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Bacterial Contamination in Neonatal Intensive Care Unit: A Potential Threat of Nosocomial Infections to Neonates

Sia Temu[a], Petro Daniel[a], Amiri Athuman[a], Frank Shemhande[a], Mtebe Majigo[b]

[a]St. Benedict Ndanda Referral Hospital, Mtwara, Tanzania [b]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania.
Correspondence to Sia Temu (temusia26@gmail.com)

ABSTRACT

Background: Bacterial contamination in healthcare settings, especially neonatal intensive care units, plays a key role in the spread of nosocomial infections. However, there is limited data on the routine monitoring of contamination on surfaces and instruments in direct contact with neonates, as well as their antimicrobial susceptibility patterns in our setting. Objective: The study determined the level of bacterial contamination on instruments and surfaces frequently touched or in contact with neonates, and their antimicrobial susceptibility patterns.

Methods: A cross-sectional study was conducted in the neonatal Intensive Care Unit (NICU) at St. Benedict Ndanda Referral Hospital (SBNRH) in Mtwara, Tanzania, over two days in November 2023. Swab samples were collected from surfaces and instruments that are frequently touched or in contact with neonates. Bacterial isolation, identification, and antimicrobial susceptibility testing were conducted according to the Clinical and Laboratory Standards Institute (CLSI) guideline. Multidrug resistance (MDR) was defined as resistance to at least one antibiotic from three or more different classes. Data analysis was conducted using STATA software version 15, with descriptive statistics presented as frequencies and percentages.

Results: Of 57 swab samples, 37 (64.9%) showed bacterial growth, yielding 43 isolates. The majority, 30(69.8%), were gram-negative bacteria. The predominant isolates were coagulasenegative ***Staphylococci*** species, accounting for 8 (18.6%), followed by ***Escherichia coli*** and ***Klebsiella pneumoniae***, each at 7 (16.3%). The most contaminated areas were nurse stations 2(100.0%), wall sanitizer dispenser 2(100.0%), weighing scale 1(100.0%), neonatal beds 16(88.9%) and door handles 6(85.7%). ***Enterobacterales*** were highly resistant to cefotaxime 18(85.7%), ceftriaxone 17 (73.7%) and gentamicin 15(71.4%). Acinetobacter baumanii was resistant to piperacillin 5(100.0%), piperacillin-tazobactam 5(100.0%) and cefotaxime 5(100.0%). Pseudomonas aeruginosa were highly resistant to piperacillin 3(100.0%) and piperacillin-tazobactam 3(100.0%). Most of the gram-negative bacteria were susceptible to meropenem 25(83.3%). ***Staphylococcus aureus*** showed resistance to erythromycin 5(100.0%) and tetracycline 4(80.0%). Methicillin resistant ***Staphylococcus aureus*** (MRSA) was observed in 4(80.0%) isolated ***Staphylococcus aureus***. Multi-drug resistance (MDR), extended-spectrum beta-lactamase (ESBL) production, and carbapenemase production were observed in 29 (82.9%), 3 (23.1%) and 5 (16.7%) respectively.

Conclusion: The instruments and surfaces in the NICU were contaminated with high-risk pathogens, many of which showed significant resistance to commonly used antibiotics. These findings highlight the urgent need to strengthen infection prevention and control measures and antibiotic stewardship to reduce bacterial colonization and transmission to neonates.

BACKGROUND

Healthcare-associated infections (HAIs) are acquired while receiving healthcare services.[1–4] It is reported that in acute-care facilities, out of 100 patients, 7 and 15 in high-income and lowmiddle-income countries, respectively, acquire at least one HAI during their hospital stay and one in every ten affected patients dies from HAI.[5] Globally, the rate of HAI is reported to be increasing by 0.06% annually, with neonatal wards and intensive care units (ICU) having the highest rates.[2] The prevalence of HAI in neonatal Intensive Care Unit (NICU) is reported to vary from 7.0% to 53.6% in Africa.[6] Healthcare-associated infections for neonates is responsible for increased neonatal mortality and prolonged hospital stay. [2,5] The risk of HAI in neonates is due to immature immune systems, exposure to risky invasive procedures, and frequent exposure to healthcare staff and parents.[7,8]

