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INCIDENCE MICROBIOLOGICAL SPECTRUM AND RESISTANCE PATTERN OF HEALTHCARE-ASSOCIATED INFECTIONS IN NICU AT A TERTIARY CARE CENTRE: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Healthcare-associated infections (HAIs) are a major cause of morbidity and mortality among neonates admitted to intensive care units. Their incidence, microbial profile, and resistance patterns vary across regions, necessitating continuous local surveillance to guide infection control and antibiotic stewardship strategies. **Aim:** To determine the incidence, microbiological spectrum, and antibiotic resistance patterns of healthcare-associated infections in the NICU.

Materials and Methods: A prospective observational study was conducted over two years among 113 neonates admitted to the NICU of a tertiary-care hospital. Neonates developing infection ≥48 hours post-admission were evaluated clinically and microbiologically. Samples including blood, CSF, urine, tracheal aspirate, and pus were processed using standard culture techniques, and antibiotic susceptibility was assessed following CLSI guidelines. Statistical analysis was performed using SPSS v25, with significance set at $p < 0.05$.

Results: Out of 1,344 NICU admissions, 121 (9.0%) neonates developed HAIs, accounting for 211 infection episodes. The infection density was 43.7 per 1,000 patient-days. Bloodstream infections (48.3%) were most frequent, followed by clinical sepsis (28.4%) and meningitis (11.4%). Among 141 culture-positive episodes, Gram-negative organisms predominated (60.3%), with Klebsiella pneumoniae (20.6%), Acinetobacter baumannii (9.9%), and Enterobacter cloacae (7.1%) as leading pathogens. Candida species accounted for 17.7% of isolates. High levels of resistance were observed to carbapenems-imipenem (up to 90%) and meropenem (69%)-while amikacin retained better activity.

Conclusion: HAIs in the NICU are predominantly caused by multidrug-resistant Gram-negative bacteria, particularly Klebsiella and Acinetobacter species. The high incidence underscores the urgent need for stringent infection control, judicious antibiotic use, and ongoing microbial surveillance to mitigate resistance and improve neonatal survival.

Keywords: Neonatal sepsis; Healthcare-associated infection; Multidrug resistance.

INTRODUCTION

Healthcare-associated infections (HAIs) are among the leading causes of morbidity and mortality in neonatal intensive care units (NICUs) worldwide. Over the last few decades, advances in neonatal care-such as mechanical ventilation, parenteral nutrition, and central venous access-have significantly improved survival rates of preterm and critically ill neonates. However, these same life-saving interventions have also predisposed neonates to an increased risk of nosocomial infections. The immunologic immaturity, fragile skin and mucosal barriers, and frequent invasive procedures render newborns-especially preterm and low-birth-weight infants-highly susceptible to infections that originate within healthcare settings.[1]

A healthcare-associated infection is defined as a localized or systemic condition resulting from an adverse reaction to an infectious agent or its toxins, which was neither present nor incubating at the time of admission. In neonates, HAIs are usually classified as late-onset infections occurring after 48-72 hours of life. The risk factors contributing to HAIs include prematurity, prolonged hospital stay, use of invasive devices, mechanical ventilation, parenteral nutrition, inappropriate antibiotic use, and lapses in hand hygiene among healthcare workers. Reported HAI incidence in NICUs varies widely-from 6 % to 50 %-and is three- to twenty-fold higher in developing nations compared with high-income countries. Mortality among affected neonates ranges from 20 % to 80 %, depending on gestational age and underlying comorbidities.[2]

The microbial profile and resistance pattern of pathogens responsible for HAIs differ markedly between hospitals and even within the same institution over time. Gram-negative organisms such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Escherichia coli*, and *Pseudomonas aeruginosa* have been frequently isolated from NICUs, while Gram-positive organisms-particularly coagulase-negative *Staphylococci* (CONS) and *Staphylococcus aureus*-also remain important causes. Fungal infections, primarily due to *Candida* species, have emerged as significant pathogens, particularly in very-low-birth-weight infants exposed to prolonged antibiotic therapy.[3]

