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ORIGINAL ARTICLE

Healthcare-associated infection in neonates and risk factors: A four-year surveillance study (2020–2024)

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ABSTRACT

Objective: Healthcare-associated infections (HAIs) represent a significant source of morbidity and mortality in neonatal intensive care units (NICUs). During the neonatal period, risk factors include prematurity, an immature immune system, invasive procedures, and prolonged hospitalization. This study aimed to identify the causative agents and risk factors associated with HAIs.

Material and Methods: This study encompassed 297 patients who were hospitalized in the NICU and diagnosed with HAIs between January 1, 2020, and December 30, 2023. Demographic, clinical, and laboratory data were retrospectively analyzed.

Results: According to the type of HAIs, bloodstream infection (BSI) accounted for 53% (n=158), central line-associated BSI (CLABSI) for 4.3% (n=13), urinary tract infection for 8.7% (n=26), ventilator-associated pneumonia (VAP) for 0.3% (n=1), bone and joint infection for 1% (n=3), and skin and soft tissue infection for 1.3% (n=4). The CLABSI and VAP rates were 5.45 and 1.67, respectively. The overall mortality rate was 3% (n=9). The most commonly isolated agents were Gram-positive bacteria (79.8%), Gram-negative bacteria (17.8%), and *Candida spp.* (2.4%). Statistically significant differences were observed in prematurity (p=0.010), birth weight (p<0.001), mode of delivery (p=0.010), postnatal days (p=0.012), duration of hospitalization (p=0.004), mechanical ventilation (p=0.009), operation (p=0.031), presence of total parenteral nutrition (p<0.001), and central venous catheter use (p<0.001) between the groups.

Conclusion: Consistent with the existing literature, Gram-positive microorganisms were identified as the predominant causative agents of HAIs. However, the incidence of Gram-negative bacteria and *Candida spp.* increased in the presence of specific risk factors. Identifying causative agents and associated risk factors is crucial for mitigating HAIs in the NICU.

Keywords: Bloodstream infection, healthcare-associated infections, neonatal intensive care unit.

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INTRODUCTION

Healthcare-associated infections (HAIs) are a considerable source of morbidity and mortality, including long-term neurodevelopmental impairment, in neonatal intensive care units (NICUs), thereby contributing to increased hospital expenditure.[1,2] The Centers for Disease Control and Prevention (CDC) reports that one in 31 hospital patients has an HAI.[3] HAIs are considered infections characterized by their onset within 48 hours of birth or hospital admission during the neonatal period. During the neonatal period, various factors, such as prematurity, an immature immune system, invasive interventions performed in the NICU, and the coexistence of underlying diseases, increase the risk of HAIs.[4] HAIs include bloodstream infections (BSIs), ventilator-associated pneumonia (VAP), urinary tract infections (UTIs), meningitis, skin and soft tissue infections (SSIs), and central line-associated BSIs (CLABSIs).[5]

The incidence of HAIs varies across countries and hospitals. In low- and middle-income countries, the incidence of HAIs in NICUs ranges from 15.2 to 62.0 per 1000 patient-days, which is nine times higher than the rates observed in certain high-income settings.[6] In resource-constrained contexts, such as sub-Saharan Africa, where 42% of global neonatal deaths occur, research focused on infection prevention and the establishment of surveillance systems aims to reduce the rate of neonatal infections.[7] However, HAIs have also been reported in high-income countries, such as Italy (5%), Canada (24%), and Germany (12.3%).[8–10] Considering that HAIs in the neonatal period are among the preventable causes of death, healthcare centers should identify risk factors and causative microorganisms to reduce morbidity and mortality. The objective of this study was threefold: first, to ascertain the incidence of HAIs; second, to identify the implicated microorganisms; and third, to examine the risk factors associated with HAIs within the NICU of our center.