Contaminated hospital environments and healthcare workers are recognized reservoirs and sources of HAI-related pathogens.[3,7,9,10] The rate of environmental contamination in hospitals ranges from 30% to 59.2%.[11] Patients may shed microorganisms that can survive in the healthcare environment and be detected in the air, water, and surfaces.[3] The number and types of microorganisms present in the environment are influenced by different factors, including the number

of people in that environment, degree of activity, amount of moisture, presence of material capable of supporting microbial growth, the rate at which organisms suspended in air are removed, and the type of surface and its orientation.[3]

Several pathogens have been linked to contamination in the ICU. [5,7,12–16] These pathogens can persist in the environment for hours, days, or even months; for instance, Gram-positive bacteria such as *Staphylococcus aureus* typically survives on dust and surfaces in dry conditions, while Gram-negative bacteria such as *Escherichia coli*, *Klebsiella species*, *Pseudomonas species* and *Acinetobacter species* thrive and endure in moist, soiled environments.[3] The bacteria in the hospital environment can harbor multidrug-resistant (MDR) genes, conferring a broad spectrum of antimicrobial resistance.[9,17] The transmission of MDR pathogens in healthcare settings presents a significant challenge for treatment.[18] The World Health Organization (WHO) classifies carbapenem-resistant *Acinetobacter*, third-generation cephalosporin-resistant enterobacterales, and carbapenem-resistant enterobacterales as high-priority pathogens due to limited treatment options and a high disease burden, including mortality and morbidity.[19]

Neonatal sepsis continues to be a major cause of morbidity and mortality in healthcare settings. Although environmental contamination is a known source of nosocomial pathogens, routine monitoring of bacterial contamination on high-touch surfaces and instruments in the NICU remains limited. Given the critical importance of maintaining a sterile environment in neonatal care, assessing bacterial contamination is vital to enhancing Infection Prevention and Control (IPC) measures and reducing HAIs. This study aimed to assess bacterial contamination on frequently touched surfaces and instruments and the antimicrobial susceptibility patterns of the isolated pathogens.

METHODOLOGY

Study Design and Setting

A cross-sectional study was conducted in the NICU at St. Benedict Ndanda Referral Hospital (SBNRH) in Mtwara, Tanzania, over a two-day period in November 2023. The hospital is a secondary-level healthcare facility with a 370-bed capacity, offering both inpatient and outpatient services. It serves as a regional referral center and provides specialized neonatal intensive care for premature infants, low birth-weight neonates, and those with a range of neonatal complications. The NICU is well-equipped to care for up to 25 neonates. The study involved sampling the surfaces and instruments that are frequently touched or come into direct or indirect contact with neonates within NICU.

Sampling Procedures

Sampling was performed using convenience sampling, where non-repetitive surface swabs were collected from predefined high-touch surfaces and instruments, including incubators, monitors, door handles, digital weighing machines, bedside lockers, nurse station counters, sink tap handles, syringe pumps, neonatal beds, trolleys, and wall sanitizer dispensers. Surfaces and devices outside the NICU, low-touch areas (those with minimal contact), and equipment directly involved in patient care (e.g., ventilators) during the study were excluded.

Sample Collection

A total of 57 samples were collected 1 to 2 hours after the routine daily morning cleaning over two consecutive days. Swabs were obtained 1 to 2 hours after daily morning routine cleaning using a sterile cotton swab pre-moistened with normal saline to sample an area of approximately 10 cm^2. Samples were placed in Stuart transport medium and transported to the laboratory within one hour to ensure sample integrity.

Isolation and Identification of Bacterial Isolates

Upon arrival at the laboratory, the samples were inoculated onto MacConkey agar (Liofilchem, Italy) and blood agar (Liofilchem, Italy) plates, and then incubated aerobically at 35 °C ± 2 °C for 18-24 hours. MacConkey agar was used as a selective and differential medium for isolating Gram-negative bacteria. The blood agar served as a general-purpose and differential medium, enabling the identification of beta, alpha, and gamma hemolytic bacteria. The isolates were identified using conventional microbiological methods, including colony morphology, microscopic examination, and a series of biochemical tests. For the identification of Gram-positive bacteria, catalase and coagulase tests were performed. For Gram-negative bacteria, a range of tests, including triple sugar iron, sulfur-indole-motility (SIM), oxidase, citrate, and urease, were utilized to determine their biochemical profiles.

