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Isolation and Identification of Methicillin-resistant Staphylococcus aureus in the Neonatal Intensive Care Unit of Zliten Medical Center

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Abstract
Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern in neonatal intensive care units (NICUs) due to its resistance to β-lactam antibiotics and its capacity to cause severe infections in vulnerable newborns. **Objective:** This study aimed to isolate and identify MRSA in the NICU of Zliten Medical Center, Libya, assess its prevalence among neonates, healthcare workers (HCWs), and equipment, and evaluate antimicrobial susceptibility patterns. **Methods:** A total of 140 samples were collected from March to May 2025, comprising swabs from neonates (n=80), HCWs (n=22), and medical equipment (n=38). Identification and antibiotic susceptibility testing (AST) were performed using standard microbiological methods and the BD Phoenix automated system. **Results:** MRSA was detected in 3.6% of total samples (5/140), including two isolates from neonates (2.5%), two from HCWs (9.0%), and one from equipment (2.7%). All isolates exhibited absolute resistance (100%) to β-lactams, while remaining fully susceptible (100%) to Vancomycin, Linezolid, Daptomycin, and Teicoplanin. **Conclusion:** The relatively low overall prevalence should not diminish the clinical significance of MRSA in high-risk populations, particularly given the higher colonization rate among staff. These findings underscore the necessity for continuous surveillance, stringent infection control measures, and antimicrobial stewardship programs in Libyan NICUs to protect neonates and prevent the spread of resistant pathogens.

Keywords: β-lactams, antibiotic susceptibility, MRSA, neonatal intensive care units, Zliten Medical Center.

Introduction
Methicillin-resistant *Staphylococcus aureus* (MRSA) is defined as any strain of *S. aureus* that has acquired resistance to methicillin and other β-lactam antibiotics, including penicillins and cephalosporins. MRSA was first identified in 1961, shortly after the introduction of methicillin, and has since become a global public health concern (Larsen et al., 2022). *S. aureus* is a Gram-positive coccus that commonly colonizes the skin and mucous membranes of humans. While often a commensal organism, it can act as an opportunistic pathogen and is capable of causing a wide array of infections. One of the major clinical challenges associated with *S. aureus* is its ability to acquire resistance to antibiotics, particularly β-lactams (Gurung et al., 2020).
The epidemiology of MRSA has evolved significantly over the decades. Initially confined to healthcare settings, where it was termed hospital-associated MRSA (HA-MRSA), the organism later emerged in community settings, giving rise to community-associated MRSA (CA-MRSA). HA-MRSA remains predominant in high-risk hospital environments such as intensive care units (ICUs), including neonatal intensive care units (NICUs). Studies have shown that MRSA colonization rates in NICUs vary globally (Esemu et al., 2024). Neonates, especially preterm and low birth weight (LBW) newborns, have underdeveloped immune systems and immature skin and mucosal barriers, making them more susceptible to colonization and infection by nosocomial pathogens such as MRSA.
Several risk factors contribute to this vulnerability, including prolonged hospitalization, the use of invasive devices such as central lines, ventilators, and urinary catheters, frequent handling by healthcare personnel, exposure to broad-spectrum antibiotics that disrupt normal flora, and close proximity to colonized or infected neonates (Rallis et al., 2025).
A large review of 37 studies found that MRSA accounts for roughly 8.8% of all neonatal sepsis cases globally, with a distinct predilection for late-onset rather than early-onset sepsis (Esemu et al., 2025). While some regions, such as Shenzhen, China, report low colonization rates of 0.8% (Lin et al., 2018), other cohorts show rates as high as 8.4%, with nearly one-quarter of colonized neonates progressing to active infection (Wu et al., 2023). However, the escalating burden of antimicrobial resistance in Libya is characterized by a high prevalence of MRSA across diverse clinical and regional settings. Over the past decade, MRSA has emerged as a dominant pathogen, with prevalence rates ranging between 54% and 68% among *S. aureus* isolates

recovered from surgical site infections and burn patients (Ghenghesh et al., 2013). This high clinical burden is closely linked to the role of healthcare professionals as transmission vectors; a large-scale screening of 569 healthcare workers across four Tripoli hospitals identified a 19% (109/569) MRSA carriage rate via PCR confirmation, indicating that clinical staff represent a significant reservoir for methicillin resistance in the country (Ahmed et al., 2012).

Beyond human vectors, the physical environment serves as a secondary reservoir. Research in Benghazi identified MRSA on 9.5% of ICU surfaces, including frequently handled items such as beds and door handles (Al-Abdli and Baiu, 2016). On a global scale, these carriage rates vary by region, with healthcare worker carriage reaching 50% in the Western Pacific compared to 17.6% in Africa (Keneh et al., 2024).

