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Prevalence and Antibiogram of MDR Pathogens Isolated from Inanimate Surfaces at Neonatal Intensive Care Unit in Khulna, Bangladesh

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Abstract

The environment in hospital, particularly the intensive care unit, is a foremost reservoir of nosocomial bacteria, including multidrug resistant (MDR) pathogens. Inanimate surfaces and objects are the crucial transmission vehicles for nosocomial bacteria. This study assesses the bacterial profile and antibiotic susceptibility patterns of the isolates found on the inanimate surfaces at neonate intensive care unit (NICU) wards in Khulna, Bangladesh. A hospital oriented, cross-sectional study was carried out with a total of 99 samples by swabbing method from the sink, door handle, bed rail, patient bed cover, table, scissor, telephone etc. which are collected from three different hospitals. Normal saline solution was used to moistened sterile cotton swabs. The isolates were characterized through bacterial culture methods, gram staining, and biochemical assays. The antimicrobial susceptibility of each isolate was evaluated using the Kirby-Bauer disk diffusion technique. Data analysis was performed using SPSS (ver.29) and GraphPad Prism (8.0.2). In this study, *Staphylococcus*, *Escherichia*, *Klebsiella* and *Pseudomonas* were the most predominant isolated bacteria, which accounted for 48.1%, 19.2%, 15.4% and 9.6% respectively. Sink, door handle, bed rail and patient bed cover are the most contaminated surfaces. Among the six isolates, 71.15% were multidrug resistant and of these, 48.0% were Gram-negative isolates. Out of 10 selected antibiotics, Colistin, Imipenem, Gentamicin and Ciprofloxacin were mostly sensitive against *Acinetobacter* and *Corynebacterium*. Although the prevention and control strategies for multidrug resistant isolates has been more challenging, but there is no alternate of implementing an active hospital infection prevention and surveillance scheme, along with systematic disinfection of surfaces to minimize bacterial colonization and the risk of infection transmission.

Keywords: Nosocomial infections, Antibiogram, Multidrug resistance

1. Introduction

A Neonatal Intensive Care Unit (NICU), also referred to as a Special Care Newborn Unit (SCANU), is dedicated to care for sick or premature newborns. It offers vital care for infants who need constant monitoring and medical intervention, an intermediate care section for those who are stable but still need specialized attention, and continued support for babies who are preparing to be discharged from the hospital [1]. The term "neonatal" refers to the first 28 days after birth. During this critical period, newborns are at the greatest risk of death, mainly due to infections, premature birth, or birth asphyxia [2].

Contaminated surfaces and patient care/ health care equipments in the NICU are a notable source of hospital-acquired infections. In developed countries, these infections impact around 5-10% of patients, while the risk is 2 to 20 times greater in developing nations. Their presence also leads to longer hospital stays [3].Worldwide, more than one million neonatal deaths are reported each year. Newborns in the ICUs are particularly susceptible to hospital-acquired infections triggered by contaminated objects and instruments[4]. Nosocomial infections, also known as healthcare-associated infections (HAIs), develop in patients while getting healthcare services in a hospital or other medical capacity. These infections were not present at the time of the patient's admission[5]. An infection is typically classified as a healthcare-associated infection (HAI) if it occurs 48 hours or more after admission or within 48 to 72 hours after discharge [6]. In NICUs, pathogens can be passed to infants through cross-transmission. This is influenced by factors such as high infant turnover, frequent visitors, poor hand hygiene, heavy workloads, insufficient staff training, ineffective disinfection practices, and surfaces that are difficult to clean [7]. Nurses serve as the main caregivers for infants in NICUs and infections can unintentionally be spread among neonates through health-care services [8]. The effects of healthcare-associated infections (HAIs) vary from minor discomfort to severe, life-threatening conditions, affecting patients' quality of life and extending their hospital stays. Moreover, HAIs place a burden on healthcare resources, increasing costs and posing risks not only to patients but also to their families through casual contact [9]. Antibiotic-resistant nosocomial infections are an emerging global issue, causing significant morbidity and mortality. Around 80% of these infections are classified as nosocomial pneumonia, nosocomial bloodstream infections, and nosocomial urinary tract infections. Neonates are especially vulnerable to nosocomial infections due to factors such as prematurity, severe illness, congenital defects, invasive monitoring, overuse of antibiotics etc. [10]. Hospital-acquired infections (HAIs) are also recognized globally as a significant public health issue, leading to potential disabilities [11].

Nosocomial infections are typically caused by bacteria, fungi, viruses, and parasites. Both gram-positive and gram-negative bacteria can survive for extended periods on dry, non-living surfaces in humid and hostile conditions. Up to 60% of surfaces in a patient's environment are believed to be contaminated with pathogens that cause healthcare-associated infections (HAIs). These pathogens can easily infect susceptible patients by contact with contaminated objects, such as bed rails, sink, table and door or through the use of patient-care equipment like stethoscopes and sphygmomanometers. Also the overuse and improper use of antibiotics aid to the increase in multidrug-resistant (MDR) bacteria in healthcare settings [12]. Now a day’s most nosocomial bacterial pathogens are evolving into superbugs, with new resistance issues emerging in hospitals.

