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Bacterial Contamination and Susceptibility Profile in Neonatal Examination Room (NER) at Al-Bayda Medical Center, Libya: A Cross-Sectional Study

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Abstract
Healthcare-associated diseases, especially in vulnerable neonates, may be complicated by environmental contamination in neonatal examination room (NER). The bacterial contamination profile and antibiotic susceptibility patterns of isolates obtained from a neonatal examination room were evaluated in this investigation. On May 13, 2025, a cross-sectional study was carried out in the neonatal unit of Al-Bayda Medical Center in Libya. Samples were collected from selected high-touch surfaces, medical equipment, and room air. The passive settle-plate method was used to sample room air, and sterile cotton swabs soaked with sterile normal saline were used to obtain surface samples. Standard microbiological techniques, such as colony morphology, Gram staining, selective media, and biochemical testing, were used to identify the bacterial isolates. The Kirby-Bauer disk diffusion method was used to test for antimicrobial susceptibility in accordance with CLSI M100 (2024) guidelines. 97% (n = 58) of the bacteria were Gram-positive, while only 3% (n = 2) were Gram-negative. *Micrococcus species* (31.7%),

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Kocuria species (30.0%), and *Bacillus species* (26.7%) were the most common isolates. Additionally, clinically significant bacteria were found, such as *Pseudomonas spp*. (3.3%), *Enterococcus spp*. (3.3%), and *Staphylococcus spp*. (5.0%). Among tested isolates, the highest sensitivity percentages were toward gentamicin (98.3%), clarithromycin (95.0%), imipenem (95.0%), ciprofloxacin (93.3%), and ceftriaxone (93.3%). Highest resistant' percentages were shown against ceftazidime (91.7%), oxacillin (68.3%), and erythromycin (58.3%). There was significant bacterial contamination in the neonatal examination room, with Gram-positive organisms predominating. cleaning, disinfection, and infection prevention and control procedures as well as routine environmental microbiological surveillance must be reinforced.

Keywords: Environmental bacterial contamination, Neonates ICU, Antibiotic sensitivity.

التلوث البكتيري ونمط الحساسية في وحدة العناية المركزة لحديثي الولادة في المركز الطبي الأبيض، ليبيا :دراسة مقطعية.

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الخلاصة

قد تتفاقم الأمراض المرتبطة بالرعاية الصحية، لا سيما لدى حديثي الولادة الأكثر عرضة للمضاعفات، نتيجة التلوث البيئي في وحدة العناية المركزة لحديثي الولادة .هدفت هذه الدراسة إلى تقييم نمط التلوث البكتيري وأنماط الحساسية للمضادات الحيوية للعزلات البكتيرية التي تم الحصول عليها من غرفة فحص حديثي الولادة .أُجريت دراسة مقطعية بتاريخ 13 مايو 2025 في وحدة حديثي الولادة بمركز البيضاء الطبي في ليبيا .جُمعت

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العينات من الأسطح الأكثر تعرضًا للمس اليدين، والمعدات الطبية، وهواء الغرفة . واستُخدمت طريقة أطباق الترسيب (Passive settle-plate method)لأخذ عينات الهواء، بينما استُخدمت مسحات قطنية معقمة مبللة بمحلول ملحي معقم لجمع العينات من الأسطح .وتم التعرف على العزلات البكتيرية باستخدام الطرق الميكروبيولوجية القياسية، بما في ذلك دراسة شكل المستعمرات، وصبغة غرام، والأوساط الزرعية الانتقائية، والاختبارات الكيميائية الحيوية .كما أُجري اختبار الحساسية للمضادات الحيوية باستخدام طريقة كيربي- باور للانتشار بالأقراص، وفقًا لإرشادات CLSI .M100 (2024)