This study focused on isolating and identifying MRSA in the NICU of Zliten Medical Center (ZMC), assessing the prevalence of MRSA among neonates, healthcare workers (HCWs), and NICU equipment, and analyzing antimicrobial resistance patterns. In addition, the study provides local data on MRSA risk and prevalence in the NICU of ZMC.

The objectives of this study were:

1. To isolate and identify MRSA in the NICU of ZMC.
2. To determine the prevalence rate of MRSA in the NICU of ZMC.
3. To determine the antibiotic susceptibility pattern of the isolated MRSA.

Materials and Methods

Sample Collection

A total of 140 samples were collected from three sources within the NICU: 80 neonatal swabs, 38 environmental swabs, and 22 swabs from medical staff. These samples were cultured to isolate MRSA over a period of three consecutive months, from March 2025 to May 2025. Random sampling was carried out two to three times per week (every third or fourth day) during the study period to ensure a representative distribution of data. All samples were collected using sterile techniques from the following sources:

Admitted neonates: Swab samples were obtained from the nasal cavity, groin, axilla, and umbilical stump to assess potential sites of colonization.

HCWs: Nasal swabs were taken from nurses, doctors, and assistants working in the NICU to investigate possible microbial carriage among staff.

NICU environment (equipment and surfaces): Swabs were collected from surfaces to evaluate environmental contamination. The samples were obtained from the surfaces of frequently used medical equipment (incubators, ventilator machines, suction machines, pulse oximeters, phototherapy units, trolleys, infusion pumps, and syringe pumps).

All collected samples were properly labeled and immediately transported to the microbiology laboratory following established procedures to ensure the integrity of the specimens and the accuracy of the results.

Process of Swabbing

A sterile dry cotton swab was properly labeled, and once opened, it was immediately moistened with a drop of sterile normal saline using a sterile syringe, followed by swabbing of the target site.

Neonatal swabbing

In the case of neonatal swabbing, a special record sheet was completed for every targeted newborn. The recorded data included code number, date of swabbing, mode of delivery, date of birth, date of admission, cause of admission, gender, birth weight, gestational age, and whether the case was inborn or referred. A moistened nasal swab was gently inserted into the anterior nares of the newborn and rotated for a few seconds. This procedure was performed for both nostrils. The swab was then reinserted into its tube. The same procedure was followed for swabbing other skin sites.

Medical staff swabbing

To ensure participant confidentiality, HCWs were identified solely by assigned code numbers. Subsequently, nasal swabs were collected from the anterior nares following the standardized procedures previously described.

Surface swabbing

For environmental and equipment surface swabbing, the code number, serial number of the equipment, and the name of the swabbed surface were recorded. A large area of the involved surface was swabbed.

Laboratory Processing

Process of culturing

Three types of media were prepared for inoculation of each sample: Blood agar, MacConkey agar, and Mannitol salt agar (MSA). Each agar plate was clearly labeled at the bottom with the swab code number, date, and time. This information was also recorded on the periphery of each plate.

The inoculation process, in accordance with Sanders, was carried out using a sterile disposable plastic loop for streaking the agar plates to achieve culture dilution and separation of colonies. Each plate was covered with its lid and placed in the incubator in an inverted position at a temperature of 35–37 °C. After an incubation period of 18–24 hours, the cultures were examined, focusing on the morphological characteristics of colonies grown on Blood and MacConkey agars, such as shape, surface texture, color, and special features like hemolysis patterns (Sanders, 2012).

For confirmation of MRSA, growth on MSA was examined for characteristic golden-yellow colonies. Next, Gram staining and microscopic examination were performed. In addition, the isolates were confirmed using the BD Phoenix™ Automated System to ensure precise taxonomic classification.

Biochemical Tests

Upon confirmation of Gram-positive cocci via microscopic examination, isolates were subjected to catalase and coagulase testing for definitive identification. Catalase-positive isolates were further evaluated using both slide and tube coagulase methods to differentiate *S. aureus* from coagulase-negative staphylococci (CoNS).

Manual and Automated AST

Pure isolates of *S. aureus* were initially screened for methicillin resistance using the cefoxitin disk diffusion method. Subsequently, definitive identification and comprehensive antimicrobial susceptibility testing (AST) were performed using the BD Phoenix™ Automated Microbiology System.

Laboratory Tests

• Gram staining

Pure, fresh isolates were subjected to the Gram stain procedure to determine their morphology and cell wall characteristics, following the standardized protocol by Smith and Hussey (2005).

• Mannitol salt agar

To selectively isolate *S. aureus*, samples were inoculated onto MSA. The yellow-pigmented colonies were tentatively identified as *S. aureus* and subsequently selected for further biochemical and automated confirmation (Shields and Tsang, 2006).

• Catalase test

The catalase test was performed following the protocol described by Reiner (2010) to differentiate staphylococci from streptococci.

