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Four-Year Bacteriological Profile and Antibiotic Resistance Rate of Neonatal Blood Cultures in a Major Neonatal Intensive Care Unit (NICU) in Shiraz, Southwest Iran

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Abstract

Background: Bacterial infections are the leading cause of death in newborns. The growing resistance of bacteria to antibiotics is a major concern. In this study, we identified isolates from blood cultures in the neonatal intensive care unit (NICU) of a major referral hospital in Shiraz in southwest Iran and determined antibiotic resistance patterns.

Methods: All newborns admitted to the NICU were evaluated in a four-year study. The blood samples were cultured using a BACTEC system. From May 2015 to March 2020, 5937 blood culture samples were analyzed. The antibacterial sensitivity test was conducted using the disk diffusion method, following the guidelines of Clinical & Laboratory Standards Institute (CLSI) 2018.

Results: In this study, 507 isolates were obtained from 5937 specimens. The most commonly isolated gram-negative bacteria were *Acinetobacter* (8.7%), *Escherichia coli* (5.7%), *Klebsiella* (1.6%), *Enterobacter* (1.4%), and *Pseudomonas* (0.9%). Among the gram-positive bacteria, coagulase-positive staphylococci (51.1%), coagulase-negative staphylococci (15.4%), *Staphylococcus epidermidis* (10.2%), non-hemolytic streptococci (2.4%), alpha-hemolytic streptococci (1.4%), and *Streptococcus pneumonia* (1.3%) were the most frequently isolated bacteria. Of the 318 *Staphylococcus aureus* isolates, 88.5% were resistant to methicillin.

Conclusion: Staphylococcus, with a high frequency in the NICU, can be an alarm for medical centers. Also, in dead infants, the most observed bacterial infection was *Acinetobacter* infection, which requires the serious attention of the hospital infection control unit.

Keywords: Neonatal, Antibiotic resistance, NICU, Blood culture, Shiraz

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Introduction

Bacterial infections such as bacteremia and sepsis are vital warning signs of neonatal mortality and morbidity (1). Gram-positive and gram-negative bacteria are present in bloodstream infections, and the frequency of each type differs depending on the time and location. Two groups can cause bloodstream infections in neonates, but *Staphylococcus* spp. are more common. These bacteria are part of the normal flora of human skin and can colonize medical devices (2,3). Due to the high mortality rate in infants, rapid identification of blood bacteria is essential. Also, an increase in bacterial resistance to antimicrobial agents is considered a significant problem in most hospitals worldwide (4,5).

Additionally, the Centers for Disease Control and Prevention (CDC) has demonstrated that numerous bacteria are resistant to multiple classes of antibiotics utilized in hospitals (6,7). Resistant isolates make treatment of these infections difficult and increase the duration of hospitalization and treatment costs. It is crucial to select the appropriate antibiotic for treatment to reduce the morbidity and mortality of patients with bloodstream infections. One of the common organisms that causes infection in neonatal intensive care units (NICUs) is *Staphylococcus spp*. *Staphylococcus aureus* can be a severe risk to infants due to its strong pathogenic factors (8,9). Although blood culture techniques take a long time, they are still the gold standard for diagnosing bloodstream infections (10). The primary purpose of this study was to investigate the prevalence of the bacteria and the antibiotic resistance of the isolates from the NICU in the primary referral center of NICU in Fars Province, Shiraz city, Iran. The second objective was to determine the relationship between bacteria and mortality rates every year.

Materials and Methods

Study design

This retrospective descriptive (historical) study examined the records of 5937 blood cultures performed for newborns aged ten days or younger admitted to Zainabieh hospital in Shiraz, Iran. This hospital is a major teaching hospital affiliated with Shiraz University of Medical Sciences and serves as the primary NICU referral center in the southwest region of Iran. The study was conducted from March 2015 to March 2020. A checklist was created to gather data on culture and antibiotic sensitivity test results.

