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Original Research Article

Bacterial Contamination of the NICU Environment: Distribution of Gram-Positive and Gram-Negative Isolates from High-Touch Surfaces

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Abstract: Neonatal intensive care units (NICUs) are specialized health care settings where neonates with critical conditions and preterm births are exposed to a high risk of healthcare-associated infections (HAI). Non-critical medical equipment and frequently touched surfaces in the NICU environment may act as a medium of bacterial transmission. It is important to know the level of bacterial contamination in the NICU environment in order to enhance infection prevention and control. The objectives of the present study were to determine the level of bacterial contamination in the NICU environment in Iraq, to determine the distribution of Gram-positive and Gram-negative bacteria and to determine the most common bacteria present in the NICU environment. A cross-sectional survey was carried out in the NICU of Al-Rifai General Hospital in Al-Rifai City in Iraq from November 2023 to March 2024. A total of 61 swab samples were collected from frequently touched surfaces and frequently used non-critical equipment in the NICU environment. These surfaces and equipment include incubators, suction tips, stethoscopes, door handles, medical equipment, and hands of healthcare workers. These samples were then cultured using standard microbiological practices and incubated at 37°C for up to 48 hours. The bacteria were identified based on their colonial characteristics and Gram staining. Among the total samples, 58 (95.1%) had evidence of bacterial growth, while 3 (4.9%) had no growth. Of the culture-positive samples, Gram-positive organisms were predominant, accounting for 40 (68.9%), while Gram-negative organisms accounted for 18 (31.0%). Among Gram-positive organisms, the most prevalent organism was Staphylococcus aureus, accounting for 13 (32.5%), followed by Bacillus cereus (6, 15.0%), Staphylococcus epidermidis (4, 10.0%), and Enterococcus faecalis (4, 10.0%). Among Gram-negative organisms, Escherichia coli was the predominant organism, accounting for 10 (55.5%), followed by Acinetobacter baumannii (3, 16.6%), and Pseudomonas aeruginosa (2, 11.1%). From the qualitative mapping, it was observed that predominant organisms were isolated from high-touch surfaces and non-critical equipment, including incubators, suction tips, stethoscopes, door handles, and healthcare workers' hands. The study revealed a high level of environmental contamination in the NICU, with a predominance of Gram-positive organisms and clinically important Gram-positive and Gram-negative organisms. The study also revealed that predominant organisms are associated with high-touch surfaces and non-critical equipment, emphasizing the need for strict hand hygiene and environmental decontamination practices in the NICU setting.

Keywords: Neonatal Intensive Care Unit (NICU), Environmental Contamination, Gram-Positive Organisms, Gram-Negative Organisms, Healthcare-Associated Infections (HAI).

1. INTRODUCTION

Neonatal Intensive Care Units (NICUs) are facilities providing lifesaving care to premature and critically ill newborns, and as such, are extremely complex environments where health care-associated infections (HAI) may occur despite best practices and efforts to provide high-quality patient care. Infants admitted to the NICU are particularly susceptible to HAIs because of their immature immune response, thin skin and mucosal barriers, invasive medical device use, exposure to broad-spectrum antibiotics, and prolonged hospital stay [1]. These factors, collectively, increase their susceptibility to colonization and subsequent infection, and as such, IPC is a fundamental cornerstone of NICU safety and quality of care. The World Health Organization (WHO) has recognized HAIs as a significant patient safety concern globally and has underscored the fact that a significant proportion of HAIs are preventable with the establishment of effective IPC programs at the national and facility levels [2-4].

The NICU is a high-touch environment characterized by a wide range of high-touch surfaces and non-critical patient-care items, such as incubators, suction machines, ventilators, stethoscopes, door handles, workstations, and weighing machines, which are frequently touched by healthcare workers, caregivers, and medical equipment. These high-touch surfaces are prone to microbial contamination with various microorganisms, which may occur as a result of direct touch, aerosolization, and indirect transfer from colonized hands and medical devices, respectively. Significantly, microbial contamination of the health care environment has been proposed as a potential factor for the transmission of various microbial agents, particularly when cleaning practices are inconsistent, compliance with hand hygiene is suboptimal, and disinfection of medical equipment between uses is not adequate [5]. As such, environmental surveillance, which is conducted by collecting and analyzing microbial cultures from high-touch surfaces, has been proposed as a valuable approach to better understand the microbial ecology of health care-associated organisms and inform IPC programs [6].

Environmental contamination in the context of the NICU is also an issue of particular concern since high-touch items have the potential to become "shared interfaces" for multiple patients and healthcare staff. The stethoscope, suction devices, surfaces of incubators, and equipment at patients' bedsides are all subject to multiple manipulations during the course of "routine" patient care activities. The presence of biofilms on dry surfaces has also been documented in frequently touched surfaces in hospital environments, where microbial communities are able to persist for longer periods than in the bloodstream or other bodily fluids and are tolerant to disinfectants. The persistence of biofilms may play **[7]**.

2. MATERIALS AND METHODS

Study Design and Setting

This study was designed as a cross-sectional environmental study conducted within the Neonatal Intensive Care Unit (NICU) of Al-Rifai General Hospital in Al-Rifai City, Iraq, from November 2023 to March 2024. This study aimed to investigate the bacterial contamination of frequently touched surfaces and non-critical patient-care equipment used within the Neonatal Intensive Care Unit environment.

