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ORIGINAL RESEARCH

Distribution of Pathogenic Bacteria in Health Care Associated Sepsis in Preterm Infants and Ten Years Variation in Their Drug Resistance

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Aim: To identify the causative bacteria of healthcare-associated sepsis in preterm infants and analyze their antibiotic resistance trends over ten years, providing evidence for infection prevention strategies.

Materials and Methods: We retrospectively analyzed blood culture data from preterm infants (<37 weeks) with healthcare-associated sepsis (onset >72 hours after birth) admitted between January 2014 and December 2023. Pathogen distribution and antibiotic resistance patterns were compared between two periods (2014–2018 vs 2019–2023).

Results: Among 9928 preterm infants, 3.3% (332 cases) had positive blood cultures, with incidence increasing from 1.4% (2014–-2018) to 2.7% (2019–2023). Gram-negative bacteria remained predominant (48.00% to 61.07%), led by *Klebsiella pneumoniae*. Gram-positive bacteria increased significantly (5.33% to 31.30%), primarily *coagulase-negative staphylococci*, while fungal infections decreased (46.67% to 7.63%). Resistance to third-generation cephalosporins persisted in *K. pneumoniae* (~80%) and increased in *Enterobacter cloacae* (60% to 90%). Emerging carbapenem resistance was observed in *E. coli* (0% to 33.33%) and *K. pneumoniae* (5.25% to 4.08%), with *Enterobacter cloacae* showing a significant rise (0% to 60%). ESBL-producing strains rose from 13.33% to 30.53%. All Gram-positive isolates remained susceptible to linezolid, except one vancomycin-resistant *Staphylococcus capsulatus*.

Conclusion: The incidence of healthcare-associated sepsis in preterm infants increased significantly, with rising carbapenem resistance in Gram-negative bacteria and a marked increase in coagulase-negative staphylococci. These trends underscore the need for enhanced infection control and judicious antibiotic use guided by blood culture results.

Keywords: sepsis, preterm infants, health care associated infections, pathogenic bacteria, drug resistance

Introduction

Neonatal sepsis is a serious infectious disease of the neonatal period, with a global incidence of 2824 cases per 100,000 live births and a mortality rate of 17.6%, based on evidence from 26 studies across 14 countries.[1] In China, neonatal sepsis patterns exhibit distinct regional characteristics.[2,3], with Gram-negative pathogens (particularly *Klebsiella* spp.) dominating late-onset sepsis (LOS) and demonstrating alarming resistance to third-generation cephalosporins (47%) and gentamicin (37%).[4] The Chinese Neonatal Network reported a 9.4% sepsis incidence among very preterm infants, with mortality and morbidity rates significantly influenced by gestational age and antibiotic exposure duration.[5,6] Globally, neonatal infections are particularly severe in hospitals, where the proportion of infections can be as high as 30% to 40%, about 2/3 of which are bloodstream infections, which have become one of the leading causes of neonatal mortality.[7] With the advancement of science and technology, especially perinatal medicine, the survival rate of preterm infants has improved significantly; however, their infection rate in hospitals exceeds that of full-term infants;[8] especially in preterm infants with gestational age <28 weeks. Once health care associated infection occurs in a preterm infant, early symptoms

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may not be apparent, making the disease difficult to detect in the early stages, but the situation can deteriorate rapidly; although confirmation of the diagnosis by blood cultures is the gold standard, this method is time-consuming and has a low positive rate.

The high incidence of sepsis in preterm infants stems from multiple interrelated risk factors, including immunological immaturity, prolonged hospitalization with invasive procedures, and specific maternal–fetal conditions.[9,10] Extremely preterm infants (<28 weeks) and very-low-birth-weight neonates (<1500g) exhibit profound immunological deficiencies, particularly in neutrophil function and complement activity, rendering them vulnerable to pathogens.[9,10] Maternal factors such as chorioamnionitis, group B streptococcal colonization, and prolonged rupture of membranes significantly increase sepsis risk.[11] Postnatally, the need for mechanical ventilation, central catheters, and extended antibiotic exposure disrupts normal microbial colonization while selecting for resistant organisms.[9,10] Notably, preterm infants undergoing internal fetal monitoring >12 hours demonstrate a 7.2-fold higher sepsis risk,[11] while cesarean delivery without labor reduces infection likelihood.[12] These factors collectively contribute to the disproportionate sepsis burden observed in preterm populations.[9–11]