In developing countries like Bangladesh, the high cost of advanced cleaning, disinfection, and sterilization methods makes it difficult to implement and this lead to contaminated patient-care equipments and inanimate objects becoming probable sources of infection. Therefore, the project aimed to assess bacterial profiles, and antibiotic resistant patterns of the isolates from inanimate surfaces at the neonate intensive care unit wards in Khulna city, Bangladesh.

2. Materials & Methods

2.1. Sample Collection and Processing

A hospital-based cross-sectional study was conducted from May 20, 2023 to January 23, 2024. The study was undertaken on inanimate objects at the NICU wards of two renowned government hospitals and one private hospital in Khulna city. Before collecting swab samples from inanimate objects (such as bed rails, bed covers, weight machines, tables, head boxes, scissors, telephones, trays, medical charts, door handles, and sinks), hands were disinfected with hand sanitizer. Powder-free disposable gloves were worn for each sample to prevent cross-contamination. A sterile cotton swab moistened with normal saline was used to collect the specimens [13]. After collection, each swab specimen was placed into a separate test tube and labeled with the sample number and date of collection. After collection, all samples were transported to Animal Biotechnology Laboratory under the Biotechnology and Genetic Engineering Discipline at Khulna University and processed within 1 hour of collection. In total, 99 swabs were collected from 3 different hospitals including 50 from private hospital, 49 from two government hospitals. The research adhered to ethical guidelines, with approval from all hospitals, ensuring no physical or mental harm to the neonates.

2.2 Microbiological Activities

The moistened swab was inoculated onto nutrient agar, blood agar (to identify fastidious and hemolytic bacteria), and MacConkey agar (to isolate gram-negative bacteria) [14]. All inoculated media were incubated at 37°C for 24 hours. Isolates were characterized based on colony morphology, gram staining results, and microscopic examination [15]. Identification of bacterial isolates was performed through different biochemical tests including catalase test, oxidase test, citrate utilization test, coagulase test, methyl red test, Triple Sugar Iron (TSI) test, and urease test [16] following the guidelines of Bergey's Manual of Determinative Bacteriology (9th edition) [17]. Stock cultures were stored in nutrient broth with glycerol at -20°C.

2.3 Antibiotic Sensitivity Test

Using the Kirby-Bauer disk diffusion method antimicrobial susceptibility test for all isolates was conducted on Muller-Hinton agar, following the guidelines ‘the Clinical and Laboratory Standards Institute’ (CLSI) [18]. Ten (10) commonly prescribed antibiotics for neonates namely, Ciprofloxacin (5µg), Amikacin, (30µg), Imipenem (10µg), Gentamicin (10µg), Cefixime (5µg), Erythromycin (15µg), Azithromycin (50µg), Cefotaxime (30µg), Colistin (10µg), and Penicillin (10µg) were used and their diameter of the zone of inhibition (mm) according to CLSI guideline 2022 was measured [19].

2.4 Data Processing and Analysis

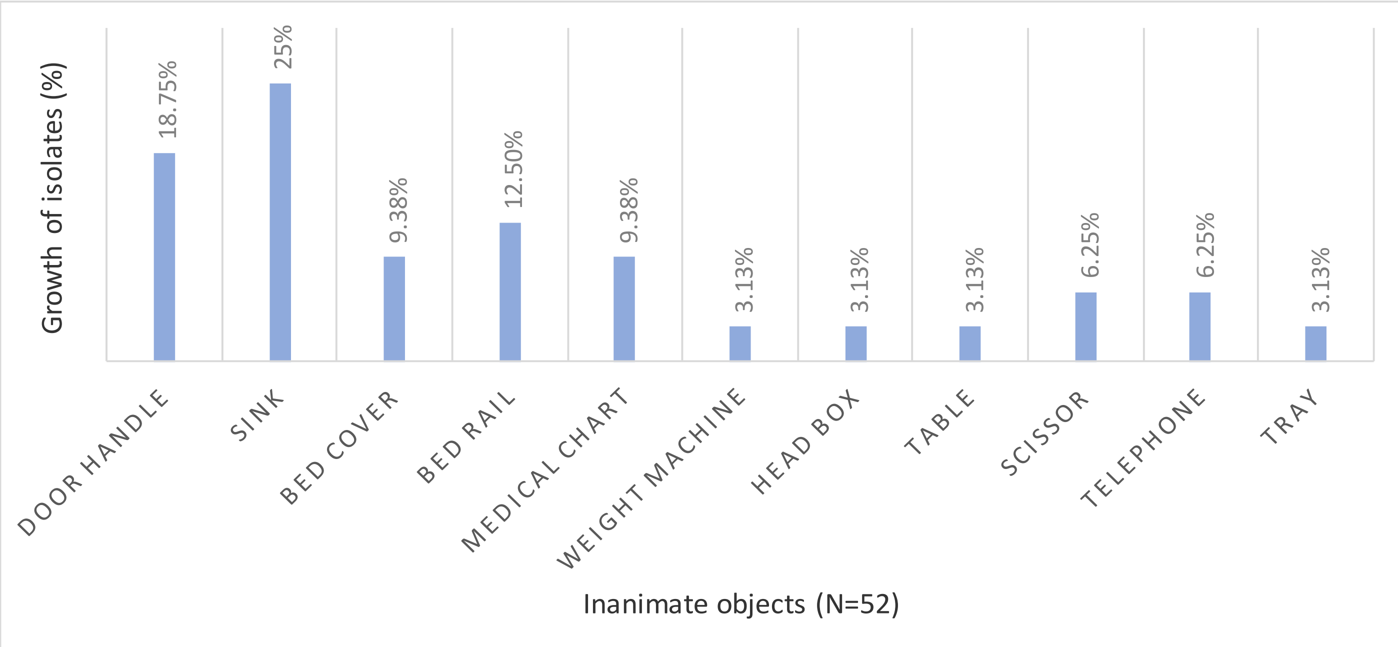
Data were analyzed by Statistical Package for Social Science (SPSS- ver.29) and GraphPad Prism (8.0.2).