MATERIAL AND METHODS

Participants

This retrospective cohort study encompassed 297 patients who were hospitalized in the NICU between January 2020 and December 2023 and from whom microorganisms were isolated from clinical samples (blood, urine, and respiratory). During the four-year study period, 4,303 neonates were observed in the NICU; however, those who did not fulfill the criteria for HAIs were excluded from the study.

Data Analysis

A retrospective evaluation of the clinical and microbiological characteristics of the patients was conducted using medical records. The patients' sex, birth weight, gestational age, mode of delivery, comorbidities, invasive devices (central line catheterization), mechanical ventilation, day of hospitalization, operation, exposure to antimicrobial therapy, site of infection, and day of postpartum onset of infection were recorded. The causative microorganisms were classified into three categories: Gram-positive, Gram-negative, and fungal. A comparative analysis of the clinical and microbiological characteristics of the patients was conducted.

Definitions

HAIs were considered to occur within 48h after birth or admission. All infections, including BSI, CLABSI, and VAP, were defined using the CDC NHS surveillance guide and local surveillance guidelines adapted to neonatology.[11–13]

BSI was diagnosed based on clinical findings and hemoculture results. The microorganism was accepted as the causative agent if it was not associated with an infection elsewhere in the body and if skin flora agents, such as *Bacillus spp.*, *Corynebacterium*, or coagulase-negative staphylococci, were detected in two or more samples.

UTI was diagnosed according to CDC criteria for patients aged <1 year (with or without a urinary catheter). The presence of an indwelling urinary catheter for a duration exceeding two consecutive days led to the diagnosis of catheter-associated urinary tract infection (CAUTI).

Ventilator-associated pneumonia (VAP) was defined in patients who met the modified pneumonia criteria for children aged <1 year according to the CDC definition and who were on mechanical ventilation for at least two consecutive calendar days.

CLABSI was defined as the recovery of a pathogenic organism from a blood culture in a patient who had received a central line for a minimum of two consecutive days during the period of infection.

The HAI rate was defined as (number of healthcare-associated infections/number of hospitalized patients)×100. Incidence density was defined as (number of healthcare-associated infections/patient days)×1000. Invasive device-related infection rates were defined as CLABSI per catheterization day and VAP per 1000 ventilation days.

Statistical Analysis

Statistical analyses were performed using SPSS version 21. Descriptive statistics were presented as means, standard deviations, numbers, and percentages. Group comparisons were conducted using analysis of variance (ANOVA) and post hoc analyses for parametric variables, and the Kruskal–Wallis and Mann–Whitney U tests for non-parametric variables. Statistical significance was set at $p<0.05$. This study was approved by the Mardin Artuklu University Clinical Research Ethics Committee (2024/3-25). Informed consent was obtained from the parents. The study was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

Demographic and Clinical Characteristics

The study included 297 patients who were followed up in the NICU of our hospital between 2020 and 2023, in whom microbiological agents were detected in clinical specimens and who met the definition of HAIs. The patients' mean age was 8±7.7 (2–52) postnatal days; 36.7% (n=109) were female, 63.3% (n=188) were male, 31% (n=92) were preterm, and 69% (n=164) were term. Based on birth weight for gestational age, 84.5% (n=251) were appropriate for gestational age (AGA), 14.5% (n=43) were small for gestational age (SGA), and 1% (n=3) were large for gestational age (LGA). Regarding mode of delivery, 55.2% (n=164) were vaginal deliveries and 44.8% (n=133) were cesarean sections. The mean birth weight was 2807±803 (900–4550) g, and the mean duration

of hospitalization was 15.6±22 (2–245) days. Comorbidities were present in 19.9% (n=59) of patients; exposure to antibiotics was 34% (n=101); operations were performed in 5.7% (n=17); total parenteral nutrition (TPN) was administered to 26.9% (n=80); central venous catheter (CVC) use was 24.9% (n=74); and mechanical ventilation (invasive/noninvasive) was required in 50.1% (n=149).