Microbiology

The microbiology database was retrospectively reviewed to identify patients with positive blood cultures. Bacterial culture results, antibiotic resistance, and sex details were collected. However, due to annual changes in the hospital, the antimicrobial agents used and tested varied yearly. This study used a BACTEC blood culture system (11,12) to diagnose bacteria in blood samples rapidly. The samples were collected in standard BACTEC culture vials containing trypticase soy or brain-heart infusion broth and were incubated aerobically at 37 °C in the BACTEC machine. Bacteria were identified through Gram staining and subcultured on MacConkey agar, chocolate agar, blood agar, Triple Sugar Iron (TSI), citrate, Sulphide Indole Motility (SIM), lysine decarboxylase, Methyl Red / Voges-Proskauer (MR/VP), etc.

Antibiotic susceptibility testing

The disk diffusion method (Kirby-Bauer) was used to determine the antimicrobial sensitivity on the Mueller-Hinton agar medium (Merck, Germany) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) (13). Antibiotic disks including ciprofloxacin (CIP, 5 μg), cefoxitin (FOX, 30 μg), gentamicin (GM, 10 μg), imipenem (IPM, 10 μg), vancomycin (V, 30 μg), amikacin (AMK, 30 μg), polymyxin B (PMB, 10 μg), cefixime (CFM 30 μg), cefoxitin (Fox 30 μg), and cotrimoxazol (TMP-SMZ 25 μg) were used.

Data analysis

Data were analyzed using SPSS ver. 16 (IBM Corp., Armonk, NY, USA) software. Percentages were used to present the descriptive results of the variables. The chi-square test was used to compare frequencies across categories. *P* values were considered statistically significant if they were less than 0.05.

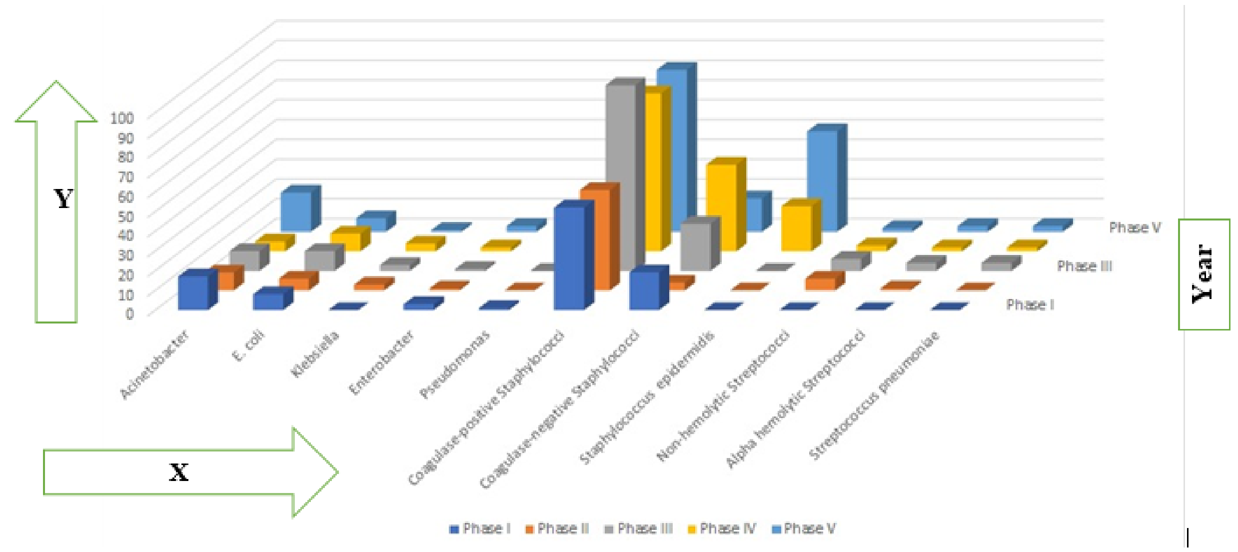
Results

We identified both gram-positive and gram-negative bacteria among the 705 isolates from blood cultures. Among the gram-negative bacteria, *Acinetobacter* was the most prevalent with 61 isolates (8.7%), followed by *Escherichia coli* with 40 isolates (5.7%), *Klebsiella* with 11 isolates (1.6%), *Enterobacter* with ten isolates (1.4%), and *Pseudomonas* with six isolates (0.9%).