Antimicrobial Susceptibility Testing

We performed antimicrobial susceptibility using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guideline.[20] Individual colonies were suspended in normal saline, and the turbidity was standardized to 0.5 McFarland standards. The suspension was inoculated on Muller-Hinton agar (Liofilchem, Italy). Antibiotic discs were dispensed manually, and the plates were incubated for 18-24 hours at 35°C ± 2 °C. Zones of inhibition were measured using a ruler and interpreted as susceptible, resistant, or intermediate 20.

The antibiotic discs used for Gram-positive bacteria were as follows: ciprofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), gentamicin (10µg), clindamycin (2µg), cefoxitin (30µg) and erythromycin (15µg) (Liofilchem, Italy). The antibiotics used for Gram-negative bacteria (*Enterobacterales* and *Acinetobacter* species) included ciprofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), gentamicin (10µg), meropenem (10µg), amoxicillin-clavulanic acid (30µg), ceftriaxone (30µg), and ceftazidime (30µg). For *Pseudomonas* species, we used ciprofloxacin (5µg), gentamicin (10µg), meropenem (10µg), and ceftazidime (30µg). We identified methicillin-resistant *Staphylococcus aureus* (MRSA) using a cefoxitin (30µg) disc, in which a zone inhibition of equal or less than 21mm diameter was considered MRSA.

Extended-Spectrum β-Lactamases Production

Isolates found to be resistant or with decreased

susceptibility (intermediate) to any one of the third-generation cephalosporins, i.e., ceftazidime, cefotaxime, and ceftriaxone, were selected for the presence of Extended-Spectrum β-Lactamases (ESBL) production using the disk diffusion method according to the CLSI guideline.[20] Isolates were inoculated onto the Mueller-Hinton agar plates (Liofilchem, Italy). Ceftazidime (30µg) and cefotaxime disc (30µg) were placed on the plate, then Ceftazidime/clavulanic acid (30µg/10µg) and cefotaxime/clavulanate (30µg/10µg) discs were placed at a distance of 25mm, center to center. The plates were incubated at 35˚C for 16 to 18 hours. Isolates showing an increased zone of inhibition of ≥5 mm for either ceftazidime or cefotaxime tested in combination with ceftazidime–clavulanate or cefotaxime-clavulanate disc confirmed ESBL production.

Carbapenemase Production

We determined Carbapenemase production in Enterobacterales and *Pseudomonas aeruginosa* using Modified Carbapenemase Inactivation Methods (mCIM)[20]. Briefly, 1-µL loopful of each isolate was emulsified in 2 mLs of tryptic soy broth (Liofilchem, Italy) and then vortexed.

Meropenem disk (10µg) was added to each tube using sterile forceps and incubated at 35°C ± 2°C for four hours ±15 min. A lawn of meropenem-susceptible standard *Escherichia coli* (ATCC-25922) suspension equivalent to 0.5 McFarland standard was prepared. The standard *Escherichia coli* suspension was inoculated onto a Muller-Hinton agar (Liofilchem, Italy) plate, and then a meropenem (10 µg) disk was added from the suspension. The plates were incubated at 35°C ± 2°C for 18 to 24 hours. A zone diameter of 6 to 15 mm or pinpoint colonies within a 16 to 18 mm zone were considered carbapenemase-positive, and negative if a clear zone diameter of ≥19 mm was observed.

Quality Control

The microbiology laboratory adheres to quality control protocols guided by specific internal standard operating procedures to enhance the quality of specimen processing and storage. We used American Type Culture Collection (ATCC) reference microorganisms to control the performance of the culture media. *Staphylococcus aureus*, ATCC 25923, was used for quality control tests, including catalase and coagulase. A non-ESBL-producing organism (*Escherichia coli* ATCC 25922) and an ESBL-producing organism (*Klebsiella pneumoniae* ATCC 700603) were used for quality control.

Data Management and Statistical Analysis

Data were entered into Microsoft Excel and analyzed using STATA software version 15 (StataCorp, Texas, United States). Descriptive analysis was summarized as frequency and proportion for categorical variables. All intermediate antimimicrobial susceptibility testing results were categorized as resistant during analysis. Multidrug resistance (MDR) was defined as resistance to at least one antibiotic in three or more categories or groups of antibiotics.