3. Results

3.1 Prevalence of Bacterial Contamination by Type of Inanimate Objects

Overall, 52.53% (52/99) of the inanimate surfaces were contaminated with diverse bacteria. Sinks in the NICUs had the highest percentage of bacterial contamination (25%), followed by door handles (18.75%), bed rails (12.50%) and bed covers (9.38%) (Figure 3.1).

Figure 3.1: Proportion of Bacterial Isolates by Type of Inanimate Objects at NICUs Ward in Khulna City.



3.2 Distribution of Bacterial Isolates by Type of Inanimate Objects

As the sinks in the NICUs was the most contaminated surface so diverse type of bacteria was found including *Staphylococcus spp., Escherichia spp., Klebsiella spp.* and *Pseudomonas spp.. Klebsiella spp.* was common gram-negative bacteria isolated from both bed covers and sinks (Table 3.2).

3.3 Identification of Isolates

Gram Staining and Biochemical Tests

Both gram positive (52%) and gram negative (48%) bacteria (Figure 3.2) were found on different inanimate surfaces. Bacteria with six (6) different genus was isolated from inanimate objects in the NICUs, among them *Staphylococcus spp.*, (48.1%), *Escherichia spp.*, (19.2%), and *Klebsiella spp.* (15.4%) were more prominent (Figure 3.3).

Figure 3.2: Gram Staining Indicating the Presence of Gram Positive and Gram Negative Bacteria on the Inanimate Objects in NICUs.

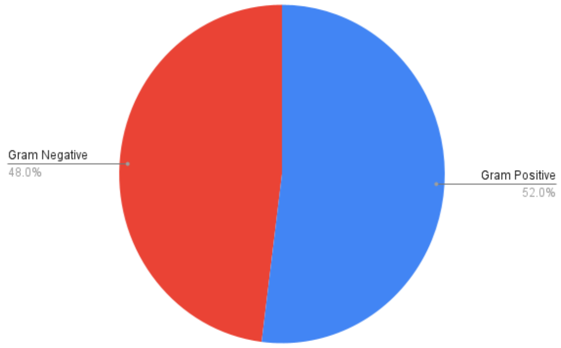


Table 3.1: Biochemical Test Results of the Isolates (N=52) Isolated from Different Inanimate Objects at NICUs Ward.

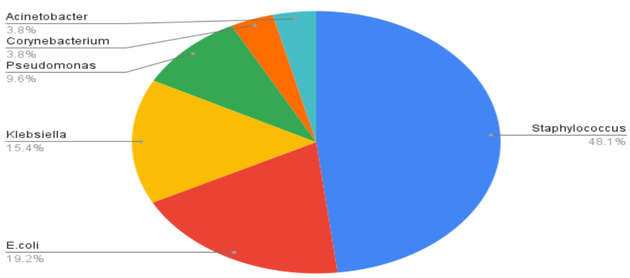
Catalase	Oxidase	Citrate	Urease	Methyl Red	Coagulase	Triple Sugar Iron (TSI) Test				
+ve	+ve	+ve	+ve	+ve	+ve	Red/ Yellow	Yellow /Yellow	Red/ Red	Black	Gas
34	5	15	33	37	25	25	18	5	2	2

Various biochemical tests confirm a wide range of bacterial presence on different inanimate objects at the NICUs ward of the hospitals. Isolated gram-positive organisms are *Staphylococcus spp.* and *Corynebacterium spp.*, whereas *Escherichia spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.*, are gram negative organisms (Table 3.1and Table 3.2).

Table 3.2: Estimated Bacteria Based on Different Biochemical Tests and Distribution of Bacterial Isolates by Type of Inanimate Objects at NICUs in Khulna City.

Types of Inanimate objects at NICUs	Organism isolated
Sink, Head Box, Door Handle, Medical Chart, Weight Machine, Table, Scissor, Tray and Bed Rail	*Staphylococcus spp.*
Medical Chart, Bed Rail and Sink	*Escherichia spp.*
Bed Cover and Sink	*Klebsiella spp.*
Telephone, Sink and Door Handle	*Pseudomonas spp.*
Bed Cover and Door Handle	*Corynebacterium spp.*
Door Handle and Telephone	*Acinetobacter spp.*

Figure 3.3: Prevalence of Isolated Bacteria on Different Inanimate Surfaces at NICUs in Khulna City.



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3.4 Antimicrobial Susceptibility Profiles of Isolated Bacteria

A total of commonly prescribed ten antibiotics from 10 classes for neonates were selected to assess the resistance profile of the isolates. Most of the bacterial isolates had high resistance to Penicillin & Erythromycin (100%), Cefotaxime (87%), Cefixime (83%), Amikacin (73%) and Azithromycin (65%) (Figure 3.4).