According to NICE guideline, standard preventive measures for preterm infants include intrapartum antibiotic prophylaxis for GBS-positive mothers, strict hand hygiene protocols, early breast milk feeding to support microbiome development, and judicious antibiotic stewardship guided by local resistance patterns. For extremely preterm infants (<28 weeks), prophylactic fluconazole may be considered in NICUs with high fungal infection rates (>10%).[13] Fluconazole remains a critical antifungal agent in neonatal care, particularly in resource-limited settings, due to its safety, oral bioavailability, cost-effectiveness, and ability to cross the blood–brain barrier.[9] However, prolonged use may lead to the emergence of azole-resistant strains, highlighting the need for judicious use and resistance monitoring.[14]

Diagnostic uncertainty of neonatal sepsis persists, as 41% of suspected LOS cases are culture-negative yet associated with neurodevelopmental risks comparable to culture-positive sepsis.[15] While molecular assays show promise, their clinical adoption remains limited in resource-constrained settings.[16] This diagnostic ambiguity often precipitates empirical broad-spectrum antibiotic use, exacerbating resistance.[17]

Recent studies state that approximately 4.95 million deaths worldwide are associated with antibiotic resistance, with about 1.27 million of these deaths directly related to resistance, and that the three most lethal types of bacteria are *Escherichia coli, Staphylococcus aureus*, and *Klebsiella pneumoniae*.[18] Therefore, understanding the distribution of pathogenic bacteria and their resistance in health care associated sepsis in preterm infants is essential for clinical work while empirically selecting antibiotic therapy to reduce morbidity and mortality.[19] For more understanding of the distribution of pathogenic bacteria and their resistance, it is better to add more data like: is a new class of broad-spectrum antibiotic, developed to treat multidrug resistant isolates. It has an extraordinarily broad spectrum of activity against most Gram-positive, Gram-negative and anaerobic pathogens.[20] However, the emergence of resistance to tigecycline in *Klebsiella pneumoniae* isolates has been reported.[21,22] For this reason, this study applied a retrospective research method to analyze the blood culture and its sensitivity to antibiotics of preterm infants suffering from sepsis in the neonatal unit of our hospital from January 2014 to December 2023, aiming to provide a theoretical basis for clinical prevention and anti-infection.

Materials and Methods

Subjects of Study

Inclusion Criteria

(1) Cases of health care associated sepsis among preterm infants (gestational age <37 weeks) treated in our neonatal unit from January 2014 to December 2023 were selected and analyzed retrospectively. (2) The study focused on late-onset neonatal sepsis (LOS), defined as sepsis occurring after 72 hours of life, with ≥48h of prior hospitalization, associated with invasive device, and excluding community-acquired infections, in accordance with the diagnostic criteria described in the fifth edition of Practical Neonatology;[23] (3) age of onset >3 days; (4) health care associated infections;[24] and (5) positive blood cultures, and the possibility of contamination of the blood cultures was ruled out in accordance with the following: a. Even if the blood cultures were positive, the children had not been treated with antimicrobial drugs, and

were in good overall health; b. The children had not undergone invasive medical procedures such as intravenous cannulation or tracheal intubation; c. Blood culture results were considered clinically significant only if the culture positivity signal occurred within 48h of incubation and correlated with clinical symptoms. Suspected sepsis cases were not included in the sepsis incidence. Suspected sepsis was defined as clinical signs of infection (eg, temperature instability, respiratory distress, feeding intolerance, or lethargy) without microbiological confirmation. Additionally, cases where the type of infection (eg, respiratory, urinary, or bloodstream) did not correspond to the detected strain (eg, a respiratory pathogen isolated from a blood culture) were excluded to ensure the accuracy of the diagnosis. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (PJ2023-06-32).

Exclusion Criteria

(1) suspected sepsis cases without microbiological confirmation; (2) cases with pathogen isolation inconsistent with infection type (eg, respiratory pathogens in blood cultures); (3) contaminated blood cultures as defined by international standards (positive cultures with typical contaminants like coagulase-negative staphylococci in asymptomatic infants); (4) infants who received antibiotics prior to blood culture collection; and (5) cases with incomplete clinical records.

Collection of Clinical Data

WHONET software was used to counter-check the data of preterm infants with positive blood culture results in the neonatal unit of our hospital, and the electronic medical records were also reviewed to collect clinical information of the infants, including: (1) perinatal information: sex of preterm infants, gestational week at birth, mode of delivery, and birth weight; (2) admission diagnosis; and (3) disease manifestations: age of onset, and the time taken for the blood culture to be positive.