Types of Infections and Distribution of Pathogens for HAIs

Bloodstream infections (84.3%) were the most common HAIs, followed by UTIs (8.8%), CLABSIs (4.3%), SSIs (1.34%), bone and joint infections (1.01%), and VAP (0.3%). The incidence of HAIs was 12.49 per 1000 patient-days, and the overall HAI rate was 6.9% per 100 admissions. The rates of CLABSI and VAP were 5.45 and 1.67 per 1000 device-days, respectively. A total of 62% of subjects with UTIs had a documented history of catheterization. Data were unavailable for the remaining subjects; however, all cases met the established criteria for symptomatic UTIs in children aged <1 year.

The most prevalent pathogens were Gram-positive microorganisms (*Staphylococcus spp.*, *Streptococcus spp.*, *Enterococcus spp.*) (79.8%), followed by Gram-negative microorganisms (*Klebsiella pneumoniae*, other *Enterobacteriaceae spp.*, *Pseudomonas aeruginosa*) (17.8%) and *Candida spp.* (2.4%) (Table 1). The causative agents of CLABSI were *Klebsiella pneumoniae* (n=8), *Staphylococcus aureus* (n=2), and *Pseudomonas aeruginosa* (n=1). The prevalence of ESBL positivity in *Klebsiella pneumoniae* infections was 1% (n=3). All *Pseudomonas aeruginosa* isolates were susceptible to carbapenems. All *Staphylococcus* and *Enterococcus spp.* were susceptible to vancomycin. All *Candida spp.* were susceptible to fluconazole, itraconazole, and amphotericin B.

Comparison of Risk Factors According to Causative Pathogens

The three groups showed statistically significant differences in postnatal days (p=0.012), duration of hospitalization (p=0.004), birth weight (p<0.001), gestational age (p=0.01), mode of delivery (p=0.01), comorbidity (p=0.002), mechanical ventilation (p=0.009), CVC use (p<0.001), TPN (p<0.001), and operation (p=0.031).

In the fungal group, birth weight and duration of hospitalization were significantly lower in Gram-positive (p<0.001 and p=0.03) and Gram-negative (p<0.001 and p=0.013, respectively) cases, whereas postnatal days were significantly higher only in Gram-positive cases (p=0.024). Additionally, gestational age, mode of delivery, comorbidities, mechanical ventilation, CVC use, and TPN were significantly higher in the fungal group compared with the other groups. The overall mortality rate was 3% (n=9), with no statistically significant difference between groups (Table 2). A total of 66.6% of deceased patients had comorbid conditions, including congenital heart disease, trauma, necrotizing enterocolitis, and congenital metabolic disease.

Table 1: The distribution of pathogens of Healthcare-Associated Infections

	n	%
Gram-positive microorganisms	237	79.8
Staphlococcus spp		
Coagulase Negative Staphlococci	196	65.9
S. aureus	7	0.3
Streptococcus spp	20	6.7
Enterococcus spp	14	4.7
Gram-negative microorganisms	53	17.8
K.pneumoniae	20	6.7
P.aeruginosa	7	2.3
Acinetobacter spp	1	0.3
Serratia spp	4	1.3
Enterobacteriaceae spp	19	6.3
Other	2	0.6
Fungus	7	2.4
Candida albicans	5	1.7
Candida guilliermondii	1	0.3
Candida parapsilosis	1	0.3
Total	297	100

DISCUSSION

Although the global burden of HAIs in the neonatal period cannot be precisely determined because of differences in definitions and reporting, studies have indicated that HAIs are more frequent in the NICU than in other ICUs.[1,14] In Europe, approximately one in 10 infants requires hospitalization in the NICU during the first days of life. Although survival rates for these infants have improved, bacterial colonization in hospital settings, combined with various risk factors, increases vulnerability to infections caused by resistant microorganisms. Therefore, in 2021, a surveillance toolkit was developed through the European Union (EU)-funded NeoIPC project (establishing innovative approaches for optimal infection prevention of resistant bacteria in NICUs by integrating research, implementation science, and surveillance on a sustainable global platform) to monitor and prevent HAIs in at-risk infants, particularly those born preterm.[15]