Of the gram-positive isolates, coagulase-positive staphylococci accounted for 359 (51.1%) of the total, followed by coagulase-negative staphylococci with 108 (15.4%), *Staphylococcus epidermidis* with 74 (10.2%), non-hemolytic streptococci with 17 (2.4%), alpha-hemolytic streptococci with 10 (1.4%), and *Streptococcus pneumonia* with 9 (1.3%). This study found that coagulase-positive *Staphylococcus* is the most prevalent among gram-positive bacteria, particularly in the NICU. Figure 1 illustrates the abundance of bacteria over the years in five phases, with a year-based overview of the data. The study also reported a total of 100 deaths in the NICU, with the highest mortality rates observed in children with *Acinetobacter*-positive cultures, followed by those with coagulase-positive staphylococci isolates (Table 1).

The resistance rates for both coagulase-positive and coagulase-negative *Staphylococcus* were highest against cefixime (Tables 2 and 3). Out of the *Staphylococcus aureus* isolates, 318 (88.5%) were resistant to cefoxitin (methicillin-resistant), and 41 (11.5%) were sensitive to it (methicillin-sensitive). Tables 4 and 5 display the resistance patterns of coagulase-positive and coagulase-negative staphylococci during different phases of the study, and there was no significant difference ($P > 0.05$) in antibiotic resistance rates between the different years (phases).

Most of the *Acinetobacter* isolates were resistant to all antibiotics except for polymyxin. Resistance to both polymyxin and imipenem was observed in *Acinetobacter* and *E. coli* (see Tables 6 and 7). The rate of antibiotic resistance in *Acinetobacter* was persistently high throughout all phases of the study, with the isolates showing an increase in resistance to almost all drugs over time. During the 5-year study, it was observed that *Acinetobacter* was the most common cause of bacterial



Y = abundance, X = years in five phases (Phase I, II, III, IV, V)

Figure 1. The trend of distribution of bacterial isolates over the years. Phase I: March 2015 to March 2016; Phase II: April 2016 to March 2017; Phase III: April 2017 to March 2018; Phase IV: April 2018 to March 2019; Phase V includes April 2019 to March 2020

Table 1. Prevalence of bacteria isolated from dead infants

Bacteria	Deaths No. (%)
Acinetobacter	37 (37%)
Escherichia coli	12 (12 %)
Klebsiella	3 (3%)
Enterobacter	3 (3%)
Pseudomonas	1 (1%)
Coagulase-positive staphylococci	25 (25%)
Coagulase-negative staphylococci	6 (6%)
Staphylococcus epidermidis	9 (9%)
Non-hemolytic streptococci	3 (3%)
Alpha hemolytic streptococci	0
Streptococcus pneumonia	1 (1%)
Total	100 (100%)

infection in infants who died. Additionally, coagulase-positive staphylococci were found to be the second most common cause (Table 1).

Discussion

Due to the importance of the methicillin-resistant *Staphylococcus* isolates obtained from infants and the infant mortality associated with these bacteria, their timely identification and treatment are crucial. Understanding the resistance patterns in each region is essential for effective treatment. This study found that the prevalence of resistance to cefoxitin (methicillin resistance) was as high as 88.5%. In a 2020 study conducted by Ahmadishoar et al in Iran, methicillin resistance was reported as 68.2% (14). Similarly, a 2018 study by Sawhney et al in India found a 95.2% resistance rate to cefoxitin in *S. aureus* isolates from NICUs, which aligns with the findings of this study (15).

Overall, the incidence of nosocomial infections is influenced by various factors. These include premature birth, premature rupture of membranes, respiratory distress syndrome, respiratory failure, seizures, cyanosis, type of birth (cesarean section or vaginal), number of births (single or twin), uterine infection, and underlying maternal diseases such as malnutrition. Furthermore, infants who receive intravenous nutrition, particularly those with central venous catheters, are at a heightened risk for circulatory infections (16).

Due to their vulnerable state, preterm infants, high-risk infants weighing less than 2 kg, or infants with acute medical problems admitted to the NICU are at a higher risk of acquiring nosocomial infections. Therefore, it is crucial for physicians and staff in this ward to have a thorough understanding of pathogenic bacteria in this unit and to continuously update their knowledge and skills in order to prevent the death of these infants. Utilizing the available tools and facilities to their fullest potential is also essential. Research from various regions worldwide has shown that the prevalence of nosocomial infections varies. In studies conducted in the US, the incidence of nosocomial infections in NICUs ranged from 12% to 26.5% (17).