Blood Collection Procedures

Upon suspicion of sepsis or before administering the second dose of antibiotics, strict aseptic technique was followed, and 3 to 5 mL of blood was drawn from the radial artery or femoral vein of the thigh of the preterm infant and placed immediately into a pediatric blood culture bottle to ensure that the blood was available for testing by a laboratory within one hour. Drawing blood after the first dose of antibiotics may lead to unreliable negative cultures and an underestimation of the incidence of confirmed sepsis.

Perform Bacterial Culture, Identification and Antibiotic Susceptibility Testing

The blood samples were placed in the BACTEC 9120 blood culture system developed by BD (USA) for automatic bacterial incubation, and after a positive alarm, the instrument continued to perform bacterial identification and drug sensitivity tests. Bacterial identification and drug susceptibility assessment were performed with a MicroScan WalkAway-96 Plus device manufactured by Siemens AG (USA) and a fully automated microbial identification system, VITEK 2 Compact from bioMérieux (France). And for analyzing fungal species and drug susceptibility, the BioMérieux ATB-FUNGUS device was selected. All experimental procedures and determination of results were performed in strict accordance with the standards developed by the American Association of Clinical Laboratory Standards Setting Committees (CLSI).[25]

Quality Control Strains

A variety of standardized test strains were used, specifically: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Enterobacter cloacae* ATCC 43560, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 27923, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 19434, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans* ATCC 90028. These standardized tests were provided by clinical laboratories affiliated with the Ministry of Health.

Multidrug-Resistant Bacteria (MDRO)

Multidrug-resistant bacteria (MDRO) were defined as resistance to three or more classes of antibiotics, including third-generation cephalosporins, carbapenems, and fluoroquinolones, as per international guidelines.[26] Extremely Low Birth Weight (ELBW) newborns were defined as having a birth weight of <1000 g.[23] Resistance to either ceftriaxone, cefotaxime, or cefoperazone sodium sulbactam refers to resistance to third-generation cephalosporin antibiotics, resistance to either imipenem, meropenem, or ertapenem refers to resistance to carbapenems,[27] and resistance to either levofloxacin or ciprofloxacin refers to resistance to quinolones.

Blinding Procedures

Given the retrospective observational nature of this study, blinding of clinicians or researchers was not applicable during data collection as all analyses were performed on existing clinical records. However, blinding was implemented during laboratory analyses: microbiologists processing blood cultures were blinded to clinical information, and statisticians analyzing the data were blinded to group allocations (2014–2018 vs 2019–2023 periods).

Statistical Methods

SPSS 19.0 statistical software was applied to analyze the data, and WHONET 5.6 software was used for drug resistance. Measures with normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$) and two independent samples *t*-test was used for comparison between groups; measures with non-normal distribution were expressed as M (Q_1, Q_3), and rank-sum test was used for comparison between groups; and counts were expressed as cases (%), and 2-test was used for comparison between groups. A post-hoc power analysis confirmed that the sample size provided ≥80% power to detect significant differences in resistance trends (α=0.05). P<0.05 was considered as statistically significant difference.

Results

General Information

A total of 9928 preterm infants were admitted to our neonatal unit from January 2014 to December 2023,332 (3.3%) had positive blood cultures. Contaminated blood cultures were defined according to international standards as positive cultures with organisms typically considered contaminants (eg, coagulase-negative staphylococci or diphtheroids) in the absence of clinical signs of infection. After excluding 17 cases with contaminated blood cultures, 45 cases with an onset date ≤3 days, and 6 cases with non-health care associated infections, 35 cases with pathogen isolation inconsistent with infection type, 30 cases with incomplete clinical records,199 cases were finally included. Among them, 69 cases occurred between 2014 and 2018 (38 males and 31 females), and 130 cases occurred between 2019 and 2023 (69 males and 61 females). The difference in incidence by gender was not statistically significant ($P > 0.05$), indicating that gender did not influence the risk of sepsis in preterm infants. The incidence of health care associated sepsis increased from 1.4% (69/5100) in 2014–2018 to 2.7%(130/4282) in 2019–2023, and this increase was statistically significant ($P < 0.05$) (Supplementary Table 1).