Figure 3.4: Antibiogram Profiling of Isolated Bacteria from Inanimate Objects at NICUs in Khulna City.

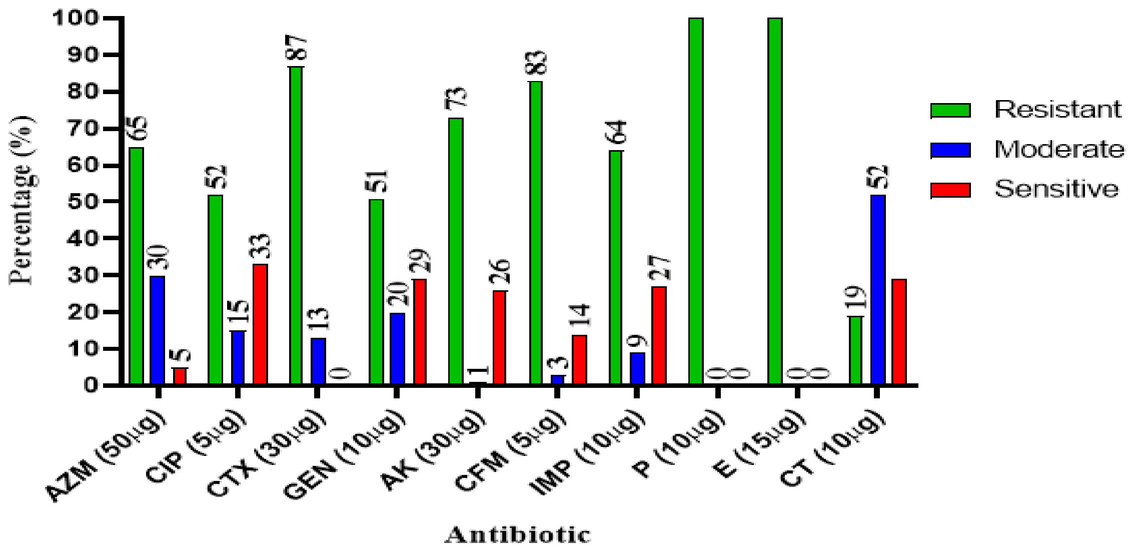


Table 3.3: Resistance Pattern of Bacteria Isolated from Different Inanimate Objects at NICUs in Khulna city.

Antibiotic Resistant Pattern										
Bacterial *Spp.*	**AZM**	**CIP**	**CTX**	**GEN**	**AK**	**CFM**	**IMP**	**P**	**E**	**CT**
Staphylococcus spp.(n=25)	92% (23)	60% (15)	100% (25)	32% (8)	52% (13)	80% (20)	64% (16)	100% (25)	100% (25)	20% (5)
Escherichia spp.(n=10)	60% (6)	60% (6)	100% (10)	100% (10)	100% (10)	100% (10)	100% (10)	100% (10)	100% (10)	0
Klebsiella spp. (n=8)	100% (8)	25% (2)	37.5% (3)	37.5% (3)	37.5% (3)	100% (8)	37.5% (3)	100% (8)	100% (8)	25% (2)
Pseudomonas spp. (n=5)	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)	0
Acinetobacter spp (n=2)	100% (2)	0	50% (1)	0	100% (2)	100% (2)	0	100% (2)	100% (2)	0
Corynebacterium spp. (n=2)	0	0	50% (1)	0	100% (2)	50% (1)	0	100% (2)	100% (2)	0

*Abbreviations: AZM- azithromycin; CIP- ciprofloxacin; CTX- cefotaxime; GEN- gentamicin; AK-amikacin; CFM- cefixime; IMP- imipenem; P- penicillin; E- erythromycin; CT- colistin

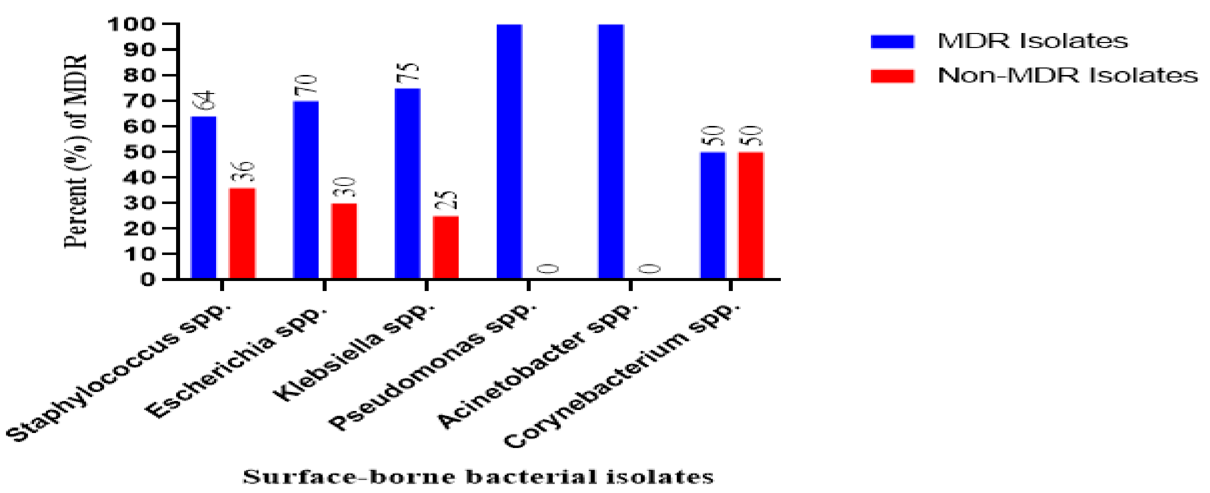
According to the resistance patterns (Table 3.3), colistin (CT) is the most effective antibiotic for *Escherichia spp., Pseudomonas spp., Acinetobacter spp. and Corynebacterium spp.* Moreover, *Acinetobacter spp* and *Corynebacterium spp.* are mostly sensitive to IMP, GEN and CIP.