Sampling Strategy and Sample Collection

A total of 61 environmental swab samples were collected from frequently touched surfaces and non-critical patient-care equipment used within the Neonatal Intensive Care Unit environment. The sampling sites included, but were not limited to, incubators, suction tips, ventilators, stethoscopes, door and window handles, digital weighing machines, oxygen masks/cylinders, instrument and staff tables, surfaces of cabinets, staff mobile phones, wall and floor surfaces, and healthcare workers' hands.

Sterile cotton swab sticks were used to collect the samples. Each sample was collected by rubbing a sterile cotton swab stick on a surface using a standardized method. The sample swab sticks were transported to the laboratory immediately.

Culture and Incubation Procedures

All swabs collected were cultured on appropriate bacteriological media following standard microbiological procedures. Plates were incubated at 37°C under aerobic conditions and observed after 24 to 48 hours. Cultures are considered positive if visible growth of bacteria is observed. Conversely, cultures are considered negative if no growth is observed after 48 hours of incubation.

Bacterial Identification

Bacterial cultures are first identified using colony characteristics and Gram stain to identify the type as Gram positive or Gram negative. Further identification of the bacteria to the species level was performed using standard biochemical procedures. These procedures are standard microbiological techniques used to identify bacteria. For instance, *staphylococci* identification was performed using the catalase and coagulase tests. Similarly, biochemical profiles for Gram-negative bacilli were performed. When more than one type of colony is observed, cultures are subcultured to identify the type of bacteria present [8].

Identification Procedures

These procedures were performed following standard microbiology procedures as indicated by microbiology reference methods [9].

Data Management and Statistical Analysis

Data collection was done and recorded using isolate count and proportion. Descriptive statistical methods were used to find:

Culture positivity rate, distribution of Gram-positive and Gram-negative isolates, and percentages of bacterial species.

Values of categorical data are expressed as n (%). Percentages of Gram-positive and Gram-negative organisms were calculated using the total number of culture-positive samples (n = 58) as the denominator. Percentages of species within Gram-positive (n = 40) and Gram-negative (n = 18) organisms were calculated individually.

Quality Assurance

In order to ensure the reliability of the data, standard aseptic techniques are followed during sampling and data collection. All cultures are allowed to incubate for the full incubation period (48 hours) before data interpretation. Gram staining and biochemical tests are done following standard quality control procedures [9].

Ethical Considerations

This study involved environmental sampling only and no direct patient interventions. The study was conducted in accordance with the institutional requirements and laboratory biosafety standards. Administrative permission to conduct an environmental sampling study in the NICU setting was obtained from the hospital.

3. RESULTS

A total of 61 environmental swabs were collected from high-touch surfaces and non-critical equipment in the NICU. Bacterial growth was detected in 95.1% (58/61) of samples, while 4.9% (3/61) showed no growth after 48 hours of incubation (Table 1; Figure 1). This implies that bacterial contamination was present in most of the samples during this period.

Out of 58 samples with bacterial growth, Gram-positive bacteria were predominant compared to Gram-negative bacteria, with a prevalence of 68.9% (40/58) compared to 31.0% (18/58), respectively (Table 1; Figures 2 and 7). This implies that Gram-positive bacteria were more commonly isolated from the NICU compared to Gram-negative bacteria.

Among Gram-positive bacteria, *Staphylococcus aureus* was the most prevalent species, with a prevalence of 32.5% (13/40), followed by Bacillus cereus with a prevalence of 15.0% (6/40). Other Gram-positive bacteria, namely *Staphylococcus epidermidis* and *Enterococcus faecalis*, accounted for 10.0% each, while other species were present in low percentages ranging from 1.0% to 5.0% (Table 2; Figure 3). This implies that most Gram-positive bacteria were concentrated in a few dominant species.

Among Gram-negative bacteria, *Escherichia coli* was predominant compared to other Gram-negative bacteria, with a prevalence of 55.5% (10/18). Other Gram-negative bacteria, namely *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, accounted for 16.6% and 11.1%, respectively. Other Gram-negative bacteria accounted for 5.5% each (Table 3; Figure 4).

The results of the qualitative organism-site mapping indicated that the most common organisms were recovered from certain high-contact surfaces and shared equipment (Table 4; Figure 5). **S. aureus** was frequently recovered from the incubator, suction tips, stethoscope, neonate bed, and tube holders, while **E. coli** was frequently recovered from staff hands, door handles, suction tips, stethoscope, and neonates' breathing tubes. Other Gram-negative organisms were associated with the weighing machine, incubator surfaces, cabinets, staff mobile phones, and the instrument table.

The distribution of all the recovered organisms (Figure 6) shows that a few organisms were responsible for a large proportion of the environmental recoveries. The distribution of the organisms in the figure shows that a few organisms were responsible for a large proportion of the recoveries.