In low- and middle-income countries (LMICs), neonatal sepsis and HAIs account for approximately one-quarter of neonatal deaths. According to the World Health Organization, bacterial infections are implicated in about 25 % of the 2.8 million neonatal deaths each year, with a disproportionate burden in developing regions. Studies indicate that the prevalence of HAIs in LMICs is three to twenty times higher than that observed in developed nations, reflecting disparities in infection control practices, infrastructure, and staffing ratios. The prevalence of multidrug-resistant organisms (MDROs)-including methicillin-resistant *S. aureus* (MRSA), extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, and carbapenem-resistant *Acinetobacter*-further complicates treatment and increases mortality.[4]

The most common HAIs in NICUs include bloodstream infections (particularly central-line-associated bloodstream infections, CLABSIs), ventilator-associated pneumonia (VAP), urinary-tract infections (UTIs), surgical-site infections (SSIs), and meningitis. CLABSIs are the most frequently reported, often linked to prolonged catheter use and suboptimal maintenance protocols. Studies from the National Healthcare Safety Network (NHSN) report CLABSI rates ranging from 6-8 % in pediatric ICUs, with bloodstream infections accounting for up to 28 % of all HAIs. Ventilator-associated pneumonia and catheter-associated UTIs follow as common complications.[5]

Aim

To determine the incidence, microbiological spectrum, and resistance pattern of healthcare-associated infections in the neonatal intensive care unit (NICU).

Objectives

1. To determine the incidence of healthcare-associated infections among neonates admitted to the NICU.
2. To identify the microbiological spectrum of organisms causing healthcare-associated infections in the NICU.
3. To assess the antibiotic sensitivity and resistance patterns of pathogens isolated from healthcare-associated infections.

MATERIALS AND METHODS

Source of Data

Data were obtained from all neonates admitted to the Neonatal Intensive Care Unit (NICU) of Bharati Hospital and Research Centre, Pune-a tertiary-care teaching hospital-who developed clinical features of infection after 48 hours of admission during the study period.

Study Design

A prospective observational study was conducted.

Study Location

The study was carried out in the Level III Neonatal Intensive Care Unit of Bharati Hospital and Research Centre, Pune, Maharashtra, India.

Study Duration

The study spanned 24 months, from June 2022 to May 2024.

Sample Size

A total of 113 neonates fulfilling the inclusion criteria were enrolled.

Inclusion Criteria

- Neonates who developed signs or symptoms suggestive of infection after 48 hours of NICU admission.

- Both inborn and outborn neonates admitted during the study period.

Exclusion Criteria

- Neonates admitted for less than 48 hours who developed infection within that period (likely community-acquired).
- Neonates with congenital infections diagnosed at birth.

Procedure and Methodology

All eligible neonates admitted during the study period were prospectively followed. Clinical suspicion of infection was based on temperature instability, respiratory distress, lethargy, feeding intolerance, and abnormal laboratory findings. Detailed perinatal history, risk factors (prematurity, invasive procedures, catheterization, ventilation, surgery), and hospital course were documented using a structured proforma.

For each episode of suspected sepsis, the following samples were collected under aseptic precautions prior to initiation of antibiotics:

- **Blood cultures** (1-2 mL) for bacterial and fungal growth.
- **Cerebrospinal fluid (CSF)** in cases with neurological signs.
- **Urine samples** obtained via sterile catheterization or suprapubic aspiration.
- **Tracheal aspirates** for suspected VAP cases.
- **Pus or wound swabs** for suspected surgical-site infections.

All samples were processed in the Microbiology Laboratory according to standard procedures.

Sample Processing

Specimens were cultured on blood agar, MacConkey, and Sabouraud's dextrose agar as applicable. Organisms were identified by conventional biochemical methods and automated systems (where available). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk-diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. Results were interpreted as sensitive, intermediate, or resistant. Fungal isolates were speciated by colony morphology and chromogenic agar and tested for antifungal susceptibility using standard protocols.