• Coagulase test

1. Slide method

The slide coagulase test was conducted following the protocol established by Katz (2010) to detect bound coagulase (clumping factor).

2. Tube method

The tube coagulase test was performed as the definitive method to detect free coagulase, following the protocol by Katz (2010).

• Antibiotic susceptibility (Disc diffusion test)

The antimicrobial susceptibility of the *S. aureus* isolates was determined using the Kirby–Bauer disk diffusion method in accordance with ASM protocols (Hudzicki, 2009). Initially, a bacterial suspension was prepared in sterile saline and adjusted to a 0.5 McFarland standard using a densitometer. This suspension was then evenly streaked over the entire surface of Mueller–Hinton agar (MHA) plates in three directions using a sterile cotton swab to ensure confluent growth. Within 15 minutes of inoculation, a 30 µg cefoxitin disk was aseptically applied to the agar surface. The plates were subsequently inverted and incubated at a controlled temperature of 33–35 °C for 16–18 hours. Post-incubation, the zones of inhibition were measured with a calibrated ruler, and results were interpreted based on the CLSI M100 criteria, where a zone diameter of ≤ 21 mm identified the isolate as MRSA.

Automated Identification

For definitive identification and AST, the BD Phoenix™ Automated Microbiology System was utilized. The system's PMIC/ID panels were inoculated with a standardized bacterial suspension (0.5 McFarland) prepared from pure, fresh cultures.

Statistical Analysis

Data were processed and analyzed using the Statistical Package for the Social Sciences (SPSS), Version 27. Descriptive statistics, including frequencies and percentages, were utilized to summarize categorical variables such as demographic data and the prevalence of MRSA isolates (Mishra et al., 2019).

Results

Microbiological profile and distribution of isolated microorganisms

The results of the present study indicated that 63 isolates showed no growth, while the rest (77 isolates) showed bacterial and fungal goth. Microbiological profile and Gram stain distribution are illustrated in Figure 1.

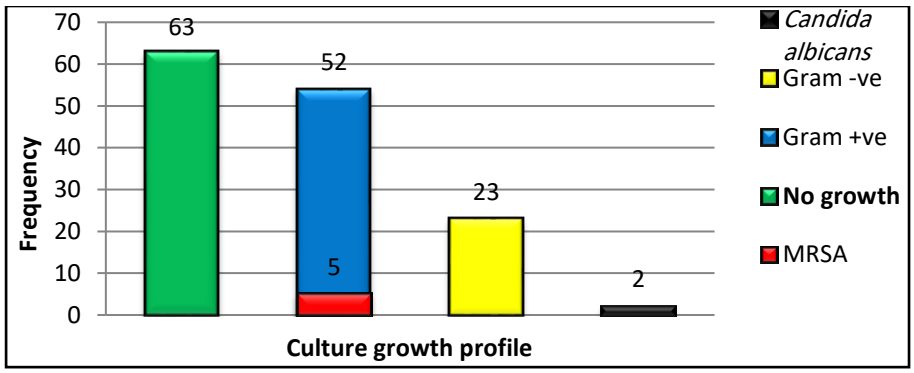

Figure 1: Frequency and distribution of microbial growth profile in the NICU.

Notably, MRSA accounted for 3.6% (5 isolates) of the total results, with a concerning distribution across all three sampling categories, including two isolates recovered from medical staff and one from the environment. In contrast, MSSA was isolated in three cases (2.1%) from neonatal swabs (table 1).

Table (1): Frequency and distribution of isolated microorganisms in the NICU.

Couture results	Frequency in neonatal swabs	Frequency in environmental swabs	Frequency in medical staff swabs	Total	Percent
No growth	24	32	7	63	45%
Staphylococcus epidermidis	12	1	9	22	15.8%
Staphylococcus haemolyticus	19	1	1	21	15%
Acinitobacter baumannii	8	3	0	11	7.8%
MRSA	**2**	**1**	**2**	**5**	**3.6%**
Klebsiella pneumonia	4	0	1	5	3.6%
Escherichia coli	3	0	0	3	2.2%
MSSA	3	0	0	3	2.1%
Moraxella catarrhalis	2	0	0	2	1.5%

Candida albicans	1	0	1	2	1.5%
Enterococcus faecalis	1	0	0	1	0.7%
Klebsiella oxytoca	1	0	0	1	0.7%
Staphylococcus capitis	0	0	1	1	0.7%
Total	80	38	22	140	100%

Neonatal screening results

Demographic and clinical characteristics of studied neonates

Clinical and demographic parameters were recorded and analyzed to characterize the study population.