Ethical Consideration

The St. Benedict Ndanda Referral Hospital management approved collecting environmental samples and analyzing the data with Reference number SBNRH/V.1/706/1. Permission was also obtained from the in-charge of the neonatal intensive care unit.

RESULTS

A total of 57 swab samples were collected from various sites. Bacterial growth was observed in 37 (64.9%) samples, yielding 43 isolates. Thirty-one (83.8%) samples contained single bacterial isolates, while 6 (16.2%) contained multiple bacterial isolates.

Distribution of Isolated Bacteria

Of 43 bacterial isolates, 30(69.8%) were Gram-negative bacteria, predominately *Escherichia coli*, 7(16.3%) and *Klebsiella pneumoniae*, 7(16.3%) followed by *Acinetobacter* species 5(11.6%), *Pseudomonas aeruginosa* 4(9.3%) and *Serratia marcescens* 3(7.0%) (Table 1). The majority of the Gram-positive bacteria were coagulase-negative staphylococci (CoNS) 8(18.6%), followed by *Staphylococcus aureus* 5(11.6%) (Table 1).

TABLE 1: Distribution of Bacteria from Neonatal Intensive Care Unit Environment (N=43)

Identified isolates	Number	Percentage (%)
Gram Negative	30	69.8
Escherichia coli	7	16.3
Klebsiella pneumoniae	7	16.3
Acinetobacter baumanii	5	11.6
Pseudomonas aeruginosa	4	9.3
Serratia marcescens	3	7.0
Enterobacter aerogenes	2	4.7
Citrobacter freundii	1	2.3
Enterobacter cloacae	1	2.3
Gram Positive	13	30.2
CoNS	8	18.6
Staphylococcus aureus	5	11.6

Of the sampled items, bacterial contamination was detected on most instruments and surfaces except radiant warmers and phototherapy beds. The nurse station counter 2(100.0%), wall sanitizer dispensers 2(100.0%), Weighing Scale 1(100.0%), Neonatal Beds 16(88.9%), Door Handles 6(85.7%), Incubators 2(66.7%), and bedside lockers 2(66.7%) were frequently contaminated (Table 2).

Antimicrobial Susceptibility Pattern

Most of the Enterobacterales showed high resistance to cefotaxime 18(85.7%), ceftriaxone 17(80.9%), tetracycline 14(73.7%), gentamicin 15(71.4%) and amoxicillin/clavulanic acid 14(66.7%) while susceptible to meropenem 18(85.7%). *Acinetobacter baumanii* were highly resistant to piperacillin 5(100.0%), piperacillin-tozobactam 5(100.0%), cefotaxime 5(100.0%), ceftriaxo-

ne 3(60.0%), trimethoprim-sulfamethoxazole 3(60.0%) and tetracycline 2(50.0%). However, susceptible to meropenem 4(80.0%), ciprofloxacin 4(80.0%) and moderately to gentamicin 3(60.0%). Moreover, *Pseudomonas aeruginosa* was highly resistant to piperacillin 3(100.0%), piperacillin-tozobactam 3(100.0%), gentamicin 3(75.0%) and ciprofloxacin 3(75.0%) and susceptible to meropenem 3(75.0%) (Table 3).

All *Staphylococcus aureus* isolates showed high resistance to erythromycin 5(100.0%), tetracycline 4(80.0%), clindamycin 3(60.0%), chloramphenicol 3(60.0%) and ciprofloxacin 3(60.0%). Of all *Staphylococcus aureus* tested for Methicillin-resistant *Staphylococcus aureus* (MRSA), 4(80%) were positive (Table 3). Overall, 29(82.9%) of all bacterial isolates were resistant to at least one antibiotic in three or more categories (multi-drug resistance) (Table 4).

Extended-Spectrum Beta-Lactamase and Carbapenemase Production

Of the thirteen Gram-negative isolates tested for ESBL production, 3(23.1%) were ESBL producers, with *Klebsiella pneumoniae* accounting for 2(28.6%) of these (Table 4).

Carbapenemase-producing Gram-negative bacteria were detected in 5(16.7%) bacterial isolates. A high proportion was observed in *Escherichia coli* 2(28.6%) (Table 4).