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Table 1: Culture positivity and Gram-stain distribution of bacterial isolates recovered from NICU environmental swabs (N = 61)

Outcome / Category	n / N	%
Swabs with bacterial growth	58 / 61	95.1
Swabs with no bacterial growth after 48 h	3 / 61	4.9
Gram-positive isolates (among positive cultures)	40 / 58	68.9
Gram-negative isolates (among positive cultures)	18 / 58	31.0

Table 2: Gram-positive bacterial species isolated from NICU environmental swabs (n = 40), ranked by frequency

Gram-positive species	n	% (of Gram-positive isolates)
Staphylococcus aureus	13	32.5
Bacillus cereus	6	15.0
Staphylococcus epidermidis	4	10.0
Enterococcus faecalis	4	10.0
Enterococcus faecium	2	5.0
Neisseria lactamica	2	5.0
Bacillus subtilis	2	5.0
Streptococcus viridans group	1	2.5

Gram-positive species	n	% (of Gram-positive isolates)
Streptococcus agalactiae	1	2.5
Streptococcus pneumoniae	1	2.5
Streptococcus pyogenes	1	2.5
Leifsonia aquatica	1	2.5
Bacillus pumilus	1	2.5
Staphylococcus xylosus	1	2.5

Table 3: Gram-negative bacterial species isolated from NICU environmental swabs (n = 18), ranked by frequency

Gram-negative species	n	% (of Gram-negative isolates)
Escherichia coli	10	55.5
Acinetobacter baumannii	3	16.6
Pseudomonas aeruginosa	2	11.1
Klebsiella pneumoniae†	1	5.5
Vibrio cholerae†	1	5.5
Paracoccus yeei	1	5.5

Table 4: Predominant organisms and their frequently reported recovery sites in the NICU (qualitative mapping)

Organism	Frequently reported recovery sites / items
Staphylococcus aureus	Incubator; suction tip; stethoscope; neonate bed; tube holder
Bacillus cereus	HCWs' hands; door handle; stethoscope; ambu bag
Escherichia coli	HCWs' hands; door handle; suction tip; stethoscope; neonates' breathing tube
Other Gram-negative isolates	Weighing machine; outer incubator surface; cabinet; staff mobile phone; instrument table

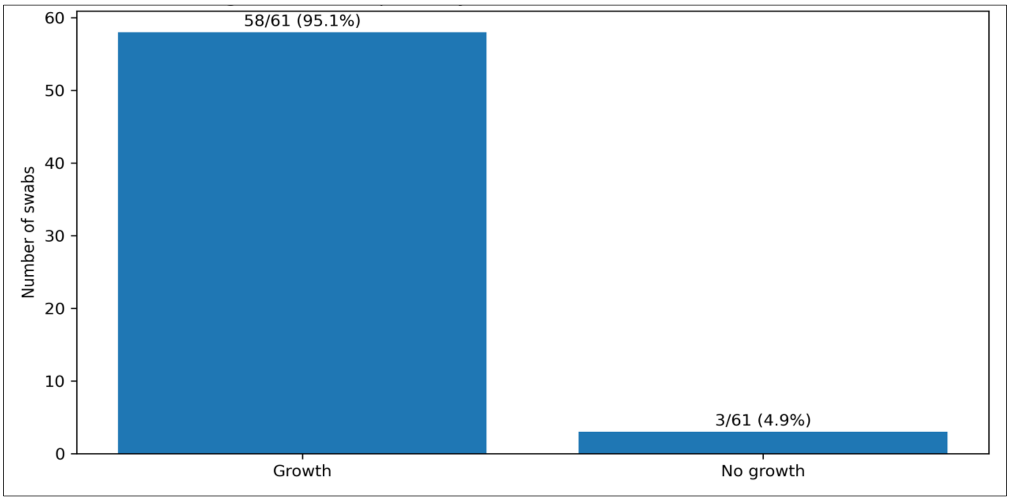

Figure 1: Culture positivity of NICU environmental swabs (N = 61)

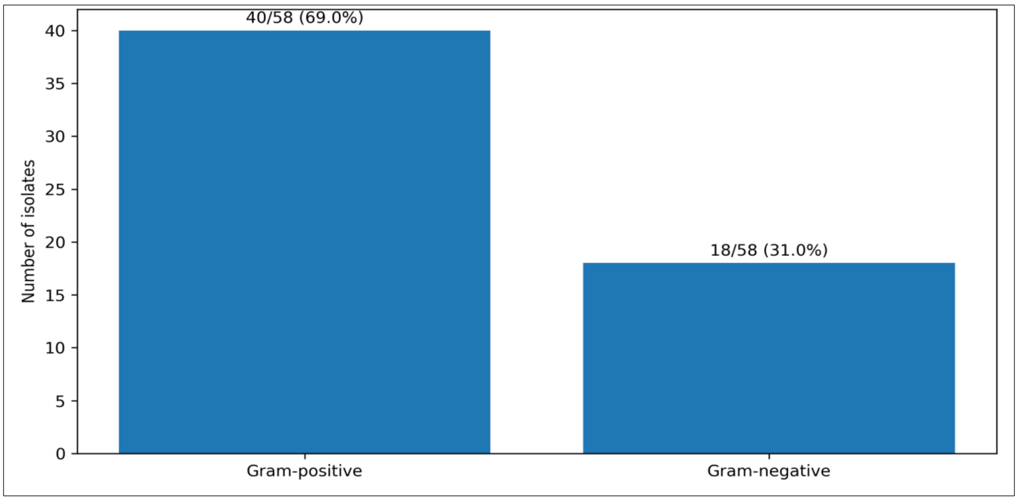

Figure 2: Gram-stain distribution among culture-positive swabs (n = 58)