Table 3.4: Multidrug-Resistance Patterns of Bacteria Isolated from Inanimate Objects at NICUs in Khulna City.

Bacterial isolates	Antibiogram Pattern					
	R0	**R1**	**R2**	**R3**	**R4**	**≥R5**
Staphylococcus spp.(n=25)	0	2 (8%)	7 (28%)	9 (36%)	4 (16%)	3 (12%)
Escherichia spp. (n=10)	0	0	3 (30%)	1 (10%)	2 (20%)	4 (40%)
Klebsiella spp. (n=8)	0	0	2 (25%)	0	2 (25%)	4 (50%)
Pseudomonas spp. (n=5)	0	0	0	0	0	5 (100%)
Acinetobacter spp. (n=2)	0	0	0	0	0	2 (100%)
Corynebacterium spp. (n=2)	0	0	1 (50%)	0	0	1 (50%)

*Notes: R0- null antibiotic resistance; R1- one class of resistance; R2- two classes of resistance; R3- three classes of resistance; R4- four classes of resistance; R5- five classes of resistance

Figure 3.5: Surface-Borne Multidrug Resistant Bacterial Isolates at NICUs in Khulna City.



The susceptibility profiles of various bacterial isolates were evaluated using 10 different classes of antibiotics. The majority of isolates demonstrated multidrug resistance to 5 or more classes (Table 3.4). Notably, *Pseudomonas spp.* and *Acinetobacter spp.* showed 100% resistance to ≥R5 classes.

Additionally, *Staphylococcus spp.*, *Escherichia spp.*, *Klebsiella spp.***,** and *Corynebacterium spp***.** exhibited multidrug resistance rates of 64%, 70%, 75%, and 50%, respectively, to ≥R3 classes (Figure 3.5). Out of 52 bacterial isolates, 37 exhibited resistance to three or more classes of antibiotics, resulting in multidrug resistance rate of 71.15%.

4. Discussion

A. Prevalence & Distribution of Isolated Bacteria

In this study 52 (52.53%) out of 99 inanimate object samples from Neonatal Intensive Care Unit (NICU) of 3 different hospitals of Khulna city showed culture positive (bacterial growth) result. In Bitew's study 51.09% growth was observed on inanimate samples from NICU of Hawassa University Comprehensive Specialized Hospital, Ethiopia [20] which is consistent with this study. Pathogens were identified 43.3% in inanimate objects at various tertiary care hospital wards in India [21]. In contrast, Manipal Teaching Hospital, Pokhara, Nepal, found higher culture positive growth (74.7%) from inanimate medical objects at the NICU [22].

The sinks (25%) and door handle (18.75%) showed more culture positive result among all inanimate objects. Notable bacterial presence was observed at NICUs on the computer mouse, drawer handles, monitor screen knob, and the surface of counters in Iran [23]. Bed (37.41%), radiant heat warmer (21.67%) and oxygen hoods (19.61%) were mostly bacterial prevalent also found by some hospitals [24]. A finding which is in line with our study, conclude that inanimate objects at the NICUs are the harbor of pathogens and these pathogens, mainly bacteria are commonly transmitted through surfaces frequently touched by healthcare workers and regularly coming into contact with patients [25].

B. Characterization of Isolated Bacteria

Six different types/ genus of bacteria were isolated in this study which are *Staphylococcus spp. (*48.12%*)*, *Escherichia spp. (*19.2%*)*, *Klebsiella spp. (*15.4%*)*, *Pseudomonas spp. (*9.6%*)*, *Corynebacterium spp. (*3.8%*)* and *Acinetobacter spp. (*3.8%*)* from inanimate objects at NICUs. In other study *Bacillus spp.* and *Acinetobacter* were the predominant bacteria found on inanimate surface of NICU [23]. A study on medical equipments, *Micrococcus spp.* (36.8%) and *Acinetobacter* (26.3%) were found most abundant bacteria [20] and are associated with different types of nosocomial infections [26]. The authors in Gambia showed the pathogenicity of *Staphylococcus aureus*, a major causes of neonatal infection through anatomical sites resulting in high morbidity and mortality for neonates [27]. Staphylococcal infections can also manifest as bacteremia, infections of the skin and soft tissues, bone and joint infections, endocarditis, meningitis, and neonatal sepsis [28]. *Escherichia spp.* is a cause of neonatal sepsis in preterm infants[29], bloodstream infections [30] & urinary tract infections [31]. Besides, *Klebsiella spp.* is a common pathogen in neonates, causing septic shock, bloodstream infections, abdominal infections, fetal distress, and various diseases for neonates [32]. Moreover, *Acinetobacter spp.* and *Corynebacterium spp.* are also linked to neonatal sepsis, diphtheria, meningitis, and severe illnesses [33] & [34].