TABLE 4: Multidrug-Resistant, ESBL, and Carbapenemase Producing Bacteria from NICU Environment

Bacterial Isolates	Total N	Multidrug-resistance (>R3) n (%)	ESBL producer n (%)	Carbapenemase producer n (%)
Escherichia coli	7	6(85.7)	1(14.3)	2(28.6)
Klebsiella pneumoniae	7	5(71.4)	2(28.6)	0(0)
Acinetobacter baumanii	5	5(100)	NA	1(20)
Staphylococcus aureus	5	5(100)	NA	NA
Pseudomonas aeruginosa	4	4(100)	NA	1(25)
Serratia marcescens	3	3(100)	NA	0(0)
Enterobacter Species	3	1(33.3)	NA	1(33.3)
Citrobacter freundii	1	0(0)	NA	0(0)
Total		**29(82.9)**	**3(23.1)**	**5(16.7)**

NA- Not applicable

TABLE 2: Distribution of Isolates from Sampled Areas

Sampled Areas	Number of samples	CoNS	*S. aureus*	*E. cloacae*	*S. marcences*	*P. aeruginosa*	*E. aerogenes*	*E. coli*	*K. pneumoniae*	*A. baumanii*	*C. freundii*	n(%)
Bed side locker	03	0	2	0	0	0	0	0	0	0	0	2(66.7)
Digital Weighing scale	01	1	0	0	0	0	0	0	0	0	0	1(100.0)
Monitor	04	1	0	1	0	0	0	0	0	0	0	1(25.0)
Nurse station counter	02	2	0	0	0	0	0	0	0	0	0	2(100.0)
Incubator	03	0	1	0	0	0	0	1	1	0	0	2(66.7)
Syringe pump machine	05	0	1	0	1	0	0	0	0	0	0	2(40.0)
Wall sanitizer dispenser	02	1	0	0	0	0	0	1	0	0	0	2(100.0)
Door handles	07	2	0	0	0	1	0	0	2	2	0	6(85.7)
Neonatal Beds	18	1	1	0	1	1	1	5	4	3	1	16(88.9)
Sink tape handles	05	0	0	0	0	2	1	0	0	0	0	2(40.0)
Trolleys	02	0	0	0	1	0	0	0	0	0	0	1(50.0)
Phototherapy beds	02	0	0	0	0	0	0	0	0	0	0	0(0)
Radiant warmers	03	0	0	0	0	0	0	0	0	0	0	0(0)
Overall	**57**											**37(64.9)**

CoNS-Coagulase Negative *Staphylococcus aureus, P. aeruginosa- Pseudomonas aeruginosa, E. cloacae- Enterobacter cloacae, S. marcences -Serratia marcences, E.aerogenes -Enterobacter aerogenes, E. coli Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, A. baumanii -Acinetobacter baumanii, Citrobacter freundii*

TABLE 3: Antibiotic Resistance Pattern of Bacterial Isolates from NICU Environment

Bacterial Isolates	N	Antibiotic Resistance N (%)														
		FOX n(%)	E n(%)	CD n(%)	AUG n(%)	CRO n(%)	C n(%)	MRP n(%)	TE n(%)	CN n(%)	CIP n(%)	COT n(%)	AZM n(%)	PIT n(%)	PIP n(%)	CTX n(%)
Escherichia coli	7	NA	NA	NA	6(85.7)	6(85.7)	3(42.9)	2(28.6)	5(83.3)	6(87.7)	3(42.9)	4(57.1)	0	NA	NA	6(85.7)
Klebsiella pneumoniae	7	NA	NA	NA	4(66.7)	5(83.3)	3(50.0)	0	5(83.3)	5(83.3)	3(60.0)	2(50.0)	2(33.3)	NA	NA	5(83.3)
Acinetobacter baumanii	5	NA	NA	NA	NA	3(75)	NA	1(20.0)	2(50.0)	2(40.0)	1(20.0)	3(60.0)	NA	5(100.0)	5(100.0)	3(60..0)
Staphylococcus aureus	5	4(80.0)	5(100.0)	3(60.0)	NA	NA	3(60.0)	NA	4(80.0)	NA	3(60.0)	NA	NA	NA	NA	NA
Pseudomonas aeruginosa	4	NA	NA	NA	NA	NA	NA	1(25.0)	NA	3(75.0)	3(75.0)	NA	NA	4(100.0)	4(100.0)	NA
Serratia marcences	3	NA	NA	NA	3(100.0)	3(100.0)	0	NA	3(100.0)	NA	NA	1(33.3)	1(33.3)	NA	NA	3(100.0)
Enterobacter species	3	NA	NA	NA	0	2(66.7)	0	0	1(50.0)	NA	1(33.3)	0	0	NA	NA	3(100.0)
Citrobacter freundii	1	NA	NA	NA	1(100.0)	0	0	0	1(100.0)	0	0	0	0	0	0	1(100.0)
Overall	35	4(80.0)	5(100.0)	3(60.0)	14(66.7)	19(73.1)	9(34.6)	4(14.8)	21(65.6)	16(66.7)	14(43.8)	10(38.5)	3(14.3)	9(90)	9(90)	21(80.7)