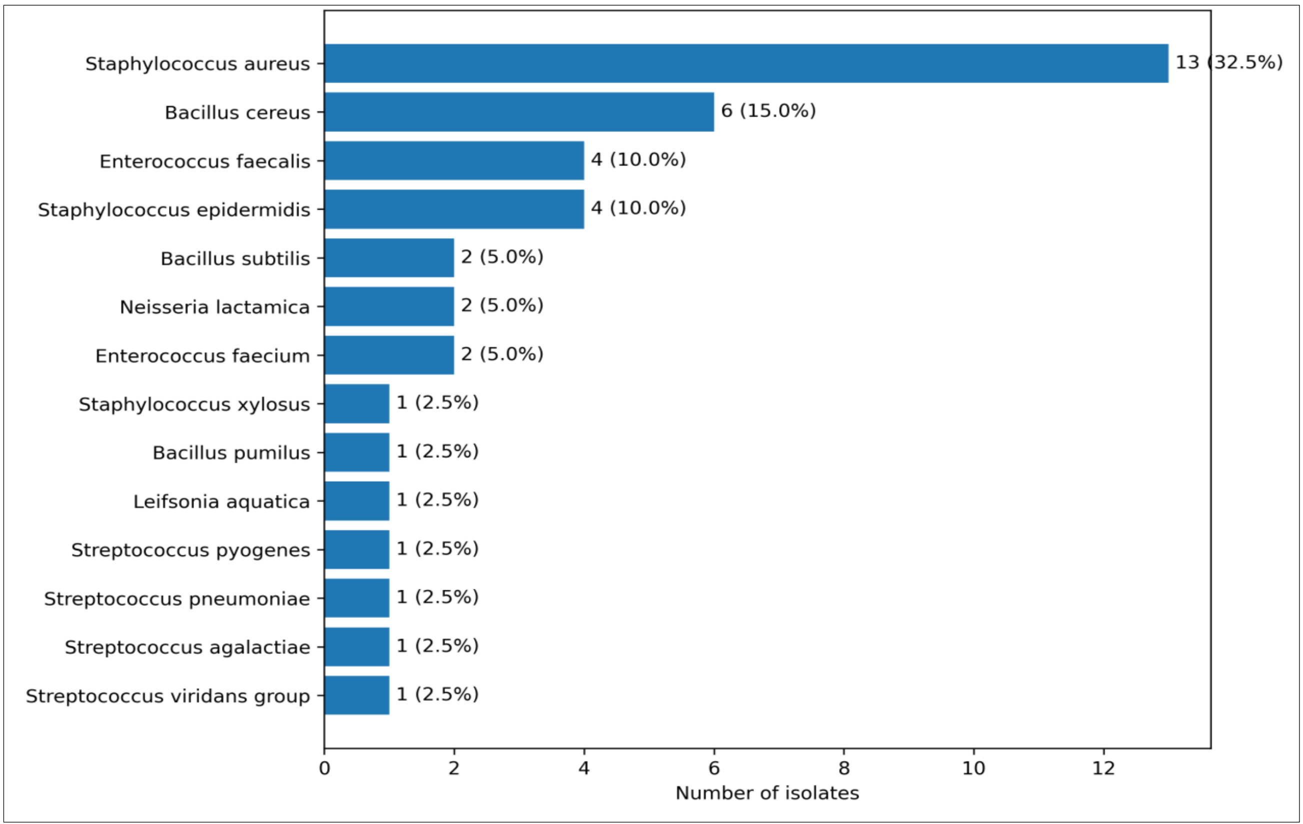

Figure 3: Distribution of Gram-positive bacterial species (n = 40)