The percentages of neonatal respiratory distress syndrome, neonatal pneumonia, neonatal hyperbilirubinemia, neonatal ischemic-hypoxic myocardial damage, neonatal hypoproteinemia, and gestational age <28 weeks, birth weight <1000 g, and cesarean section were higher in 2019–2023 than 2014–2018 children, and the age of the day of the onset of the disease, gestational age, birth weight, and time to report positive blood cultures were lower than those of children in 2014–2018 ($P < 0.05$) (Table 1).

Detection of Pathogenic Bacteria

A total of 206 pathogenic strains were isolated, 191 were single strains, and in six blood cultures, two germs were isolated at the same time. In a case of sepsis with two health care associated infections in a preterm infant, *Klebsiella pneumoniae* was isolated for the first time, and health care associated infections recurred 2 weeks after discontinuation of anti-infective treatment, and one strain of each of *Klebsiella pneumoniae* and *Enterobacter aerogenes* was isolated in the

Table 1 Comparison of General Data of Children with Health Care Associated Sepsis in Preterm Infants in the Pre and Post Groups

Programs	2014—2018 (n=69)	2019—2023 (n=130)	Test Value (Z/χ²)	P-value
Sex (m/f)	38/31	69/61	0.072	0.788
Cesarean section/normal delivery	40/29	98/32	6.430	0.011
Average gestational age (P25-P75, weeks)	31 (30–33)	30 (28–32)	2.621	0.009
Gestational age <28 weeks [cases (%)]	2 (2.9)	23 (17.7)	8.981	0.003
Average birth weight (P25-P75, g)	1450 (1280–1700)	1230 (990–1545)	3.734	<0.001
ELBW [cases (%)]	4 (5.8)	33 (25.4)	11.426	0.001
Mean age at onset (P25-P75, d)	15.0 (11.0–24.8)	20.5 (13.0–31.0)	2.538	0.011
Mean time to positive blood culture report (P25-P75, h)	120 (108–120)	14.2 (8.7–24.2)	9.886	<0.001

Abbreviation: ELBW: Extremely Low Birth Weight (<1000g).

blood cultures. From 2014 to 2018, the main pathogenic bacteria detected were *Klebsiella pneumoniae, Pseudomonas* spp. with smooth colonies, *Escherichia coli, Candida albicans*, and *Enterobacter cloacae*, and a total of 10 (13.33%) strains of ultrawide-spectrum β-lactamase-producing (extended spectrum βlactamases (ESBLs) were identified during this time. Subsequently, the top pathogens changed to *Klebsiella pneumoniae, coagulase-negative staphylococci* (CNS), *Enterobacter cloacae, Enterobacter aerogenes*, and *Candida albicans* from 2019 to 2023, and 40 (30.53%) *ESBLs-producing strains* were detected during this period. Comparison of the data from the two five-year periods showed a significant increase in the frequency of Gram-positive (G^+) organisms and an increase in the detection rate of Gram-negative (G^-) organisms, which continued to lead the way, particularly in the case of *Klebsiella pneumoniae*; meanwhile, there was a significant decrease in the rate of detection of fungi. Pathogenic bacteria isolated from the anterior and posterior groups were dominated by *Candida albicans* and *Pseudomonas* spp. with smooth colonies. Comparison of the distribution of pathogenic species of sepsis due to infections in hospitals in premature infants showed a statistically significant difference when comparing the two groups before and after (χ^2=49.430, P<0.001) (Table 2).

Table 2 Distribution of Pathogenic Bacteria in Blood Cultures of Health Care Associated Sepsis in Preterm Infants in the Pre and Post Groups

Pathogen	2014—2018		2019—2023		Test Value	P
	Number of Strains	Component Ratio (%)	Number of Strains	Component Ratio (%)		
Gram-positive bacteria	4	5.33	41	31.30	49.430	<0.001
Staphylococcus epidermidis	0	0.00	23	17.56		
Hemolytic staphylococcus	1	1.33	2	1.53		
Human staphylococcus	0	0.00	1	0.76		
Staphylococcus sciuri	0	0.00	2	1.53		
Staphylococcus aureus	1	1.33	0	0.00		
Staphylococcus head	0	0.00	1	0.76		
Staphylococcus capitatus	0	0.00	1	0.76		
Staphylococcus pasteurus	0	0.00	1	0.76		
Staphylococcus caprae	0	0.00	1	0.76		
Staphylococcus aureus	1	1.33	2	1.53		
Enterococcus faecalis	0	0.00	3	2.29		
Enterococcus faecalis	0	0.00	4	3.05		

(*Continued*)

Table 2 (Continued).