In intensive care units, bacteria, particularly staphylococci, are the primary cause of neonatal bloodstream infections. According to the current study, coagulase-positive staphylococci were the most frequently isolated bacteria. In the United States and Europe, coagulase-negative staphylococci were reported as the most common bacteria in these units, accounting for 48% of bloodstream infections (18). However, in a similar study conducted at hospitals in Ghana and India, gram-positive bacteria were found to be the predominant isolates (19,20). In contrast, in Nepal and Ethiopia,

Table 2. Resistance profile of gram-positive bacteria for different tested antibiotics

	Coagulase-positive staphylococci	*S. epidermidis*	CONS	Non-H strep	*Streptococcus pneumonia*	Alpha-H strep
Amikacin	49.8 %	60.8 %	50.9 %	88.2 %	88.8 %	60 %
Cefixime	75.7 %	41.8 %	81.4 %	76.4 %	55.5 %	30 %
Ciprofloxacin	65.4 %	70 %	54.6 %	94.1 %	66.6 %	50 %
Cotrimoxazol	64 %	60.8 %	59.2 %	64.7 %	77.7 %	40 %
Cefoxitin	88.5%	84%	87%	-	-	-
Gentamicin	62.6 %	74.3 %	70.3 %	88.2 %	88.8 %	40 %
Vancomycin	-	-	-	41.1 %	11.1 %	20 %

Table 3. Resistance profile of gram-negative bacteria to different tested antibiotics

	Acinetobacter	*E. coli*	*Klebsiella*	*Pseudomonas*	*Enterobacter*
Amikacin	80.3 %	17.5 %	81.8 %	33.3 %	90 %
Cefixime	83.6 %	55 %	63.6 %	16.6 %	40 %
Ciprofloxacin	81.9 %	22.5 %	36.3 %	0 %	30 %
Cotrimoxazole	83.6 %	62.5 %	45.4 %	33.3 %	30 %
Gentamicin	81.9 %	25 %	18.1 %	33.3 %	100 %
Imipenem	81.9 %	17.5 %	54.5 %	0 %	20 %
Polymyxin B	47.5 %	12.5 %	18.1 %	0 %	0 %

Table 4. The trend of antibiotic resistance for coagulase-positive staphylococci over the years

Antibiotic	Phase I (%)	Phase II (%)	Phase III (%)	Phase IV (%)	Phase V (%)	*P* value
Amikacin	55.7%	50.9 %	45.7 %	55%	45.1	0.2786
Cefixime	84.6 %	92.1	62.7 %	87.5 %	63.4 %	0.2871
Ciprofloxacin	69.2 %	74.5 %	59.5 %	67.5 %	62.1 %	0.2582
Cotrimoxazole	69.2 %	62.7 %	60.6 %	67.5 %	62.1 %	0.4239
Gentamicin	65.3%	76.4 %	56.3 %	62.5 %	59.7 %	0.3009
Cefoxitin	88%	91%	84%	78%	87%	0.3359

Phase I: March 2015 to March 2016; Phase II: April 2016 to March 2017; Phase III: April 2017 to March 2018; Phase IV: April 2018 to March 2019; Phase V: April 2019 to March 2020.

Table 5. The trend of antibiotic resistance for coagulase-negative staphylococci over the years

Antibiotic	Phase I	Phase II	Phase III	Phase IV	Phase V	*P* value
Amikacin	36.8 %	50 %	33.3 %	63.6 %	58.8 %	0.1698
Cefixime	84.2 %	75 %	79.1 %	79.5 %	88.2 %	0.4376
Ciprofloxacin	52.6 %	25 %	50%	59 %	58.8 %	0.2952
Cotrimoxazole	47.3 %	0 %	54.1 %	68.1 %	70.5 %	0.2041
Gentamicin	68.4%	50 %	58.3 %	77.2 %	76.4 %	0.2460
Cefoxitin	89%	92%	96%	88%	81%	0.2537

Phase I: March 2015 to March 2016; Phase II: April 2016 to March 2017; Phase III: April 2017 to March 2018; Phase IV: April 2018 to March 2019; Phase V: April 2019 to March 2020.

mainly gram-negative bacteria were isolated from infants (21, 22). In a study by Yusef et al, similar to the present study, *Acinetobacter* was the most commonly identified bacterium in neonatal bloodstream infections and was often associated with neonatal mortality (23).