C. Multidrug Resistant (MDR) Bacteria

Overall antibiogram profiling of the six bacterial isolates show resistant against Penicillin (100%), Erythromycin (100%), Cefixime (83%), Cefotaxime (87%), Azithromycin (65%), Amikacin (73%). In a different study most of the strains showed resistant to Azithromycin, Cefixime, Ceftazidime [35] & susceptible to Imipenem (73%), Ciprofloxacin (61.5%) [36].

This research work revealed, *Acinetobacter spp.* & *Klebsiella spp.* showed complete resistance against

Azithromycin, Penicillin, Erythromycin and Cefixime. Additionally, *Pseudomonas spp.* showed sensitivity to only Colistin and resistance against other 9 antibiotics used in this study which is in line with the findings of hospital in China [34] & [37]. Methicillin-resistant Staphylococcus aureus (MRSA) are challenging to treat, resulting in extended hospital stays, with biofilm production contributing to antibiotic resistance [38]. In a similar study, *Pseudomonas* exhibited sensitivity to Amikacin and Colistin but demonstrated resistance to Gentamycin, Ciprofloxacin, Cefotaxime, and Imipenem, which can be attributed due to its carbapenem resistance and biofilm-forming ability [39]. In this study, *Staphylococcus spp* demonstrated the highest resistance to Penicillin, Erythromycin, Azithromycin, and Cefotaxime, whereas *E. coli* showed sensitivity only to Colistin, Azithromycin, and Ciprofloxacin. Conversely, a separate study reported that *Staphylococcus aureus* was resistant to Cefotaxime, Amikacin, Ciprofloxacin, Imipenem, and Colistin, while *E. coli* exhibited resistance to Ampicillin and Vancomycin [40].
This study found that 71.15% of the isolates were resistant to five or more drug classes. Pseudomonas spp. and Acinetobacter spp. exhibited complete resistance (100%) to five or more drug classes. Moreover, multidrug resistance rates against three or more drug classes were observed in Staphylococcus spp. (64%), Escherichia spp. (70%), Klebsiella spp. (75%), and Corynebacterium spp. (50%). This indicates that the commonly used first-line drugs are largely ineffective [41]. *Saha et al.* (2024) conducted a relevant study revealing that most bacterial isolates from the pediatric ward in Khulna, Bangladesh, demonstrated multidrug resistance to seven or more classes of antibiotics [42].

5. Conclusions

Inanimate objects especially, sinks (25%), door handles (18.75%) and bed rails (12.50%) are vitiated with various types of bacteria. Of these, *Staphylococcus spp* (48.12%) is the predominant bacterial type, followed by *Escherichia spp.* (19.2%) and *Klebsiella spp* (15.4%). Gram-negative isolates specially *Acinetobacter spp.* and gram-positive isolate *Corynebacterium spp.* are sensitive to Ciprofloxacin, Gentamycin, Colistin and Imipenem. The frequency of MDR (71.15%) bacteria both in gram-negative and gram-positive was worryingly high. This might be the cross-contamination between patients and inanimate objects, indiscriminate use of antibiotics, or absence of a guide-line regarding the selection of drugs and disinfection techniques. These data might be important for the development of future guidelines for chemical -based and / microbiome-based sanitation approaches.

References

1. K. A. Simonsen, A. L. Anderson-Berry, S. F. Delair, and H. D. Davies, “Early-onset neonatal sepsis,” *Clin Microbiol Rev*, vol. 27, no. 1, pp. 21–47, Jan. 2014, doi: 10.1128/CMR.00031-13.
2. H. Tasew, M. Zemicheal, G. Teklay, T. Mariye, and E. Ayele, “Risk factors of birth asphyxia among newborns in public hospitals of Central Zone, Tigray, Ethiopia 2018,” *BMC Research Notes*, vol. 11, no. 1, p. 496, Jul. 2018, doi: 10.1186/s13104-018-3611-3.
3. D. R. Bhatta *et al.*, “Bacterial contamination of neonatal intensive care units: How safe are the neonates?,” *Antimicrob Resist Infect Control*, vol. 10, no. 1, p. 26, Jan. 2021, doi: 10.1186/s13756-021-00901-2.
4. L. M. Muhe *et al.*, “Major causes of death in preterm infants in selected hospitals in Ethiopia (SIP): a prospective, cross-sectional, observational study,” *Lancet Glob Health*, vol. 7, no. 8, pp. e1130–e1138, Aug. 2019, doi: 10.1016/S2214-109X(19)30220-7.