Pathogen	2014—2018		2019—2023		Test Value	P
	Number of Strains	Component Ratio (%)	Number of Strains	Component Ratio (%)		
Streptococcus mutans	1	1.33	0	0.00		
Gram-negative bacteria	36	48.00	80	61.07		
Klebsiella pnenmoniae	19	25.33	50	38.17		
Escherichia coli	10	13.33	3	2.29		
Enterobacter cloacae	5	6.67	9	6.87		
Acinetobacter baumannii	0	0.00	2	1.53		
Enterobacter aerogenes	1	1.33	6	4.58		
Acid-producing Krebs	1	1.33	3	2.29		
Serratia marcescens	0	0.00	2	1.53		
Bacillus citriodora Yangstoffii	0	0.00	1	0.76		
Dispersed Pantothenium	0	0.00	1	0.76		
Morganella morganii	0	0.00	1	0.76		
Pseudomonas aeruginosa	0	0.00	1	0.76		
Burkholderia cenocepacia	0	0.00	1	0.76		
Fungi	35	46.67	10	7.63		
Smooth pseudofilamentous yeast	18	24.00	3	2.29		
Candida albicans	9	12.00	5	3.82		
Candida flexneri	3	4.00	0	0.00		
Pseudomonas tropicalis	2	2.67	1	0.76		
Nearly smooth pseudohyphae	0	0.00	1	0.76		
Sake pseudo-saccharomyces cerevisiae	1	1.33	0	0.00		
Pseudohyphae	1	1.33	0	0.00		
Candida kefyr	1	1.33	0	0.00		
Sum	75	100.00	131	100.00		

Notes: "Component ratio" refers to the percentage of each pathogen among the total isolates.

Detection of Multiple Pathogenic Bacteria

Methicillin-resistant CNS and *Staphylococcus aureus* were not detected among Gram-positive bacteria during the five-year period from 2014 to 2018; however, 12 strains of methicillin-resistant CNS and one strain of methicillin-resistant *Staphylococcus aureus* (MRSA) were detected during the next five years. Vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecalis* were not detected in the 2 groups before and after. The detection rates of penicillin-resistant CNS in the 2 groups before and after were essentially equal, with 100% and 96.88%, respectively. The erythromycin-resistant CNS detection rate in the 2 groups before and after showed an increasing trend, which was 50% and 87.5%, respectively. Among the results of gram-negative bacteria, no carbapenem-resistant *Enterobacter cloacae* and *Escherichia coli* were found from 2014 to 2018, but one carbapenem-resistant strain of *Klebsiella pneumoniae* was found. And in the subsequent period from 2019 to 2023, a total of five strains of carbapenem-resistant *Enterobacter cloacae*, 1 strain of carbapenem-resistant *Escherichia coli*, and two carbapenem-resistant *Klebsiella pneumoniae* strains were detected.

Drug Resistance of Major Pathogenic Bacteria

After comparing and analyzing the data over the past ten years, none of the differences were statistically significant when comparing the resistance of commonly used antibiotics to common pathogenic bacteria (all $P > 0.05$). The susceptibility of Gram-positive bacteria to linezolid was maintained at 100.00%, and only one strain of *Enterococcus faecalis* was resistant to tigecycline and one strain of *Staphylococcus capitis* was resistant to vancomycin; while for penicillin, erythromycin and clindamycin, the resistance was as high as 97.78%, 84.44% and 75.56%, respectively. Comparison of data from different time periods showed that the resistance rate of *Klebsiella pneumoniae* to third-generation cephalosporin antibiotics was basically stable, at 78.95% and 79.59%; however, *Escherichia coli* showed a significant decrease in the resistance rate to this series of drugs, from 90% to 33.33%; in contrast, there was a significant increase in the rate of resistance to *Enterobacter cloacae*, which surged from 60% to 90%. *Enterobacter cloacae* is a member of the Enterobacteriaceae family, typically associated with urinary tract infections (UTIs) and other opportunistic infections. It was also detected in *Klebsiella pneumoniae* and *Enterobacter cloacae*, which also showed an increasing trend of resistance to cefoperazone/sulbactam, from 10.53% to 20.41% and from 40.00% to 50.00%, respectively. The quinolone resistance rate of *Klebsiella pneumoniae* and *Enterobacter cloacae* increased from 10.53% and 0% to 60% and 33.33% in the before and after groups, respectively, and the quinolone resistance rate of *Escherichia coli* showed a decreasing trend, which was 60% and 33.33%, respectively.