النتائج: شكّلت البكتيريا موجبة الغرام نسبة 58) %97 عزلة)، في حين لم تتجاوز البكتيريا سالبة الغرام نسبة) %3 عزلتان .(وكانت أكثر الأنواع شيوعًا هي Micrococcus بنسبة (31.7%)، و Kocuria بنسبة (30.0%)، و Bacillus بنسبة .(26.7%) كما تم عزل أنواع بكتيرية ذات أهمية سريرية، منها *Pseudomonas spp.* بنسبة (3.3%)، و*Enterococcus spp.* بنسبة (3.3%)، و *Staphylococcus spp.* بنسبة .(5.0%)وأظهرت العزلات أعلى معدلات الحساسية تجاه الجنتاميسين (98.3%)، والكلاريثروميسين (95.0%)، والإيميبينيم (95.0%)، والسيبروفلوكساسين (93.3%)، والسيفترياكسون .(93.3%) في المقابل، سُجلت أعلى معدلات المقاومة تجاه السيفتازيديم (91.7%)، والأوكساسيلين (68.3%)، أظهرت نتائج الدراسة وجود تلوث بكتيري ملحوظ في غرفة فحص حديثي الولادة، مع predominance للبكتيريا موجبة الغرام .توصي الدراسة بتعزيز إجراءات التنظيف والتطهير، وتطبيق برامج فعالة للوقاية من العدوى ومكافحتها، إلى جانب إجراء المراقبة الميكروبيولوجية البيئية بشكل دوري لضمان سلامة بيئة الرعاية الصحية.

الكلمات المفتاحية: التلوث البكتيري البيئي، وحدة العناية المركزة لحديثي الولادة، الحساسية للمضادات الحيوية.

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1.1.Introduction

Neonates are particularly susceptible to opportunistic and healthcare-associated infections (HAIs). Sepsis is an important cause of morbidity and mortality during first 28 days of life. Untreated bacterial infections can quickly lead to many life-threatening diseases, such as sepsis, meningitis, pneumonia, septic shock and multi-organ failure (Bano et al., 2026, Liang and Yan, 2023). Due to their immature immune systems and frequent need for hospital-based treatment, HAIs, also known as named nosocomial infections are significant contributors to morbidity, mortality, extended hospital stays, and higher medical expenses, especially in countries with low resources (Dramowski et al., 2022, Organization, 2022). The risk of bacterial colonization and infection in neonatal units may be increased by extended hospital stays, invasive procedures, excessive antibiotic exposure, inadequate infection prevention strategies, and contact with contaminated medical equipment (Dramowski et al., 2022).

Hospitalization is not a prerequisite for a newborn to develop a bacterial infection as many infections originate from the mother during pregnancy or delivery, and others are acquired later from the home, community, or healthcare environment. But hospitalization mainly increases the risk of hospital-acquired infections, especially in premature infants or those requiring invasive procedures in the NER. Bacteria may be accumulated on environmental surfaces and neonatal care equipment, particularly in locations where hand contact and repeated clinical usage occur frequently (Porter et al., 2024, Hartz et al., 2015). Bacterial contamination of surfaces and equipment in NER, including incubators, suction devices, weighing machines, and other regularly touched things, has been documented in earlier research (Van de Vuurst and Escobar, 2023).

Cleaning, disinfection, and infection control procedures in neonatal care environments can be guided by the assessment of bacterial contamination from high-touch surfaces, medical equipment, room air, and healthcare worker contact points (Belay et al., 2024). Antibacterial resistance may limit therapeutic options and

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lead to treatment failure, which makes it a serious concern in infections linked to healthcare. Both commensal and clinically significant bacteria, such as those linked to skin, dust, air, water sources, and contaminated equipment, can be found in hospital settings. Gram-positive bacteria, such as *Micrococcus species, Kocuria species, Bacillus species, and Staphylococcus species*, are commonly found on dry hospital surfaces and may endure environmental stress (Chawla et al., 2023, Belay et al., 2024). Gram-negative organisms like *Pseudomonas species*. are clinically significant despite being less common in many environmental surveys and are linked to serious illnesses in susceptible patients (Schwartz et al., 2024).
This study aimed to examine the bacterial contaminants of the air and inanimate surfaces inside the NER at Al-Bayda Medical Center. Furthermore, to determine the antibacterial susceptibility pattern of the bacterial isolates recovered from investigated samples. Limited studies found investigated the NER at the targeted hospital, and this study helps provide a baseline data on environmental bacterial contamination and resistance patterns, which may inform infection control policies and environmental hygiene protocols in neonatal care settings. Significantly, this study helps provide a baseline for upcoming monitoring, infection control regulations, and multi-center research in Libyan healthcare facilities. The gathered data will close a significant knowledge gap at the local and national levels.