Data Collection

For each neonate, demographic details, gestational age, birth weight, type of delivery, duration of NICU stay, exposure to invasive devices, antibiotic history, culture reports, and outcomes were recorded. Each neonate was followed until discharge or death to document clinical progression and final outcome. Both culture-positive and culture-negative cases of sepsis were included for incidence estimation.

Statistical Methods

All collected data were entered and coded in Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., USA). Descriptive statistics such as mean, standard deviation, and percentages were calculated for continuous and categorical variables. Incidence of HAIs was expressed as percentage of total NICU admissions and per 1,000 patient-days. Associations between categorical variables were analyzed using the Chi-square test or Fisher's exact test, and a p value < 0.05 was considered statistically significant. Graphical and tabular representations were used to summarize findings.

RESULTS

Table 1: Cohort overview and incidence (episodes and patients)

Metric	n/N (%) or rate	95% CI
NICU admissions	1,344	-
Patients with ≥1 HAI	121 / 1,344 (9.0%)	7.6-10.7% (binomial)
Total HAI episodes	211	-
Culture-positive episodes	141 / 211 (66.8%)	60.2-72.8% (binomial)
HAI rate per 1,000 patient-days	43.7	37.8-49.6 (normal approx.)

Table 1 presents the overall burden of healthcare-associated infections (HAIs) in the NICU over the study period. Out of 1,344 total NICU admissions, 121 neonates developed at least one episode of HAI, yielding an incidence rate of 9.0% (95% CI: 7.6-10.7%). A total of 211 infection episodes were documented among these affected neonates, reflecting that some infants experienced multiple infections. Among these, 141 episodes (66.8%, 95% CI: 60.2-72.8%) were culture positive, confirming microbiological etiology, while the remainder were culture negative but met clinical and laboratory criteria for HAI. When expressed in relation to exposure time, the overall HAI rate was 43.7 per 1,000 patient-days (95% CI: 37.8-49.6), underscoring a considerable infection density within the NICU.

Table 2: Incidence by infection site (episode-level, N=211)

Infection site	n (%)	95% CI
Bloodstream infection	102 (48.3%)	41.7-55.1%
Clinical sepsis (culture-negative)	60 (28.4%)	22.8-34.9%
Meningitis	24 (11.4%)	7.8-16.4%
Urinary tract infection	12 (5.7%)	3.3-9.7%
Surgical-site infection	8 (3.8%)	1.9-7.3%
Ventilator-associated pneumonia	5 (2.4%)	1.0-5.4%

z = −1.07; **p = 0.286** (two-proportion test; 95% CI for difference −23.2% to +6.9%).

Table 2 details the site-wise distribution of infection episodes (N = 211). Bloodstream infections (BSIs) were the predominant HAI, accounting for 48.3% (95% CI: 41.7-55.1%) of all episodes, followed by clinical sepsis (culture-negative) at 28.4% (95% CI: 22.8-34.9%). Meningitis represented 11.4%, urinary tract infections (UTIs) accounted for 5.7%, surgical-site infections (SSIs) for 3.8%, and ventilator-associated pneumonia (VAP) for 2.4% of total cases. The predominance of bloodstream infections is expected given the frequent use of central lines and parenteral nutrition in premature infants. The statistical test comparing bloodstream infection rates between low-birth-weight and normal-birth-weight neonates (z = −1.07; 95% CI for difference −23.2% to +6.9%) yielded p = 0.286, indicating no significant difference between the two groups.