5. A. Sikora and F. Zahra, "Nosocomial Infections," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 23, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK559312/
6. M. Haque, M. Sartelli, J. McKimm, and M. Abu Bakar, "Health care-associated infections – an overview," *Infect Drug Resist*, vol. 11, pp. 2321–2333, Nov. 2018, doi: 10.2147/IDR.S177247.
7. K. D. Pegu, H. Perrie, J. Scribante, and M. Fourtounas, "Microbial contamination of the hands of healthcare providers in the operating theatre of a central hospital," *S Afr J Infect Dis*, vol. 36, no. 1, p. 221, Apr. 2021, doi: 10.4102/sajid.v36i1.221.
8. J. Johnson, I. C. Akinboyo, and J. K. Schaffzin, "Infection Prevention in the Neonatal Intensive Care Unit," *Clin Perinatol*, vol. 48, no. 2, pp. 413–429, Jun. 2021, doi: 10.1016/j.clp.2021.03.011.
9. M. G. Gozel, C. H. Hekimoglu, E. Y. Gozel, E. Batir, M.-L. McLaws, and E. A. Mese, "National Infection Control Program in Turkey: The healthcare associated infection rate experiences over 10 years," *Am J Infect Control*, vol. 49, no. 7, pp. 885–892, Jul. 2021, doi: 10.1016/j.ajic.2020.12.013.
10. C. C. Wang, K. Li, and S. Gaylord, "Wang et al. Respond," *Am J Public Health*, vol. 109, no. 9, pp. e5–e6, Sep. 2019, doi: 10.2105/AJPH.2019.305239.
11. K. Gidey, M. T. Gidey, B. Y. Hailu, Z. B. Gebreamlak, and Y. L. Niriayo, "Clinical and economic burden of healthcare-associated infections: A prospective cohort study," *PLoS One*, vol. 18, no. 2, p. e0282141, Feb. 2023, doi: 10.1371/journal.pone.0282141.
12. A. Sikora and F. Zahra, "Nosocomial Infections," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 23, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK559312/
13. K. E. Harr, "Sample Collection," *Vet Clin North Am Exot Anim Pract*, vol. 21, no. 3, pp. 579–592, Sep. 2018, doi: 10.1016/j.cvex.2018.05.008.
14. B. Jung and G. J. Hoilat, "MacConkey Medium," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 23, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK557394/
15. N. Tripathi and A. Sapra, "Gram Staining," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 23, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK562156/
16. K. Laffineur, M. Janssens, J. Charlier, V. Avesani, G. Wauters, and M. Delmée, "Biochemical and susceptibility tests useful for identification of nonfermenting gram-negative rods," *J Clin Microbiol*, vol. 40, no. 3, pp. 1085–1087, Mar. 2002, doi: 10.1128/JCM.40.3.1085-1087.2002.
17. *Bergey's manual of determinative bacteriology*. Baltimore : Williams & Wilkins, 1993. Accessed: Feb. 23, 2025. [Online]. Available: http://archive.org/details/bergeysmanualofd0000unse_l7d4
18. R. Humphries, A. M. Bobenchik, J. A. Hindler, and A. N. Schuetz, "Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition," *J Clin Microbiol*, vol. 59, no. 12, p. e0021321, Nov. 2021, doi: 10.1128/JCM.00213-21.
19. M. L. Bayot and B. N. Bragg, "Antimicrobial Susceptibility Testing," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 23, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK539714/
20. A. Bitew, A. Mengist, H. Belew, Y. Aschale, and A. Reta, "The Prevalence, Antibiotic Resistance Pattern, and Associated Factors of Bacterial Vaginosis Among Women of the Reproductive Age