The resistance of certain bacteria to carbapenem antibiotics changed over time in both periods, with resistance in *Klebsiella pneumoniae* decreasing from 5.25% to 4.08%, *Escherichia coli*, which had never been resistant, surging to 33.33%, and *Enterobacter cloacae* similarly spiking from zero resistance to 60.00%. In contrast, the percentage of resistance to fluconazole by non-mucoid *Pseudomonas aeruginosa* also increased, from 10.53% to 33.33%, although resistance to itraconazole remained at one level, 31.58% versus 33.33%, respectively), and resistance to voriconazole declined, from 5.26% to 0.00%. On the other hand, the elevation of resistance to fluconazole in *Candida albicans* was also noteworthy, increasing from no resistance to 20.00%, and to itraconazole and voriconazole, both of which increased from 11.11% and 0.00%, to a level of 20.00% in both cases. It is worth mentioning that all detected fungi remained sensitive to amphotericin B and flucytosine (Table 3–4).

Table 3 Antimicrobial Resistance of Major Gram-Negative Bacilli to Antimicrobial Drugs in Blood Cultures of Health Care Associated Sepsis in Preterm Infants in Both Groups (Number of Strains, %)

Antibacterial Drug	Klebsiella pnenmoniae		Escherichia coli		Enterobacter cloacae	
	2014—2018 (n=19)	2019—2023 (n=49)	2014—2018 (n=10)	2019—2023 (n=3)	2014—2018 (n=5)	2019—2023 (n=10)
Ampicillin	19 (100.00)	49 (100.00)	10 (100.00)	2 (66.67)	5 (100.00)	10 (100.00)
Ampicillin sulbactam	15 (78.95)	43 (87.76)	7 (70.00)	1 (33.33)	5 (100.00)	9 (90.00)
Piperacillin tazobactam	1 (5.26)	4 (8.16)	0 (0.00)	0 (0.00)	2 (40.00)	9 (90.00)
Cefepime	7 (36.84)	25 (51.02)	1 (10.00)	0 (0.00)	2 (40.00)	6 (60.00)
Cefazolin	13 (68.42)	42 (85.71)	9 (90.00)	1 (33.33)	5 (100.00)	9 (90.00)
Cefotaxime	14 (73.68)	40 (81.63)	10 (100.00)	1 (33.33)	3 (60.00)	8 (80.00)
Ceftriaxone	15 (78.95)	38 (77.55)	9 (90.00)	1 (33.33)	5 (100.00)	9 (90.00)
Ceftazidime	7 (36.84)	12 (24.49)	0 (0.00)	0 (0.00)	3 (60.00)	9 (90.00)
Cefoperazone sodium sulbactam sodium	2 (10.53)	10 (20.41)	0 (0.00)	0 (0.00)	2 (40.00)	5 (50.00)
Cefotetan	1 (5.26)	1 (2.04)	0 (0.00)	0 (0.00)	5 (100.00)	9 (90.00)
Meropenem	0 (0.00)	1 (2.04)	0 (0.00)	1 (33.33)	0 (0.00)	5 (50.00)
Imipenem	1 (5.26)	2 (4.08)	0 (0.00)	0 (0.00)	0 (0.00)	6 (60.00)
Gentamycin	4 (21.05)	7 (14.29)	3 (30.00)	1 (33.33)	0 (0.00)	0 (0.00)
Amikacin	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Levofloxacin	1 (5.26)	5 (10.20)	6 (60.00)	0 (0.00)	0 (0.00)	0 (0.00)
Ciprofloxacin	2 (10.53)	30 (61.22)	4 (40.00)	1 (33.33)	0 (0.00)	3 (30.00)
Tigecycline	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Voltaren	6 (31.58)	36 (73.47)	3 (30.00)	1 (33.33)	3 (60.00)	9 (90.00)
Cotrimoxazole	4 (21.05)	13 (26.53)	4 (40.00)	0 (0.00)	3 (60.00)	5 (50.00)

(*Continued*)

Table 3 (Continued).