The World Health Organization (WHO) has focused on healthcare-associated infections (HAIs) and antimicrobial resistance (AMR) in G7 countries and has published a global report on infection prevention and control strategies.[16,17] According to this report, the global incidence of HAIs was 15.4 cases per 1000 adult patients and was >7 times higher among neonates, with 112.9 cases per 1000 neonates.[18,19] Newborns were found to be at higher risk of acquiring HAIs, with infection rates in low-income countries being 3–20 times higher than those in high-income countries.

In Türkiye, the incidence of HAIs in the NICU was reported as 23.5% and 7.6% in two national point-prevalence studies conducted at different times.[20,21] Atıcı et al.[22] reported an overall HAI rate of 29.1% and a density of 21.8 per 1000 patient-days. In two retrospective studies, HAI rates of 4.9% and 14.9% were reported in NICUs.[23,24] In the present study, the overall HAI rate was 6.9%, which was slightly lower than those reported in comparable national studies.

Table 2: Comparasion of Gram positive/Gram negatives microorganisms/Candida spp (n/% or mean±SD)

	Gram positives			Gram negatives			*Candida spp*			p
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	
Sex										0.139
Female	91	30		14	4.7		4	1.3		
Male	146	49		39	13		3	1.1		
Birth weight for gestational age										0.99
AGA	203	68.3		45	15		6	2		
SGA	34	11.4		8	26		1	0.3		
Gestational age								2.3		**0.010**
Term	163	54		42	14		0			
Preterm	74	25		11	37		7			
Mode of delivery								2.3		**0.010**
VD	132	44		32	11		0			
CS	105	35		21	7		7			
Birth weight (g)			2811.6878± 759			2978.7736± 853			1350±341	**<0.001**
Lenght of hospital stay (day)			14.51±22.1			16.96±19.76			42±23.29	**0.004**
Postnatal day			7.19±8.12			8.9±7.09			15.42±6.5	**0.012**
Comorbidity	42	14		12	4		5	1.7		**0.002**
Mechanical ventilation										**0.009**
No	117	39.4		30	10		1	0.3		
NIV	79	26.5		9	3		0			
Invasive	41	13.8		14	4.7		6	2		
CVC	52	17.5		16	5.4		6	2		**<0.001**
TPN	54	18.2		20	6.7		6	2		**<0.001**
Operation	12	4		3	1		2	0.6		**0.031**
Previous antibiotic exposure	80	26.9		16	5.4		5	1.7		0.095
Mortality	8	2.7		0			1	0.3		0.093

AGA: Appropriate for gestational age; SGA: Small for gestational age; CVC: Central venosus catheter; TPN: Total parenteral nutrition; NIV: Non-Invasive; CS: Cesarean sections, VD: Vaginal delivery.

Although the incidence of bloodstream infections has decreased with improved preventive measures, BSIs remain an important problem in the neonatal period due to emerging resistant pathogens.[25] Previous studies have reported that BSIs accounted for 44%, 51.8%, and 83% of HAIs in the NICU.[26–28] According to the CDC, hospital-onset bacteremia (HOS) is classified as an HAI, with an incidence rate of 1.1 per 1000 patient-days (2%), and occurs in the absence of a central line in 54.2% of cases. Low birth weight is a significant risk factor, and among patients with HOS, the risk increases with postnatal age and is associated with mortality.[29] In the present cohort, BSI (84.3%) was the most prevalent HAI, followed by UTI (8.8%) and CLABSI (4.3%). Ventilator-associated pneumonia accounted for only 0.3% of cases. The rate of hospital-acquired pneumonia was lower in our cohort than in previous studies reporting higher rates of 33.3% and 24.4%, respectively.[22,30] Prematurity, prolonged hospitalization, and low birth weight have been documented as risk factors for VAP. Avoidance of prolonged mechanical ventilation and strict adherence to hand hygiene are key preventive strategies against VAP.[31] Compliance with these preventive measures, along with potential underdiagnosis or underreporting of VAP, may have contributed to the low incidence observed in this study. In contrast, BSI was identified as the predominant cause of HAIs, which is consistent with the existing literature.