Group from Felege Hiwot Referral Hospital, Ethiopia," *Infect Drug Resist*, vol. 14, pp. 2685–2696, 2021, doi: 10.2147/IDR.S305329.
21. D. Saikeerthana, P. Prabha, V. Vijayashree, and G. N. M. Krishna, "Overview of Pathogens causing Nosocomial Infections in Various Wards of a Tertiary Health Care Hospital and their Antimicrobial Susceptibility Pattern - A Prospective Surveillance Study," *J. Pure Appl. Microbiol.*, vol. 15, no. 4, pp. 1940–1946, Dec. 2021, doi: 10.22207/JPAM.15.4.15.
22. D. R. Bhatta *et al.*, "Bacterial contamination of neonatal intensive care units: How safe are the neonates?," *Antimicrob Resist Infect Control*, vol. 10, no. 1, p. 26, Jan. 2021, doi: 10.1186/s13756-021-00901-2.
23. N. Sultan, I. Shchors, M. V. Assous, and M. Bar-Meir, "The NICU flora: An effective technique to sample surfaces," *PLOS ONE*, vol. 16, no. 9, p. e0257821, Sep. 2021, doi: 10.1371/journal.pone.0257821.
24. D. R. Bhatta *et al.*, "Bacterial contamination of neonatal intensive care units: How safe are the neonates?," *Antimicrob Resist Infect Control*, vol. 10, p. 26, Jan. 2021, doi: 10.1186/s13756-021-00901-2.
25. D. R. Bhatta *et al.*, "Bacterial contamination of neonatal intensive care units: How safe are the neonates?," *Antimicrob Resist Infect Control*, vol. 10, p. 26, Jan. 2021, doi: 10.1186/s13756-021-00901-2.
26. B. M. Aiesh *et al.*, "Nosocomial infections in the surgical intensive care unit: an observational retrospective study from a large tertiary hospital in Palestine," *BMC Infect Dis*, vol. 23, no. 1, p. 686, Oct. 2023, doi: 10.1186/s12879-023-08677-z.
27. A. Bojang *et al.*, "Genomic approach to determine sources of neonatal Staphylococcus aureus infection from carriage in the Gambia," *BMC Infect Dis*, vol. 24, no. 1, p. 941, Sep. 2024, doi: 10.1186/s12879-024-09837-5.
28. A. C. Blanchard, C. Quach, and J. Autmizguine, "Staphylococcal Infections in Infants: Updates and Current Challenges," *Clinics in Perinatology*, vol. 42, no. 1, pp. 119–132, Mar. 2015, doi: 10.1016/j.clp.2014.10.013.
29. O. Villavicencio-Carrisoza *et al.*, "Advancing Understanding of Escherichia coli Pathogenicity in Preterm Neonatal Sepsis," *Microorganisms*, vol. 13, no. 2, Art. no. 2, Feb. 2025, doi: 10.3390/microorganisms13020219.
30. S. P. Bergin *et al.*, "Neonatal Escherichia coli Bloodstream Infections: Clinical Outcomes and Impact of Initial Antibiotic Therapy," *Pediatr Infect Dis J*, vol. 34, no. 9, pp. 933–936, Sep. 2015, doi: 10.1097/INF.0000000000000769.
31. A. Rafferty, R. J. Drew, R. Cunney, D. Bennett, and J. F. Marriott, "Infant Escherichia coli urinary tract infection: is it associated with meningitis?," *Arch Dis Child*, vol. 107, no. 3, pp. 277–281, Mar. 2022, doi: 10.1136/archdischild-2021-322090.
32. X. Wei *et al.*, "Clinical features and risk factors of Klebsiella pneumoniae infection in premature infants: a retrospective cohort study," *BMC Infectious Diseases*, vol. 24, no. 1, p. 1311, Nov. 2024, doi: 10.1186/s12879-024-10201-w.
33. H. N. R. Jesus *et al.*, "Pan-genomic analysis of Corynebacterium amycolatum gives insights into molecular mechanisms underpinning the transition to a pathogenic phenotype," *Front. Microbiol.*, vol. 13, Nov. 2022, doi: 10.3389/fmicb.2022.1011578.

34. J. Xiao, C. Zhang, and S. Ye, "Acinetobacter baumannii meningitis in children: a case series and literature review," *Infection*, vol. 47, no. 4, pp. 643–649, Aug. 2019, doi: 10.1007/s15010-018-1234-1.
35. F. A. Nobel *et al.*, "Isolation of multidrug resistance bacteria from the patients with wound infection and their antibiotics susceptibility patterns: A cross-sectional study," *Ann Med Surg (Lond)*, vol. 84, p. 104895, Nov. 2022, doi: 10.1016/j.amsu.2022.104895.
36. Md. Al Amin, M. N. Hoque, A. Z. Siddiki, S. Saha, and Md. M. Kamal, "Antimicrobial resistance situation in animal health of Bangladesh," *Vet World*, vol. 13, no. 12, pp. 2713–2727, Dec. 2020, doi: 10.14202/vetworld.2020.2713-2727.
37. W. Imtiaz, Z. Syed, Z. Rafaque, S. C. Andrews, and J. I. Dasti, "Analysis of Antibiotic Resistance and Virulence Traits (Genetic and Phenotypic) in Klebsiella pneumoniae Clinical Isolates from Pakistan: Identification of Significant Levels of Carbapenem and Colistin Resistance," *Infect Drug Resist*, vol. 14, pp. 227–236, 2021, doi: 10.2147/IDR.S293290.
38. D. R. Bhatta *et al.*, "Bacterial contamination of neonatal intensive care units: How safe are the neonates?," *Antimicrob Resist Infect Control*, vol. 10, no. 1, p. 26, Jan. 2021, doi: 10.1186/s13756-021-00901-2.
39. M. Y. Memar *et al.*, "In-vitro Effect of Imipenem, Fosfomycin, Colistin, and Gentamicin Combination against Carbapenem-resistant and Biofilm-forming Pseudomonas aeruginosa Isolated from Burn Patients," *Iran J Pharm Res*, vol. 20, no. 2, pp. 286–296, 2021, doi: 10.22037/ijpr.2020.111824.13380.
40. I. F. Jaja *et al.*, "Antimicrobial Resistance Phenotype of Staphylococcus aureus and Escherichia coli Isolates Obtained from Meat in the Formal and Informal Sectors in South Africa," *Biomed Res Int*, vol. 2020, p. 3979482, Sep. 2020, doi: 10.1155/2020/3979482.
41. M. Roney, A. M. Huq, and M. F. F. Mohd Aluwi, "Combat against antibiotic resistance is a challenge in Bangladesh," *Intelligent Pharmacy*, vol. 2, no. 3, pp. 305–307, Jun. 2024, doi: 10.1016/j.ipha.2024.02.002.
42. B. K. Saha, A. Ashraf, T. A. Sm, and S. Siddiqua, "Magnitude, Diversity and Prevalence of Antibiotic Resistant Bacteria in Pediatric Patients of Coastal Area at Khulna Region, Bangladesh," *International Journal of Research and Scientific Innovation*, vol. 11, no. 15, pp. 713–729, 2024.