Antibacterial Drug	Klebsiella pnenmoniae		Escherichia coli		Enterobacter cloacae	
	2014—2018 (n=19)	2019—2023 (n=49)	2014—2018 (n=10)	2019—2023 (n=3)	2014—2018 (n=5)	2019—2023 (n=10)
Ampicillin	19 (100.00%)	49 (100.00%)	10 (100.00%)	2 (66.67%)	5 (100.00%)	10 (100.00%)
Cefotaxime	14 (73.68%)	40 (81.63%)	10 (100.00%)	1 (33.33%)	3 (60.00%)	8 (80.00%)
Ceftriaxone	15 (78.95%)	38 (77.55%)	9 (90.00%)	1 (33.33%)	5 (100.00%)	9 (90.00%)
Meropenem	0 (0.00%)	1 (2.04%)	0 (0.00%)	1 (33.33%)	0 (0.00%)	5 (50.00%)
Ciprofloxacin	2 (10.53%)	30 (61.22%)	4 (40.00%)	1 (33.33%)	0 (0.00%)	3 (30.00%)

Table 4 Resistance of Major Fungi to Antifungal Drugs in Blood Cultures of Hospital-Acquired Sepsis in Preterm Infants in Both Groups (Number of Strains, %)

Antibacterial Drug	Smooth Pseudofilamentous Yeast		*Candida albicans*	
	2014—2018 (n=19)	2019—2023 (n=3)	2014—2018 (n=9)	2019—2023 (n=5)
Fluconazole	2 (10.53)	1 (33.33)	0 (0.00)	1 (20.00)
Itraconazole	6 (31.58)	1 (33.33)	1 (11.11)	1 (20.00)
Voriconazole	1 (5.26)	0 (0.00)	0 (0.00)	1 (20.00)
Amphotericin B	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Flucytosine	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Discussion

Sepsis remains a significant threat to preterm infants, particularly those with extremely low birth weight or gestational age <28 weeks. While advances in neonatal care have improved survival rates, the rise in health care associated infections and antibiotic resistance poses a growing challenge. This study highlights several critical trends and factors contributing to the increase in infections and resistance, as well as potential strategies to mitigate these issues. The data from this study indicate a significant increase in the incidence of health care associated sepsis from 1.4% in 2014–2018 to 2.7% in 2019–2023. This rise can be attributed to several factors. First, the increased survival of extremely preterm infants, who are more vulnerable to infections due to their immature immune systems and frequent need for invasive procedures such as mechanical ventilation, central venous catheters, and prolonged hospitalization. These procedures, while life-saving, create opportunities for pathogens to invade and colonize.[7] Recent multicenter studies demonstrate that device-associated infections dominate HAI epidemiology, with respiratory infections and bloodstream infections being most prevalent in ICUs, particularly among Extracorporeal Membrane Oxygenation (ECMO) patients where tracheostomy increased HAI risk 28.6-fold.[28] Notably, 72.4% of ECMO-related HAIs involved multidrug-resistant Gram-negative bacteria, while 71.3% of non-fermentative Gram-negative isolates in Ukrainian hospitals showed carbapenem resistance,[29] underscoring the critical intersection of invasive devices and antimicrobial resistance in neonatal sepsis. Second, the overuse of broad-spectrum antibiotics, particularly third-generation cephalosporins and carbapenems, has disrupted the normal microbial flora, promoting the selection of resistant strains. The proportion of ESBL-producing bacteria, for example, increased from 13.33% to 30.53% over the study period, reflecting the impact of antibiotic pressure on microbial resistance.[26]

To address these challenges, several measures must be implemented. First, strict adherence to infection control protocols is essential. This includes hand hygiene, aseptic techniques during invasive procedures, and regular surveillance of hospital-acquired infections.[24] Second, antibiotic stewardship programs should be strengthened to promote the rational use of antibiotics. Empirical therapy should be guided by local resistance patterns, and de-escalation should be practiced once culture results are available.[27] Third, the use of probiotics and other strategies to restore normal flora in preterm infants may help reduce the risk of opportunistic infections.[7] The study identified *Klebsiella pneumoniae* as the predominant pathogen, with high resistance rates to third-generation cephalosporins and an emerging trend of carbapenem resistance. This is particularly concerning given the limited therapeutic options for carbapenem-resistant *Enterobacteriaceae* (CRE).[30] The detection of vancomycin-resistant *Staphylococcus capitis* further underscores the need for vigilance in monitoring and managing resistant strains.[18] The spread of resistance genes, particularly through plasmids, highlights the importance of genomic surveillance to track and contain resistant pathogens.[31] The observed rise in ESBL-producing *Enterobacteriaceae* aligns with reports linking prolonged device use to nosocomial transmission.[32] Strict adherence to catheter bundle protocols and hand hygiene compliance, as demonstrated in the Smart Use of Antibiotics Program, reduced antibiotic days by 30% without compromising outcomes,[33] underscoring the synergy between stewardship and infection control.