2. Materials and Methods

2.1. Study Design and Setting

This cross-sectional investigation was carried out in 2025, and the area of study was the neonatal examination room of Al-Bayda Medical Centre in Libya.

2.2. Sample Collection

sampling was conducted on the 13th of May (2025). Samples were taken once from 22 high-touch locations in the neonatal examination room prior to routine cleaning and disinfection, while

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the room was operating normally (Table 1). Sterile cotton swabs soaked with sterile normal saline were used to capture environmental surface samples; to enhance microbiological recovery, the swabs were rolled over roughly 10 × 10 cm surface regions. The sampling site was swabbed once. The passive settle-plate method was used to sample the air in the room. To evaluate the possible contribution of airborne bacteria, the plates were exposed for more than an hour at a height of around 1.2 meters above the ground close to a window. The colony was regarded as an indication of the presence/absence of growth.

Table (1): Sample Collection Sites

Site category	Sampled sites
Neonatal equipment	Infant weighing scale, infant radiant warmers, neonatal transport crib
Monitoring and electronic devices	Patient monitors, computer monitor screen, computer keyboard, computer mouse
Respiratory and resuscitation equipment	Resuscitation face mask, suction machine outer surface, suction tubing
Room environment	Door handle, window handle/latch, room air settle plates

2.3. Isolation and Identification of Bacteria

Swab samples were inoculated into nutrient agar, incubated aerobically at 37°C for 24 hours, and subsequently sub-cultured for purification. Bacterial isolates were identified by traditional methods, including colony morphology, Gram staining, different selective media, and biochemical assays. Colony morphology was evaluated on blood agar according to colony size, shape, pigmentation, surface texture, and hemolytic characteristics. Gram staining was employed to distinguish between Gram-positive and Gram-negative bacteria. Cetrimide agar was employed for the selective isolation of *Pseudomonas aeruginosa*, whilst Mannitol Salt Agar was utilized for the preliminary differentiation of *staphylococcal species*. Catalase and oxidase assays were employed as initial differentiation tests. Coagulase and DNase assays were employed to distinguish *Staphylococcus aureus* from other

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Staphylococcal species, whereas spore staining was utilized to verify *Bacillus species*. The entire study was conducted with quality-control procedures in place. Bacterial isolation and sample collection were done using sterile cotton swabs and culture media. To reduce contamination, normal microbiological protocols were followed during specimen processing, and all culture mediums were examined for sterility before use.

2.4. Ethical considerations

Since the study only used environmental samples and excluded human participants, patient data, and animal subjects, ethical approval was not necessary.

2.5. Antibiotic Susceptibility Testing

In accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI M100, 2024), the Kirby-Bauer disk diffusion method with Mueller-Hinton agar were used to test the susceptibility of isolated bacteria towards a panel of references belonging to different antibiotic classes: ciprofloxacin, clarithromycin, imipenem, amoxicillin–clavulanic acid, ceftriaxone, trimethoprim–sulfamethoxazole, ceftazidime, gentamicin, oxacillin, cefaclor, cefoxitin, erythromycin, and cefixime. The antibiotic discs were placed on the surface of Mueller-Hinton agar plates and incubated at 37°C for 18 hours. Inhibition zones were measured in (mm).

2.6. Statistical analysis

Data were entered into Microsoft Excel 2021 and analyzed descriptively. Bacterial isolates and antimicrobial susceptibility results were summarized as frequencies and percentages.

3. Results

Following purification and bacterial identification, 60 bacterial isolates were recovered and examined from a total of 22 culture plates.

3.1. Distribution of Environmental Bacterial Isolates

The distribution of environmental bacterial isolates revealed that *Micrococcus luteus* constituted the highest proportion of isolates (31.7%), followed by *Kocuria varians* (30.0%) and *Bacillus cereus*

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(26.7%). Other environmental organisms were detected at lower frequencies; *Staphylococcus aureus* accounting for (5.0%) of the isolates, while *Enterococcus* spp. and *Pseudomonas aeruginosa* each represented (3.3%) of the total environmental isolates (Table 2).