All isolated bacteria showed high resistance rates to many antibiotics, which poses a serious warning in treating neonatal infections in the NICU. In a similar study conducted in Nepal and Ethiopia, *Staphylococcus* spp. was highly resistant to different antibiotics (21,22). A similar study by Liu et al also reported that polymyxin was the most effective antibiotic for treating gram-negative infections, including those caused by *Acinetobacter*, in neonates (24).

The high level of antibiotic resistance in our study's isolates is associated with a significant number of neonatal deaths. However, using broad-spectrum antibiotics as empirical therapy for a prolonged period

Table 6. The trend of antibiotic resistance for *Acinetobacter* over the years

Antibiotic	Phase I	Phase II	Phase III	Phase IV	Phase V	*P* value
Amikacin	76.4 %	88.8 %	60 %	80 %	90 %	0.6306
Cefixime	82.3 %	88.8 %	70%	100 %	90 %	0.4469
Ciprofloxacin	82.3 %	88.8 %	70 %	100 %	85 %	0.6287
Cotrimoxazole	82.3 %	88.8%	70%	100 %	90 %	0.4469
Gentamicin	82.3 %	88.8 %	70%	100 %	85 %	0.6287
Imipenem	76.4 %	88.8 %	70 %	100 %	90 %	0.3062
Polymyxin	52.9 %	55.5 %	30 %	40 %	50 %	0.5232

Phase I: March 2015 to March 2016; Phase II: April 2016 to March 2017; Phase III: April 2017 to March 2018; Phase IV: April 2018 to March 2019; Phase V: April 2019 to March 2020.

Table 7. The trend of antibiotic resistance for *E. coli* over the years

Antibiotic	Phase I	Phase II	Phase III	Phase IV	Phase V	*P* value
Amikacin	25 %	16.6 %	20 %	11.1 %	14.2 %	0.1101
Cefixime	50 %	33.3 %	80 %	55.5 %	42.8 %	0.8883
Ciprofloxacin	25 %	16.6 %	30 %	22.2 %	14.2%	0.4269
Cotrimoxazole	25 %	66.6 %	100 %	66.6 %	42.8 %	0.6910
Gentamicin	50 %	33.3 %	20 %	11.1 %	14.2 %	0.0640
Imipenem	25 %	16.6 %	30 %	11.1 %	0 %	0.1366
Polymyxin	12.5 %	16.6 %	20 %	0 %	14.2%	0.5837

Phase I: March 2015 to March 2016; Phase II: April 2016 to March 2017; Phase III: April 2017 to March 2018; Phase IV: April 2018 to March 2019; Phase V: April 2019 to March 2020.

can be harmful. In addition, our results suggest that local practitioners should carefully select antibiotics to treat neonatal bacteremia, especially when treatment needs to be initiated before the blood culture results are available.

Conclusion

In this study, the most commonly isolated bacteria from blood cultures in the NICU were coagulase-positive staphylococci, which showed high methicillin resistance rates, and *Acinetobacter*, which exhibited a high resistance rate to almost all antibiotics studied. It is important to note that devices, such as catheters, incubators, and suctions, and vectors, such as the hospital staff's hands and washing solutions, can transfer bacterial infections to infants. Therefore, the high percentage of isolated bacteria from blood cultures in this study and their high antibiotic resistance rates highlight the need for strict control of the sterility of hospital equipment and staff. Continuous training of personnel and encouraging them to adhere to hygienic principles are also crucial in preventing the spread of infections.

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Authors' Contribution

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Resources: Navid Omidifar, Mohammad Motamedifar.
Software: Mohsen Moghadami.
Supervision: Mohammad Motamedifar.
Validation: Farshad Kakian.
Visualization: Navid Omidifar.
Writing–original draft: Farshad Kakian.
Writing–review & editing: Farshad kakian, Mohammad Motamedifar.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Ethical Approval

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (ethics code*:* IR.SUMS.MED.REC.1398.1048*)*. We followed all the ethical principles of the World Medical Association Declaration of Helsinki for medical research involving human subjects. We obtained written informed consent from all study participants before their recruitment. Participants were assured that their data would be processed per Iran's data protection laws, ensuring their confidentiality.

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