The observed antibiotic resistance trends in septic preterm infants reflect competing selective pressures. Rising resistance to third-generation cephalosporins and carbapenems stems from their overuse, prolonged hospitalization facilitating nosocomial spread, and plasmid-mediated resistance gene transmission.[26,31,32] Conversely, decreased resistance to fluoroquinolones and aminoglycosides correlates with targeted antibiotic stewardship and improved infection control, while reduced fungal sepsis diminished antifungal selection pressure.[7,24,27] These opposing trends highlight how prescribing practices and microbial ecology interact in NICUs. The observed increase in fluconazole resistance among *Pseudomonas spp*. and *Candida albicans* underscores the challenges of antifungal stewardship. While fluconazole is a cornerstone therapy in NICUs, especially where resources are constrained, its overuse may drive resistance through efflux pump overexpression.[34] This trend calls for enhanced surveillance and alternative strategies, such as combination therapy or targeted prophylaxis in high-risk infants. The emergence of carbapenem-resistant *Enterobacter cloacae* and *E. coli* limits therapeutic options, as highlighted by Ding et al.[35] This necessitates reserved use of last-line agents and rapid implementation of contact isolation for CRE carriers.[13]

While this study focused on pathogen profiles and resistance trends, neonatal sepsis is associated with significant complications including septic shock, necrotizing enterocolitis in preterm infants,[8] and neurodevelopmental impairment in survivors.[15] Future prospective studies should correlate specific pathogens (eg, carbapenem-resistant *Klebsiella*) with clinical outcomes to guide risk stratification.

While our study relied on conventional culture methods, emerging molecular diagnostics (eg, PCR, mass spectrometry) offer faster pathogen detection and resistance gene profiling, with reported sensitivity of 91% and specificity of 88%.[16] Integrating such tools could reduce empirical antibiotic overuse, particularly for culture-negative sepsis cases,[15] though cost and technical barriers remain challenges in resource-limited settings.

This study has several limitations. First, its retrospective design may introduce selection bias, particularly in blood culture collection and antibiotic exposure documentation. Second, the single-center nature limits generalizability to other NICUs with different resistance patterns. Third, we could not assess clinical outcomes (eg, mortality, complications) associated with resistant infections due to data constraints. Future studies should prospectively evaluate pathogen-resistance-outcome relationships, incorporate genomic surveillance of resistance mechanisms, and test targeted interventions like antibiotic cycling in high-risk units.

Conclusion

In conclusion, the rise in health care associated sepsis and antibiotic resistance among preterm infants is a multifactorial problem that requires a comprehensive approach. The emergence of carbapenem-resistant *Enterobacter cloacae* and *E. coli*, alongside rising fluconazole resistance in *Candida albicans*, underscores the urgent need for alternative treatment strategies and enhanced surveillance in NICUs. By strengthening infection control measures, promoting antibiotic stewardship, integrating rapid molecular diagnostics for early pathogen detection, and investing in research on novel therapies and diagnostics, we can mitigate the impact of these infections and improve outcomes for this vulnerable population.[24] These findings highlight the importance of updating NICU protocols to include routine resistance monitoring and tailored empirical therapy based on local resistance patterns. The data presented in this study highlight the need for a multidisciplinary approach to address the rising incidence of health care associated sepsis and antibiotic resistance in preterm infants. The findings should be reviewed by an epidemiologist to further analyze the trends and differences, and discussed by clinicians to critically evaluate the procedures that may contribute to infections and identify corrective solutions. Future studies should focus on the clinical outcomes associated with these resistant pathogens to guide risk stratification and therapeutic decisions. In view of this, when applying antibiotics, healthcare professionals should strictly follow the principles of use and pay great attention to the problem of bacterial resistance; before implementing empirical treatments, blood cultures and drug sensitization should be executed as much as possible, and the results of the blood cultures should be used to determine which antimicrobial drug to choose and to reduce or avoid the development of drug-resistant strains of bacteria.

Data Sharing Statement

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Qucik-PJ 2023-06-32). Informed consent was obtained from the parents or legal guardians of all infant participants. All methods were carried out in accordance with Declaration of Helsinki.

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Author Contributions

Shen-wang Ni and Li Wang are co-first authors. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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