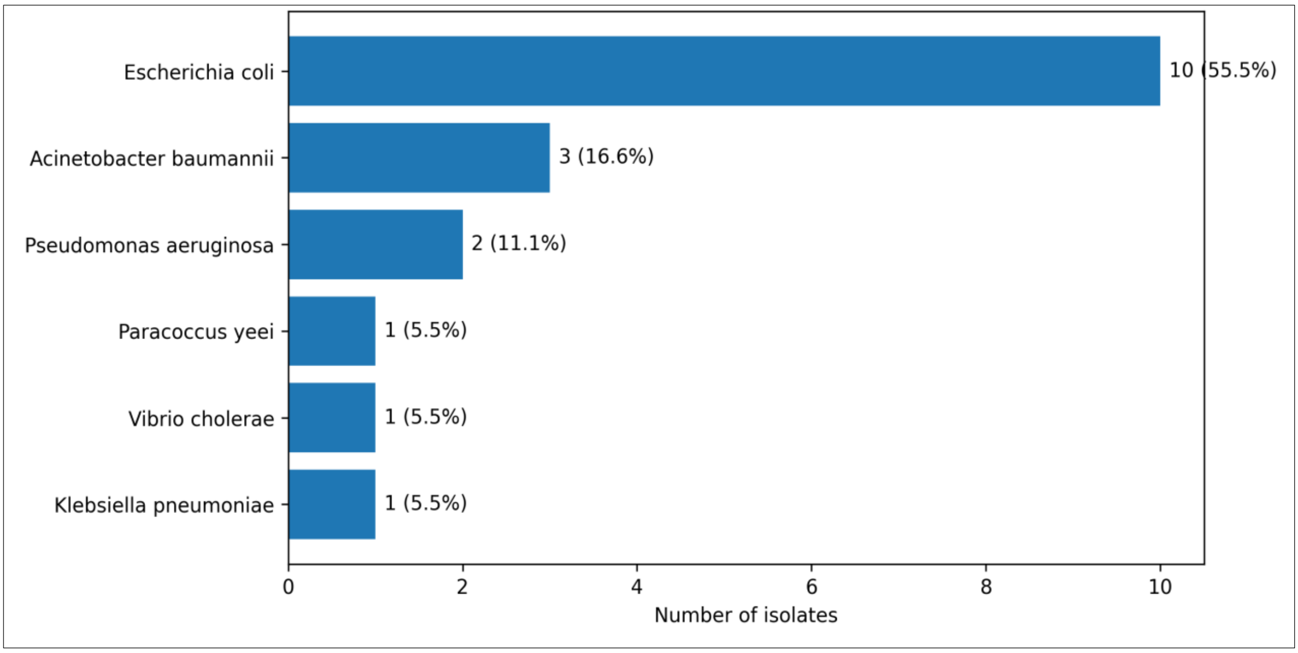

Figure 4: Distribution of Gram-negative bacterial species (n = 18)

Figure 5: Qualitative mapping of predominant organisms to frequently reported recovery sites in the NICU

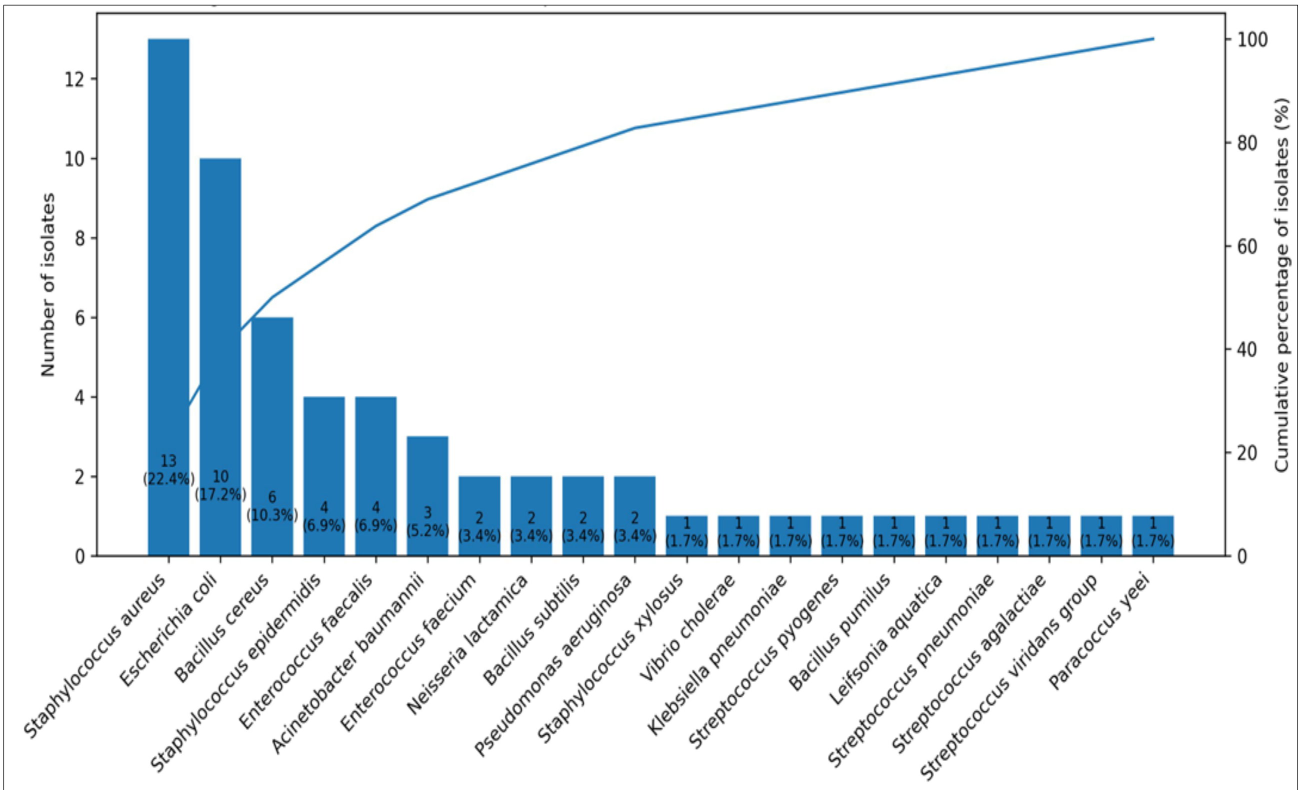

Figure 6: Pareto chart of bacterial species recovered from NICU environmental swabs (n = 58)