Table (2): Distribution of Bacterial Isolates Recovered from Environmental Samples

Organism	**Frequency**	**Percentage** (%)
Micrococcus luteus	19	31.7
Kocuria varians	18	30.0
Bacillus cereus	16	26.7
Staphylococcus aureus	3	5.0
Enterococcus spp.	2	3.3
Pseudomonas aeruginosa	2	3.3

3.2. Gram-Staining Classification of Environmental Isolates

The overall classification of environmental bacterial isolates demonstrated a marked predominance of Gram-positive organisms, accounting for 97% (n = 58) of the total isolates, whereas Gram-negative bacteria were identified at a considerably lower frequency, representing only 3% (n = 2) of the total isolates (Figure 1).

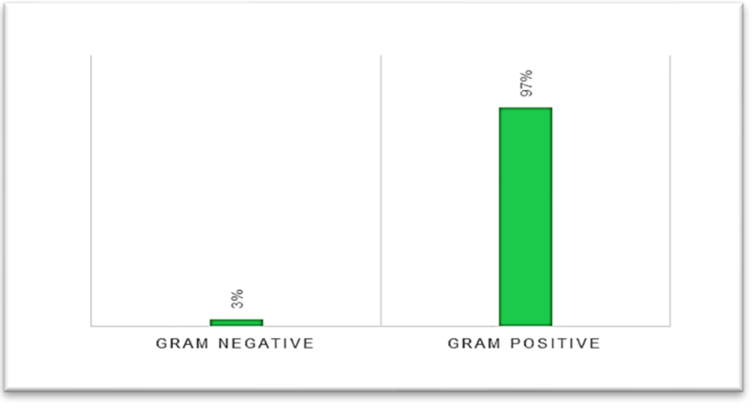

Figure (1): The percentages of Gram-Positive and Gram-Negative Environmental Isolates.

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3.3. Environmental Contamination Pattern

The number of isolates recovered per culture plate, demonstrating mixed and polymicrobial contamination patterns (Figure2). Three bacterial mix contamination per plate was the most frequent pattern, accounting for 48% of the plates, followed by two-isolate contamination at 26%. Single-isolate and four-isolate contamination were each observed in 13% of the samples. Table (3) represents the distribution of identified bacteria among the collection sites.

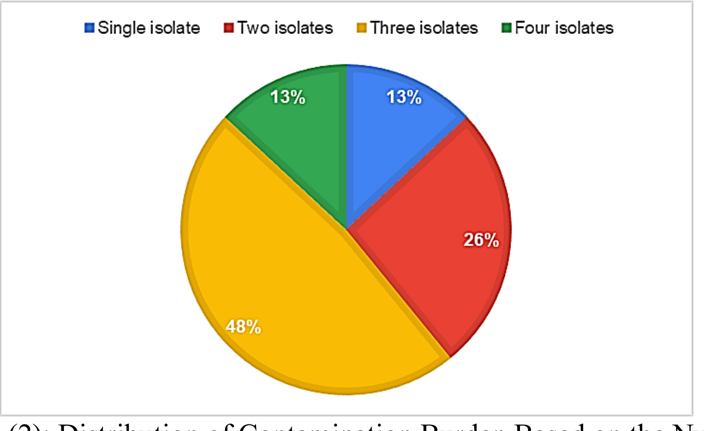

Figure (2): Distribution of Contamination Burden Based on the Number of Isolates Recovered per Culture

Table (3): Distribution of Bacterial Isolates According to Sampled Sites

Sampled site	Specific sampled part	Bacteria recovered
Infant radiant warmer (1,2,3)	Bed handle / front rail	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus*
Infant radiant warmer	Control panel	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus*
Infant radiant warmer	Mattress	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus*, *Pseudomonas aeruginosa* *Staphylococcus aureus*
Infant radiant warmer	Transparent side panels	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus*