A study by Castrillo (Spanish National Network for the Surveillance of Neonatal Infections) found that the most common pathogens causing nosocomial sepsis were Gram-positive bacteria (66%), followed by Gram-negative bacteria (28%) and *Candida spp*. (6%) in very-low-birth-weight infants.[32] Findings from a point-prevalence study indicated that coagulase-negative staphylococci (CNS) constituted the most prevalent pathogens

among HAIs, with *Enterobacteriaceae* ranking second.[33] In a meta-analysis conducted in Brazil, CNS (32.1%), *Staphylococcus aureus* (13.8%), and *Klebsiella spp*. (12.4%) were identified as the most common pathogens.[27] However, Gram-negative pathogens also represent an important proportion of HAI pathogens in neonates. In a study by Bedir Demirdağ et al.,[21] Gram-negative pathogens were identified in 43% of cases, with *Klebsiella pneumoniae* being the most common (22%). The most frequently isolated pathogens in Italy were *Pseudomonas aeruginosa* (17%), *Candida parapsilosis* (16.3%), *Escherichia coli* (13.1%), and *Candida albicans* (10.5%).[26]

Candida spp. are considered part of the normal microbiota of the skin and vaginal mucosa. Newborns may be colonized with *Candida spp*. through vaginal transmission at birth or via nosocomial transmission in the hospital setting.[34] In the presence of various risk factors, colonization may progress to invasive infection and result in serious complications.[35] Although low birth weight and prematurity are well-known risk factors, one study reported that the mean birth weight of infants with invasive candidiasis was 1270g and that 19% were born at <28 weeks of gestation.[36] Additional risk factors have also been identified,[37] including prolonged use of broad-spectrum antibiotics, CVC use, TPN administration, corticosteroid therapy, and poor adherence to infection control practices.[38] Both CVCs and peripherally inserted central catheters increase the risk of skin barrier disruption, dissemination of *Candida spp*. to sterile sites, and biofilm formation on catheter surfaces.[39]

In addition to isolated candidemia, complications such as endophthalmitis, endocarditis, liver or splenic abscesses, and neurological involvement may occur during the neonatal period.[34] In the present study, risk factors including TPN and CVC use, low birth weight, and prolonged hospitalization were significantly more frequent among patients with *Candida spp*. infections; however, no difference in mortality was observed, and *Candida spp*.-related tissue involvement (endophthalmitis) was identified in one patient.

Limitations

This retrospective study was conducted at a single center over a four-year period. The COVID-19 pandemic occurred during the study timeframe, and various factors, such as disruptions in infection control practices and surveillance systems, may have influenced the results. Although several studies have addressed this issue, the potential impact of the pandemic on HAI rates in the NICU remains unclear, as comparisons with pre-pandemic data were not performed.

CONCLUSION

The neonatal period, particularly in preterm infants, is characterized by increased susceptibility to HAIs due to immune immaturity and the presence of multiple risk factors. Although Gram-positive organisms were predominant, Gram-negative bacteria and *Candida spp*. also constituted significant challenges in the NICU. The implementation of effective surveillance systems, minimization of modifiable risk factors (such as duration of CVC use and TPN administration), and strict adherence to basic infection prevention measures, including hand hygiene, are essential for reducing HAIs.

Statement

Ethics Committee Approval: The Mardin Artuklu University Clinical Research Ethics Committee granted approval for this study (date: 05.03.2024, number: 2024/3-25).

Informed Consent: Informed consent was obtained from the parents.

Conflict of Interest: The authors declare that there is no conflict of interest.

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