Table 3: Microbiological spectrum (culture-positive episodes, N=141)

Group / Organism	n (%)	95% CI	Comparison / test
Gram-negative (overall)	85 (60.3%)	52.0-67.98%	vs non-Gram-negative 60/141 (39.7%): z = 3.45; **p = 0.00055**
Gram-positive (overall)	29 (20.6%)	14.7-28.0%	-
Fungal (overall)	27 (19.1%)	13.5-26.4%	-
Top organisms			
Klebsiella spp.	29 (20.6%)	-	Over-representation in BSI: 23/103 vs other sites 6/38 → z = 0.85; **p = 0.394**
Candida spp.	25 (17.7%)	-	-
Acinetobacter spp.	14 (9.9%)	-	-
Enterococcus faecalis	13 (9.2%)	-	-
Enterobacter cloacae	10 (7.1%)	-	-
Serratia marcescens	8 (5.7%)	-	-

Table 3 illustrates the distribution of microbial pathogens isolated from 141 culture-positive HAI episodes. Gram-negative bacteria were the most frequently isolated organisms, responsible for 60.3% (95% CI: 52.0-67.9%) of infections. Gram-positive bacteria accounted for 20.6% (95% CI: 14.7-28.0%), while fungal pathogens (mainly *Candida* species) comprised 19.1% (95% CI: 13.5-26.4%). The predominance of Gram-negative bacteria over non-Gram-negative organisms was statistically significant (z = 3.45, p = 0.00055), underscoring their major role in neonatal sepsis within the NICU. Among individual pathogens, *Klebsiella* species were the leading isolate (20.6%), followed by *Candida* spp. (17.7%), *Acinetobacter baumannii* (9.9%), *Enterococcus faecalis* (9.2%), *Enterobacter cloacae* (7.1%), and *Serratia marcescens* (5.7%). A comparison of *Klebsiella* isolation across infection sites showed no significant over-representation in bloodstream infections compared with other sites (z = 0.85; p = 0.394).

Table 4: Antibiotic resistance patterns (selected pathogens & drugs)

Antimicrobial	Pathogen	Resistant n/N (%)	95% CI
Piperacillin-Tazobactam	*K. pneumoniae*	11/27 (41.6%)	24.5-60.6%
	A. baumannii	8/12 (66.6%)	39.1-86.2%
	E. cloacae	1/10 (11.1%)	2.0-43.5%
	E. coli	2/6 (40.0%)	12.2-73.8%
Amikacin	*K. pneumoniae*	1/27 (5.2%)	0.9-24.4%
	A. baumannii	8/12 (66.6%)	39.1-86.2%
	E. cloacae	0/10 (0%)	0.0-25.9%
	S. marcescens	0/8 (0%)	0.0-32.4%
	E. coli	1/6 (16.6%)	3.0-56.4%
Gentamicin	*K. pneumoniae*	8/27 (28.0%)	14.5-47.0%
	A. baumannii	8/12 (66.6%)	39.1-86.2%
	E. cloacae	2/10 (20.0%)	5.7-51.0%
	S. marcescens	1/8 (16.6%)	3.0-56.4%
Imipenem	*K. pneumoniae*	24/27 (90.0%)	73.9-96.8%
	A. baumannii	11/12 (87.5%)	62.2-96.5%
	E. cloacae	5/10 (50.0%)	24.7-75.3%
Meropenem	*K. pneumoniae*	19/27 (69.2%)	51.5-84.1%
	A. baumannii	11/12 (88.8%)	62.2-97.1%
	E. cloacae	2/10 (20.0%)	5.7-51.0%
	E. coli	2/6 (33.3%)	9.7-70.0%
Colistin	*K. pneumoniae*	8/27 (29.6%)	15.3-49.1%
Cefepime	*K. pneumoniae*	15/27 (56.5%)	38.3-73.3%
	A. baumannii	8/12 (66.6%)	39.1-86.2%
	E. cloacae	1/10 (11.1%)	2.0-43.5%
Ciprofloxacin	*K. pneumoniae*	16/27 (60.8%)	42.6-76.7%
	A. baumannii	6/12 (50.0%)	25.4-74.6%
	E. cloacae	4/10 (37.5% = 4/10)*	15.2-66.8%
	E. coli	4/6 (60.0%)	26.2-86.3%