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Sampled site	Specific sampled part	Bacteria recovered
Patient monitor (1,2,3)	Screen	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus* , *Staphylococcus aureus*
Computer monitor	Screen	*Micrococcus luteus*, *Kocuria varians*, *Bacillus cereus*
Computer keyboard	Keyboard keys	*Micrococcus luteus*, *Kocuria varians*
Resuscitation face mask	Inner surface	*Kocuria varians*, *Micrococcus luteus*, *Enterococcus* spp.
Window	Window handle / latch	*Kocuria varians*, *Enterococcus* spp.
Suction machine	Outer surface	*Kocuria varians*, *Micrococcus luteus*
Suction machine	Suction tubing	*Bacillus cereus*
Infant weighing scale	Weighing scale surface	*Micrococcus luteus*, *Kocuria varians*
Door	Door handle	*Bacillus cereus*, *Micrococcus luteus*, *Kocuria varians*
Room air	Settle plate (1,2,3)	*Bacillus cereus*, *Micrococcus luteus*, *Kocuria varians*
Neonatal transport crib	Bed surface / mattress	*Bacillus cereus*, *Micrococcus luteus*, *Kocuria varians*, *Pseudomonas aeruginosa*

3.4 Antibiotic Susceptibility Analysis of Environmental Samples

Antimicrobial susceptibility testing of the environmental isolates showed variable responses to the tested antibiotics. Gentamicin demonstrated very high efficacy (98.3%), alongside clarithromycin (95.0%), ciprofloxacin (93.3%), and ceftriaxone (93.3%). In contrast, the lowest efficacy rates were observed for ceftazidime (91.7%), oxacillin (68.3%), and erythromycin (58.3%), Percentages were calculated based on a total of 60 bacterial isolates (Table 4).

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Table (4): Number and percentages of isolated samples, sensitive, resistant and intermediate to Antibiotic Susceptibility Patterns of Environmental Bacterial Isolates

Antibiotic	Number of Sensitive samples (%)	Number of Resistant (%)	Number of Intermediate n (%)
Gentamicin	59 (98.3)	0 (0.0)	1 (1.7)
Ciprofloxacin	56 (93.3)	0 (0.0)	4 (6.7)
Clarithromycin	57 (95.0)	0 (0.0)	3 (5.0)
Imipenem	57 (95.0)	0 (0.0)	3 (5.0)
Amoxicillin-Clavulanic acid	50 (83.3)	8 (13.3)	2 (3.3)
Ceftriaxone	56 (93.3)	2 (3.3)	2 (3.3)
Trimethoprim-Sulfamethoxazole	45 (75.0)	11 (18.3)	4 (6.7)
Ceftazidime	2 (3.3)	55 (91.7)	3 (5.0)
Oxacillin	17 (28.3)	41 (68.3)	2 (3.3)
Cefaclor	30 (50.0)	26 (43.3)	4 (6.7)
Cefoxitin	35 (58.3)	25 (41.7)	0 (0.0)
Erythromycin	22 (36.7)	35 (58.3)	3 (5.0)
Cefixime	32 (53.3)	24 (40.0)	4 (6.7)

3.5. DISCUSSION

This study aimed to identify the bacterial profile and antimicrobial susceptibility patterns of environmental isolates recovered from a neonatal examination room. The results demonstrated bacterial contamination, with a predominance of Gram-positive bacteria and a high frequency of polymicrobial contamination. This finding is important because neonatal-care surfaces and equipment can act as reservoirs for healthcare-associated bacteria, particularly in units caring for vulnerable neonates (Bhatta et al., Dramowski et al., 2022). The high percentage of polymicrobial contamination suggests the presence of multiple bacterial sources within the neonatal examination room environment. This may reflect contamination from high-touch surfaces, shared equipment, room air, and repeated contact by healthcare personnel. Similar concerns have been reported in neonatal units, where shared equipment, inadequate environmental

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cleaning, and suboptimal hand hygiene may contribute to bacterial transmission pressure (Dramowski et al., 2022).

This study revealed that Gram-positive bacteria represented 97% of the identified isolates. This predominance may reflect the ability of skin and environment associated Gram-positive organisms to persist on dry hospital surfaces. *Micrococcus*, *Kocuria*, and *Bacillus* species were the most common isolates, accounting for the majority of recovered bacteria (Ribeiro et al., 2019, Bano et al., 2026, Centeleghe et al., 2022).

