), a weak inducer of AmpC beta-lactamases and inhibitor of ESBLs may be a valuable treatment option for BSIs²².

This study had several limitations. First, the present study was performed at a single center. Second, the sample of this study was relatively small, which could be a source of bias. Third, we may have overestimated the incidence of CNS because its diagnosis was not based on two simultaneous blood cultures.

Conclusion

Our study showed that BSI was a serious clinical challenge in the China. Gram-negative bacteria gradually became the main pathogenic bacteria, among which *E. coli* and *K. pneumoniae* accounted for the largest proportion. The phenomenon of multi-drug resistance of bacteria is serious, and the first-line drug can't meet the practical needs. Late onset sepsis and antibiotic exposure were significantly associated with MDR infection. Other antibiotics such as TZP should be the first choice in empirical antimicrobial therapy.

Declarations

Declarations

Ethics approval and consent to participate

For this study, samples were collected at the microbiology laboratory of our hospital, with no contact with the patient. This study was retrospective and there was no patient identification performed during data collection. Therefore, the ethics committee determined that informed consent was not required.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HZ and QH designed the study, HZ, QH, CL and YS performed the experiments. LG and YS analyzed data. HZ, QH and CL wrote this manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

The distribution trend of pathogen of neonatal bloodstream infection from 2014 to 2019

Figure 2

Distribution of pathogens of bloodstream infection in neonates of different body weight

Figure 3

Changes in first-line drug susceptibility from 2014 to 2019