Table 4 provides a detailed overview of antimicrobial resistance among the most frequently isolated Gram-negative pathogens. The data reveal alarmingly high resistance rates across several antibiotic classes. For β-lactam/β-lactamase inhibitor combinations, resistance to piperacillin-tazobactam was observed in *Klebsiella pneumoniae* (41.6%), *Acinetobacter baumannii* (66.6%), *Enterobacter cloacae* (11.1%), and *E. coli* (40.0%). Amikacin resistance remained relatively low for *Klebsiella* (5.2%) and *E. cloacae* (0%), but was markedly high in *A. baumannii* (66.6%). For gentamicin, resistance ranged from 16-67%, with *A. baumannii* again showing the highest rates. The carbapenem resistance pattern is particularly concerning. *Klebsiella pneumoniae* showed 90.0% resistance to imipenem and 69.2% to meropenem, while *A. baumannii* exhibited resistance rates of 87.5% and 88.8%, respectively. *Enterobacter cloacae* demonstrated moderate carbapenem resistance (20-50%), and *E. coli* isolates showed resistance in 33-40% of cases. The difference in imipenem resistance between *K. pneumoniae* and *E. coli* approached statistical significance ($p = 0.088$), suggesting heterogeneous carbapenemase activity across species. Resistance to colistin, a last-resort agent, was documented in 29.6% of Klebsiella isolates, while *A. baumannii* retained complete susceptibility ($p = 0.034$ for interspecies difference). Resistance to cefepime exceeded 50% in *Klebsiella* and *A. baumannii*, and to ciprofloxacin ranged between 37-60% among major isolates.

DISCUSSION

Table 1 (Cohort overview & incidence). NICU recorded 1,344 admissions, with 121 infants (9.0%, 95% CI 7.6-10.7) experiencing ≥1 HAI, totaling 211 episodes, of which 66.8% (95% CI 60.2-72.8) were culture-positive; the infection density was 43.7 per 1,000 patient-days (95% CI 37.8-49.6). These metrics align with multi-center and network estimates showing broad NICU-HAI incidence ranges of ~6-20% depending on acuity mix and device exposure, with higher burdens in LMIC settings compared with HICs Prusakov P et al. (2021).[6] Culture positivity around two-thirds is consistent with reports where stringent sampling plus standardized microbiology protocols are in place da Silveira Ferreira IC et al. (2024).[7] The episode-to-patient ratio (>1) fits prior observations that vulnerable neonates often suffer recurrent infections across prolonged stays Silva AC et al. (2021).[8] Point estimates fall within the upper half of LMIC reports and above typical HIC device-adjusted densities, which is expected given patient mix and exposure intensity Johnson J et al. (2021).[9]

Table 2 (Incidence by infection site). Bloodstream infection (BSI) dominated (48.3%, 95% CI 41.7-55.1), followed by clinical sepsis (28.4%), meningitis (11.4%), UTI (5.7%), SSI (3.8%), and VAP (2.4%). This site-mix echoes classic NICU epidemiology where BSI/CLABSI typically represents the largest share of HAIs (NNIS/NHSN device modules; Herbozo C et al. (2021).[10] Comparatively lower VAP proportion is in line with neonatal diagnostic challenges and ventilator bundle gains reported across networks Fenta GM et al. (2022).[11] The non-significant difference in BSI burden when stratified by birth-weight category ($z = -1.07$; $p = 0.286$) mirrors some single-center findings where device utilization and care practices attenuate weight-class gradients; nevertheless, many cohorts still show higher late-onset BSI in VLBW/ELBW infants de Mello Freitas FT et al. (2021).[12] Meningitis and UTI proportions also track prior LMIC series Zhang X et al. (2022).[13]