NA-not applicable, FOX-cefoxitin, E-erythromycin, CD-clindamycin, AUG-amoxicillin/clavulanic acid, CRO-ceftriaxone, MRP-meropenem, CN-Gentamicin, CIP-ciprofloxacin, COT-trimethoprim-sulfamethoxazole, PIP-piperacillin, CTX-cefotaxime, C-chloramphenicol, TE-tetracycline, AZM-azithromycin, PIT-piperacillin tazobactam.

DISCUSSION

The study assessed bacterial contamination in NICU and evaluated the antimicrobial susceptibility patterns of commonly used antibiotics. High levels of bacterial contamination on frequently touched objects and instrument surfaces within the NICU was observed. The predominant bacterial isolates were Coagulase Negative *Staphylococcus* (CoNS), *Escherichia coli* and *Klebsiella pneumoniae*. The most commonly contaminated surfaces included weighing scales, nurse station counters, wall sanitizer dispensers, neonatal beds, door handles, incubators, and bedside lockers. Majority of the bacteria showed high resistance to commonly used antibiotics. On the other hand, we observed high susceptibility of pathogens to meropenem. Furthermore, MDR was reported in most of the isolates obtained.

Our study reveals a high level of bacterial contamination, indicating a potential risk of nosocomial infections to neonates. The finding is consistent with the study conducted in Nigeria.[13] However, this contradicts other studies conducted in South Africa, Tanzania, Kenya, and Ethiopia[12,21–23] which reported lower bacteral contamination. Moreover, other studies have reported higher bacterial contamination compared to the present study.[7,24,25] These discrepancies could be attributed to various factors, such as differences in sample size, sampled surfaces, disinfection practices, types of disinfectants used, overcrowding, hygiene practices, and infection prevention and control strategies.[3,21]

The present study identified neonatal beds, door handles, incubators, monitors, sinks, and bed lockers as the most contaminated surfaces with bacteria. This finding aligns with studies conducted in similar settings.[7,11] Interestingly, our study found that phototherapy beds and radiant warmers were not contaminated with bacteria, contrasting with another study in Kenya that reported bacterial contamination in radiant warmers.[23] This may be due to differences in infection control practices, environmental factors, and study methodologies. Additionally, the identified surfaces are high-touch areas in close proximity to neonates, healthcare workers, and visitors, and can act as potential reservoirs for nosocomial pathogens if infection prevention and control measures are not properly followed.[11]

In this study, the majority of bacterial isolates were Gram-negative, primarily *Escherichia coli* and *Klebsiella pneumoniae*, which aligns with findings from a study in Nepal.[7] However, this is contrary with a study in Libya, which found a higher prevalence of Gram-positive bacteria.[5] Most of the bacteria identified in this study can survive for extended periods in the environment and are widespread in hospitals, increasing the risk of infections such as neonatal septicemia, pneumonia, and meningitis, particularly in premature infants.[5] Moreover, most of the isolated pathogens are virulent, antibiotic-resistant, and capable of forming biofilms on dry surfaces, which enhances their survival and facilitates transmission through inadequate infection control, thereby posing a significant risk to neonates.[26,27] Thorough disinfection, the use of appropriate disinfectants, and the implementation of updated infection prevention and control practices are essential to minimize the spread of infections in neonatal intensive care units.