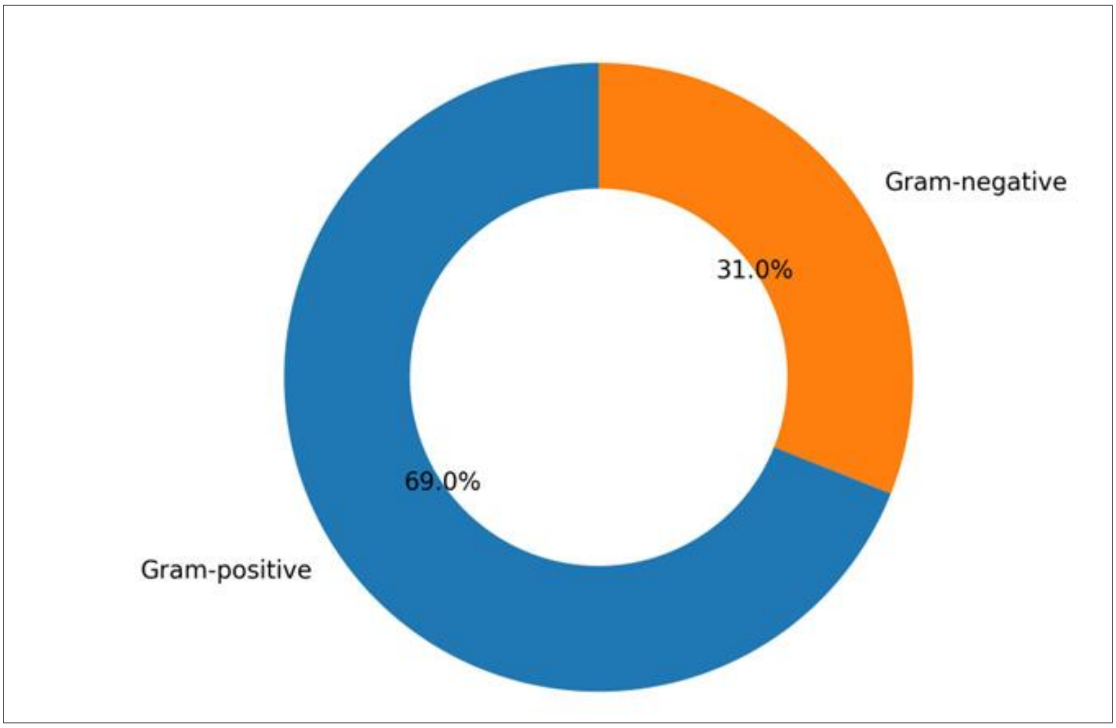

Figure 7: Proportion of Gram-positive versus Gram-negative isolates among culture-positive swabs (n = 58)

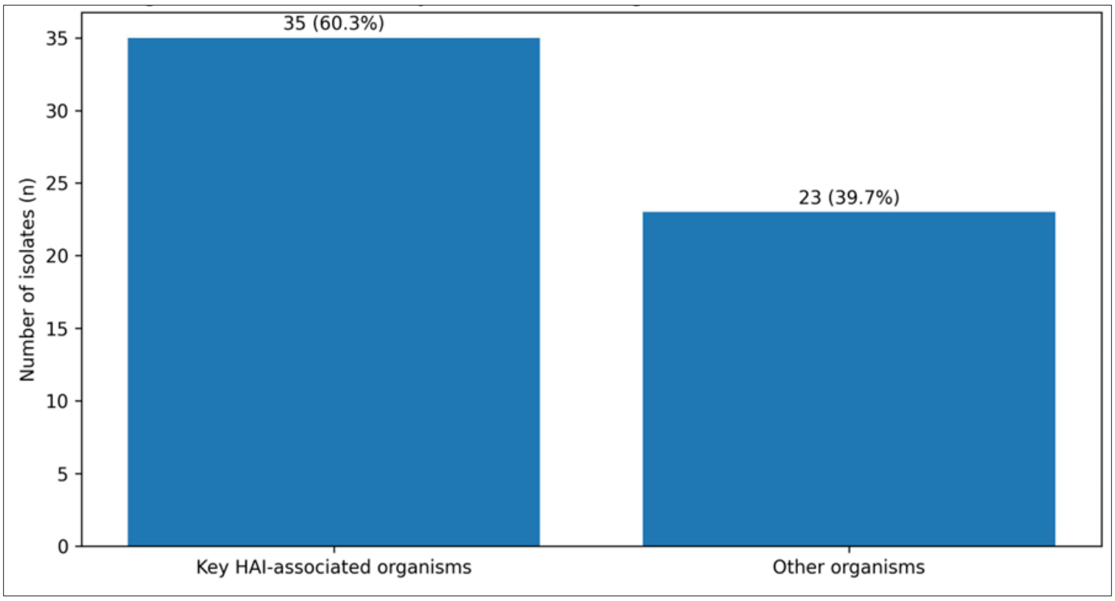

Figure 8: Distribution of key HAI-associated organisms versus other isolates (n = 58)