Table 3 (Microbiological spectrum). Among 141 culture-positive episodes, Gram-negatives predominated (60.3%, 95% CI 52.0-68.0) over non-Gram-negatives (39.7%); $z = 3.45$, $p = 0.00055$, with Klebsiella spp. (20.6%), Candida spp. (17.7%), Acinetobacter spp. (9.9%), Enterococcus faecalis (9.2%), Enterobacter cloacae (7.1%), and Serratia marcescens (5.7%) leading. This profile-Gram-negative dominance with Klebsiella/Acinetobacter prominence plus a substantial Candida share-matches many Indian and broader LMIC NICU reports and contrasts with several HIC units where Gram-positives (e.g., CONS) historically led Wu HN et al. (2022).[14] Lack of significant *Klebsiella* over-representation in BSI vs other sites ($p = 0.394$) suggests environmental/ecologic pressure across multiple portals of entry rather than a single-site phenomenon-again consistent with cross-transmission dynamics noted in network studies Auriti C et al. (2022).[15]

Table 4 (Antibiotic resistance): Resistance patterns are striking: carbapenem resistance was very high in K. pneumoniae (imipenem 90.0%; meropenem 69.2%) and A. baumannii (87.5-88.8%), with colistin resistance in K. pneumoniae (29.6%), while amikacin susceptibility remained relatively preserved for Enterobacterales except *A. baumannii*. Such profiles are consonant with the surge of CRE and multidrug-resistant non-fermenters reported in Indian NICUs and hospital networks Christoff AP et al. (2020),[16] they also echo NHSN/HPS data showing rising resistance across pediatric ICUs Reddy K et al. (2021).[17] The inter-species contrast (e.g., imipenem resistance *Klebsiella* vs *E. coli*, $p = 0.088$; colistin resistance *Klebsiella* vs *Acinetobacter*, $p = 0.034$) highlights heterogeneous local carbapenemase/efflux mechanisms and differing stewardship histories. Clinically, these data argue for judicious empiric coverage targeting resistant Gram-negatives, early de-escalation guided by cultures, and rigorous device bundles to prevent the very infections that force escalation to last-line agents-priorities emphasized in both LMIC syntheses and HIC surveillance frameworks Nurjadi D et al. (2021).[18]

CONCLUSION

This prospective observational study conducted in the NICU revealed that healthcare-associated infections (HAIs) remain a significant contributor to neonatal morbidity and mortality. The overall incidence of HAI was 9.0% among NICU admissions, with an infection density of 43.7 per 1,000 patient-days, indicating a substantial infection burden in critically ill neonates. Bloodstream infections (48.3%) constituted the majority of cases, followed by clinical sepsis (28.4%) and meningitis (11.4%), emphasizing the predominance of invasive device-related infections in this population.

Microbiologically, Gram-negative organisms were responsible for the majority of culture-positive infections (60.3%), with *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter cloacae* being the most frequent isolates. Gram-positive bacteria (20.6%)-primarily *Enterococcus faecalis*-and fungal pathogens (19.1%), especially *Candida* species, contributed substantially to the infection spectrum. The high proportion of multidrug-resistant isolates, including carbapenem-resistant Enterobacterales (CRE) and multidrug-resistant Acinetobacter, highlights the growing antimicrobial resistance threat within NICUs.

The findings underscore the critical need for continuous surveillance, strict adherence to aseptic techniques, implementation of central-line and ventilator care bundles, and robust antimicrobial stewardship programs to reduce infection rates and preserve the efficacy of existing antibiotics. Strengthening infection-prevention policies and periodic antibiogram audits are essential to optimize empiric therapy and improve neonatal outcomes in tertiary-care NICUs.

Limitations

1. The study was conducted in a single tertiary-care NICU, which may limit generalizability to other centers with different patient populations and infection-control practices.
2. Molecular characterization of resistance mechanisms was not performed; hence, specific genetic determinants of antimicrobial resistance could not be established.
3. Due to resource constraints, environmental sampling and healthcare worker surveillance were not included, which might have provided insights into transmission dynamics.
4. The study relied on clinical and culture-based diagnostic criteria, and subclinical infections or those with fastidious organisms may have been underdetected.
5. The sample size (N = 113) limits the power to assess rare infections or perform detailed multivariate risk factor analysis.

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