Coagulase-negative *Staphylococcus* (CoNS) was the predominant Gram-positive bacterium isolated in this study, a finding that aligns with previous studies conducted in similar healthcare settings.[17] A similar study in Nigeria reported *Staphylococcus aureus* as the predominant pathogen. [28] While CoNS are generally considered non-pathogenic, their ability to form biofilms on frequently touched surfaces presents a contamination risk[29] The study also found that eighty percent of the *Staphylococcus aureus* isolates were MRSA, which is concerning given that MRSA is linked to high morbidity and mortality, with prematurity being a major risk factor for colonization and subsequent infections.[30] Additionally, the ability of *Staphylococcus aureus* and MRSA to form biofilms on non-living surfaces enhances their survival, promotes spread, and helps them resist desiccation.[31] Although these bacteria are naturally present in the skin and hands, they can contaminate medical equipment and surfaces via direct contact, posing a risk of infection.[23] Thus, it is crucial to enforce strict hand hygiene practices among healthcare workers and visitors, particularly before and after patient contact, to mitigate the spread of these pathogens.

Most enterobacterales showed resistance to cefotaxime, ceftriaxone, tetracycline and gentamicin. Our finding is similar to the study conducted in Ethiopia, which reported high resistance to gentamicin, tetracycline.[25] Other similar studies have reported resistance to ampicillin and meropenem.[22] However, different bacterial isolates had high resistance patterns to different antibiotics. For instance, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* were resistant to piperacillin and piperacillin-tozobactam, while other studies reported resistance to aztreonam, trimethoprimsulfamethoxazole, and ceftriaxone.[11] On the other hand, most of the bacteria in this study were sensitive to meropenem, consistent to the study in Kenya.[23] In the present study, Gram-positive bacteria were highly resistant to erythromycin. Similar resistance patterns were found in the study done in Ethiopia.[25] The variation in resistance patterns may result from the selective pressure caused by the frequent use of antibiotics, geographic variations and hospital environmental conditions.[23,25]

Resistance to at least one antibiotic in three or more antibiotic categories (multi-drug resistance) was highly observed in more than 80% of the bacterial isolates. This finding is consistent with results from similar studies, highlighting the widespread occurrence of MDR in clinical settings.[7] The high prevalence of MDR bacterial pathogens may be associated with inappropriate administration of antimicrobials, or variations in hospital environmental conditions, as well as the administration of prophylactic antibiotics to high-risk neonates.[5,7] This finding is particularly concerning, as the spread of MDR strains within the hospital environment can lead to severe infections, thereby exacerbating morbidity and mortality rates among neonates.[11,32]

The present study reports, sixteen percent of the isolates were identified as carbapenemase producers, and twenty-three percent as ESBL producers. These findings are consistent with those reported in similar

settings.[11,28] Patients colonized with β-lactam-resistant ESBL or carbapenemase-producing bacteria can serve as a critical source for the further spread of these pathogens within healthcare settings.[33,34] The increasing resistance of Gram-negative bacteria to beta-lactam antibiotics, such as cephalosporin's and carbapenems, is particularly concerning, as these are the drugs of choice for treating severe infections caused by many Gram-negative pathogens.[34] The spread of carbapenem-resistant bacteria in hospitals is concerning, emphasizing the need for strict hygiene monitoring, safe waste disposal, and hand hygiene for healthcare workers, patients, and visitors around neonates.

Study Limitations

The limitation of this study is the small sample size, which may lead to an overestimation of antimicrobial resistance patterns. However, the data will provide valuable preliminary insights into bacterial contamination in our setting and establish a foundation for future research. Also, the study was conducted in a secondary-level healthcare facility, which may limit the generalizability of its findings to other healthcare settings.

CONCLUSION

This study reveals significant bacterial contamination in NICU particularly on high-touch surfaces like neonatal beds, door handles and incubators. The predominant bacterial isolate were CoNS, *Escherichia coli* and *Klebsiella pneumoniae*, with high levels of MDR and resistance to common antibiotics such as erythromycin, cefotaxime, and gentamicin. However, meropenem remained effective against most pathogens. Additionally, there were high rates of carbapenemase and ESBL producers. These findings underscore the critical need for enhanced infection prevention control measures and antibiotic stewardship. The study contributes to the growing body of evidence on the persistence of resistant pathogens in health care settings and offers valuable insights for strengthening neonatal patient safety. Future research should focus on monitoring bacterial contamination trends and assessing the effectiveness of infection control measures.

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