Their high frequency may be related to their common presence on skin, dust, air, hospital surfaces, and frequently handled equipment. Hospital built environments are recognized as reservoirs of diverse microorganisms, including opportunistic and healthcare-associated bacteria (Christoff et al., 2019).

The detection of clinically significant bacteria, including *Staphylococcus* species, *Enterococcus* species, and *Pseudomonas* species, is noteworthy. Although these organisms were recovered at low frequencies, their presence in a neonatal examination room warrants attention because of their potential clinical relevance. Similarly reported potential pathogens from NER surfaces and equipment, supporting the importance of environmental monitoring in neonatal-care areas (Johnson et al., 2021).

Antimicrobial susceptibility testing showed high in vitro susceptibility rates to gentamicin, ciprofloxacin, clarithromycin, imipenem, and ceftriaxone. These findings should be interpreted as laboratory susceptibility patterns of environmental isolates, not as evidence of clinical treatment effectiveness. The observed susceptibility may reflect lower antibiotic selective pressure among some environmental isolates compared with clinical pathogens. Rashid reported higher resistance rates among clinical isolates than environmental isolates, while Bhatta, also reported susceptibility of many NER environmental isolates to aminoglycosides as this study revealed with gentamicin (Riaz et al., 2012). Conversely, resistance was observed to ceftazidime, oxacillin, and erythromycin. These findings indicate the presence of resistant environmental isolates and support the need for routine cleaning, disinfection, and

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microbiological surveillance. However, antimicrobial susceptibility results should be interpreted cautiously because some antibiotics and clinical breakpoints may not be applicable to all environmental organisms when results are analyzed collectively.

Antibiotic susceptibility testing, performed on pooled environmental isolates, showed higher resistance rates ceftazidime, oxacillin, and erythromycin. However, because these percentages were calculated from pooled isolates of diverse genera, species-specific interpretations cannot be drawn from these data. Nevertheless, they highlight that the environment within the NER is contaminated, and necessary precautions and treatments must be taken to protect children from potential exposure to these contaminants, especially given their weakened immune systems. These findings support routine environmental monitoring and strengthened cleaning and disinfection practices in neonatal care areas.

Several limitations should be considered. The study was conducted in a single medical facility, sampling done once, the colonies were not calculated, and only regarded as an indication for either presence or absence of growth. Bacterial identification was based on conventional methods without molecular confirmation. Therefore, the true microbial diversity may have been underestimated, as culture-dependent methods can miss organisms that are difficult to cultivate (Christoff et al., 2019). Despite these limitations, this study identified polymicrobial contamination, predominance of Gram-positive organisms, clinically significant bacteria, and antimicrobial susceptibility patterns among environmental isolates recovered from a neonatal examination room. These findings support the need for continued environmental surveillance and strengthened infection-control measures in neonatal-care areas.

3.6. Conclusion

Gram-positive bacteria, such as *Micrococcus species, Kocuria species, and Bacillus species,* were the most common type of bacteria found in the neonatal examination room. At lower

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frequencies, the study also detected potentially pathogenic bacteria (including *Staphylococcus aureus*, *Enterococcus spp*., and *Pseudomonas aeruginosa*) in the environment, which underscores the need for enhanced infection control measures to prevent possible transmission to vulnerable neonates. Polymicrobial contamination was seen in a significant percentage of culture plates, indicating contamination from many environmental sources.

3.7. Recommendation:

These results reinforce cleaning, disinfection, and infection prevention and control procedures as well as routine environmental microbiological surveillance. Also, the study output recommends studying, evaluating, and approving the fewest possible number of the most effective antibiotics to reduce the spread of bacterial resistance to antibiotics. Also, the study advised: molecular identification to confirm species-level taxonomy, quantitative CFU counts to enable comparison with established hygiene benchmarks; repeated sampling across multiple time points, and multi-center designs to improve generalizability. Additionally, studies linking environmental contamination patterns to actual neonatal infection rates (surveillance studies) are needed to establish the clinical impact of these environmental findings.

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