4. DISCUSSION

This research offers a comprehensive description of bacterial contamination in the NICU hospital environment through environmental swab surveillance of high-touch surfaces and non-critical equipment. The most notable result was the extremely high culture positivity rate, recorded at 95.1% (58/61). This indicates a high level of presence of microorganisms on these sampling sites. Among these culture-positive samples, Gram-positive organisms dominated, with a percentage of 68.9%, compared with 31.0% Gram-negative organisms (Table 1, Figures 2 and 7). The species identified showed that *Staphylococcus aureus* was the most commonly identified Gram-positive organism, with a percentage of 32.5% in Gram-positive culture-positive samples, while *Escherichia coli* was the most commonly identified Gram-negative organism, with a percentage of 55.5% in Gram-negative culture-positive samples (Tables 2-3, Figures 3-4). Qualitative site mapping showed that the most dominant organisms were present on high-touch surfaces and shared equipment in the hospital environment (Table 4, Figure 5). The Pareto curve in Figure 6 shows that a small number of organisms dominated in these samples.

Collectively, these findings support the assertion that the NICU environment is significantly susceptible to routine microbial contamination, and that the pattern of contamination includes microorganisms with established healthcare relevance. Environmental contamination does not, in itself, indicate transmission to neonates, but rather suggests possible reservoirs and interfaces of contact that may facilitate cross-transmission, especially in such an intense care environment where contact with equipment and surfaces is unavoidable [10-15].

The WHO acknowledges that healthcare-associated infections are one of the most important global patient safety issues, pointing out that "many of these infections are preventable, and effective IPC interventions can significantly reduce their occurrence" [16-20]. NICUs, in particular, are environments in which neonates, having underdeveloped immune systems, are at high risk of infection due to their need for intensive care support. Environmental contamination has increasingly been recognized as an integral part of the "infection ecology" of intensive care environments, in particular, due to the repeated likelihood of high-touch surfaces and equipment being repeatedly contaminated and re-contaminated during the course of care. A risk-based approach to environmental cleaning is emphasized by the CDC, in which "the frequency and stringency of cleaning increase in proportion to patient vulnerability and contamination risk, especially in high-risk areas such as intensive care units" [21]. The high rate of positivity in this study correlates with the expectation that high-touch surfaces in busy care environments will accumulate bacterial contamination, especially when these surfaces are frequently contacted and in which the efficacy and regularity of cleaning/disinfecting vary in their effectiveness or regularity [22, 23].

While previous literature may have concentrated on more "obvious" reservoirs, more recent literature emphasizes that many "routine" items, such as shared stethoscopes, incubator surfaces, suction devices, and mobile devices, may all contribute to microbial persistence and dissemination within a hospital setting. The NICU setting is particularly critical because of the use of many devices, some of which have complex geometries that make disinfection more difficult. Recent UK guidelines on incubator disinfection and handling of neonatal equipment emphasize the critical issue of decontamination processes for incubators and shared use of equipment, with a focus on evidence gaps and a need to generate new data to inform such processes. In that context, the current study's approach to mapping organisms to incubators, suction tips, and stethoscopes is particularly relevant to the emerging focus of new guidelines [24, 25].

The preponderance of Gram-positive organisms in this study (Table 1, Figures 2 and 7) is consistent with other environmental surveys of healthcare settings, which frequently report an abundance of these organisms, likely as a result of their ease of transfer and persistence on dry surfaces. Among these Gram-positive organisms, **Staphylococcus aureus** was predominant (Table 2, Figure 3), and these organisms were recovered from several high-touch sites, including the incubator, suction tip, stethoscope, neonate bed, and tube holder (Table 4, Figure 5). This is of considerable epidemiologic significance, as **S. aureus** has long been recognized as a cause of colonization and infection in the NICU and has been linked with both sporadic cases and outbreaks of disease in these settings.

The CDC has established evidence-based recommendations that are specifically aimed at the prevention and control of *S. aureus*, including MRSA and MSSA, in NICU patients, and this is a reflection of the continued burden and importance of this pathogen in the context of neonatal care [26, 27]. The recovery of *S. aureus* from HTS and equipment in this study highlights the potential of the environment as a reservoir that may contribute to the overall pressure of this organism in the healthcare setting and supports the rationale for targeted prevention and control strategies in this setting [28].

Other Gram-positive isolates obtained, such as **Staphylococcus epidermidis** and **Enterococcus faecalis** *, are also clinically relevant. Coagulase-negative staphylococci and enterococci are often colonizers but may be important opportunistic pathogens in neonates, particularly in the setting of invasive devices. This may indicate that environmental contamination can include organisms that are commonly associated with contact-mediated pathways and may highlight the importance of adherence to standard precautions in the routine disinfection of non-critical devices and high-touch surfaces [27, 28].

The Gram-negative fraction (31.0%) (Table 1) included several organisms strongly associated with the healthcare environment. The predominance of *E. coli* (Table 3; Figure 4) is of interest in the context of neonatal sepsis as well as the organism's known ability to colonize equipment and high-touch sites under the influence of contamination or poor disinfection practices. The presence of *Acinetobacter baumannii* and *P. aeruginosa* is of particular interest in the NICU environment, as these organisms have been strongly associated with environmental persistence, as well as transmission in the healthcare environment. Current reviews of these organisms highlight *A. baumannii* as being highly adapted for survival in the hospital environment. Persistence of this organism on surfaces is of particular concern as it complicates outbreak investigation and increases the risk of transmission in the healthcare environment. *P. aeruginosa* is a classic opportunistic pathogen known for persistence in moist reservoirs as well as on surfaces. This organism is of particular interest as it is known for device-associated infections [29].

The presence of *Klebsiella pneumoniae*, even if it is a low percentage, may also be of significance because of its common association with outbreaks in NICUs and its potential to develop extended-spectrum beta-lactamase (ESBL) and carbapenem resistance. Although antimicrobial susceptibility was not assessed in the present study, the presence of these organisms represents a contamination pattern with clinically significant organisms of known concern and reiterates the potential utility of surveillance and cleaning [30-32].

It is noteworthy that the occurrence of *Vibrio cholerae* and *Paracoccus* is less common in the general datasets of NICU environmental surveillance studies. A clear methodological explanation may be required for inclusion in the manuscript. In writing the manuscript for submission, it is important to highlight the identification method, which is biochemical or phenotypic. Additionally, it is important to recognize that the occurrence of less common bacteria may be related to unusual sources of contamination or limitations of identification [33].

Qualitative results in Table 4 and Figure 5 show that important bacteria were consistently related to high-touch surfaces or equipment. For example, *S. aureus* was related to incubators and stethoscopes. *E. coli* was related to hands of staff, door handles, and breathing tubes. Other Gram-negative bacteria were related to weighing machines and mobile phones of staff. This is consistent with the conceptual framework for environmental surface cleaning guidance. This guidance includes surface type according to the frequency of contact or the level of risk of exposure. The CDC clearly describes a risk-based approach to the frequency of surface cleaning and processes. This includes the probability of contamination, the level of risk of exposure, and the level of vulnerability of the patient. All of these elements come together in NICUs.

Furthermore, recent studies on incubator decontamination revealed that incubator surfaces may be contaminated with healthcare-associated pathogens, and effective decontaminating strategies may vary depending on their feasibility and workflow constraints. The isolation of microorganisms from incubators and surrounding equipment in this study further emphasizes their scientific and practical implications of incubator-based cleaning and disinfecting processes [33, 34].

The inclusion of suction tips and breathing tubes as frequently contaminated items is particularly relevant, as these items are directly connected or manipulated during airway-related care. While these items may be considered as being of single-patient or semi-critical nature, external surfaces and accessories can also become contaminated and act as a point of contact. The recovery of organisms from stethoscopes is also consistent with the overall literature, which also indicates that non-critical equipment can act as a potential source of contamination. This supports the argument that non-critical items, which are frequently shared or reused, need to be included as part of a systematic approach of decontamination [35].

The Pareto chart (Figure 6) shows that a small number of organisms, such as S. aureus and E. coli, dominate the total number of organisms isolated in the NICU environment. Such a distribution of organisms is not unusual in environmental microbiology and can be helpful in prioritization, as all organisms and their sites are not equally important in infection control, and a more targeted approach can be applied by addressing the organisms that are most commonly present and their associated sites. From a publication viewpoint, the Pareto chart adds interpretive value and creates a narrative that can be logically drawn: that the NICU environment is dominated by a small number of organisms that have clinically relevant profiles.

Additionally, Figure 8 supports the clinical approach by distinguishing between key HAI-associated organisms and those that are not. The observation that a large number of isolates cluster in key HAI-associated organism groups supports the external validity of the results to the risk of healthcare-associated infections.

The results of this research align with those of other environmental NICU studies. For example, this research sampled items such as incubators, ventilators, suction devices, stethoscopes, door handles, etc. Other studies also reported high-touch site contamination. Gram-positive bacteria were also dominant in this research. Other environmental sampling studies in NICUs also reported Staphylococcus spp., *Enterococci* spp., Gram-negative bacilli, etc. This supports the external validity of the results. This research also had an extremely high percentage of positive cultures. This is 95.1 percent. This is very high. This result may be related to the context of the NICU. For example, it may be related to workload or sampling strategy. To assist in the submission of the research to a journal, it is important to note that the Methodology section of the manuscript should specify that sampling was for high-touch items where a high percentage of cultures are expected to be positive. This is important for providing context for the high percentage of positive cultures. This is indicated by [35-37].

CONCLUSIONS

The NICU environment showed a high degree of bacterial contamination, as evidenced by a high culture positivity rate of 95.1% in high-touch surfaces and non-critical devices. The Gram-positive bacteria dominated in this environment, accounting for 68.9% of all isolated bacteria. The most commonly isolated Gram-positive bacteria was Staphylococcus aureus, suggesting its high prevalence in this environment during this particular period. The presence of clinically relevant Gram-negative bacteria was also noted in this environment, with 31.0% of all isolated bacteria belonging to this group. The most commonly isolated Gram-negative bacteria in this environment was *Escherichia coli,* followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The qualitative analysis showed that the most predominant bacteria were consistently related to high-touch surfaces and devices, including incubators, suction tips, stethoscopes, door handles, healthcare workers' hands, and neonates' breathing tubes. The isolate distribution in this environment showed a high degree of concentration, with a small number of species representing a high percentage of the total number of isolated bacteria.

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