1- Sex distribution

Figure (2) illustrates the distribution of neonates by gender and the corresponding prevalence of MRSA colonization. The vast majority of the study population were males, accounting for 63.7% (n=51) of the total sample, while females constituted 36.3% (n=29). one MRSA isolate was recovered from a male neonate, Similarly, one MRSA isolate was detected among the females. statistical analysis indicates that sex does not significantly influence the risk of MRSA colonization in the NICU setting (P > 0.05)

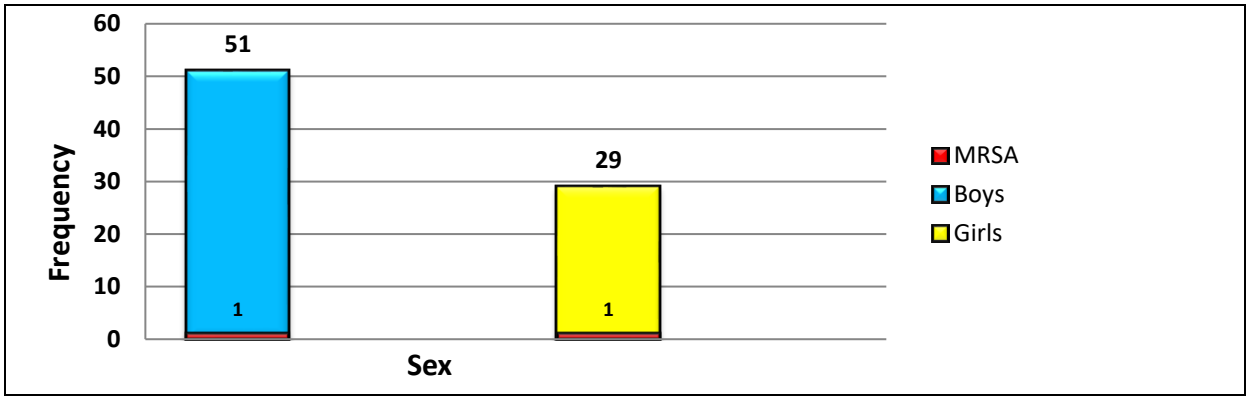

Figure 2: Distribution of MRSA colonization by sex.

2 - Referral status (inborn/outborn)

Microbiological analysis as illustrated in Figure 3, revealed that MRSA colonization was equally distributed between the two groups, with one isolate detected in the inborn group (2.0% subgroup prevalence) and one in the outborn group (3.3% subgroup prevalence). Statistical evaluation using Fisher's Exact Test confirmed that there was no significant association between referral status and the risk of MRSA acquisition (P = 1.00). These findings indicate that MRSA colonization was not significantly influenced by whether the infant was born within the facility or referred from an external source.

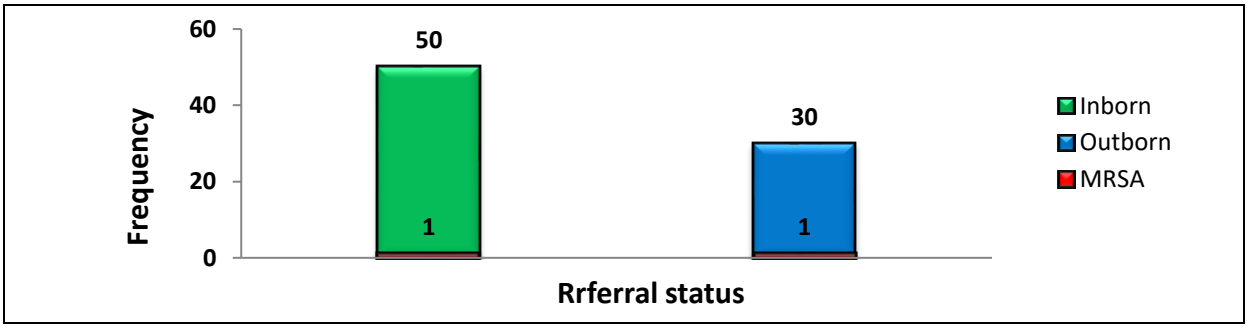

Figure 3: Distribution of MRSA colonization by referral status.

3- Birth weight categories

The distribution of neonates according to birth weight categories is presented in the provided figure 4. The vast majority of the study sample, 92.5% (n=74), were classified as Normal Birth Weight (NBW) with a

weight of ≥2.5 kg. LBW infants (1.5 - < 2.5 kg) accounted for 5% (n=4), while Extremely Low Birth Weight (ELBW) infants (< 1 kg) represented 2.5% (n=2) of the total cohort. Regarding microbiological findings (as shown in Figure 5), the two MRSA isolates identified in this study were recovered exclusively from the NBW group, resulting in a subgroup prevalence of 2.7%. No MRSA cases were detected in the LBW or ELBW categories. Statistical analysis using Fisher's Exact Test showed no significant correlation between birth weight and MRSA colonization (P = 1.00), indicating that birth weight was not a significant risk factor for MRSA acquisition in this sample.

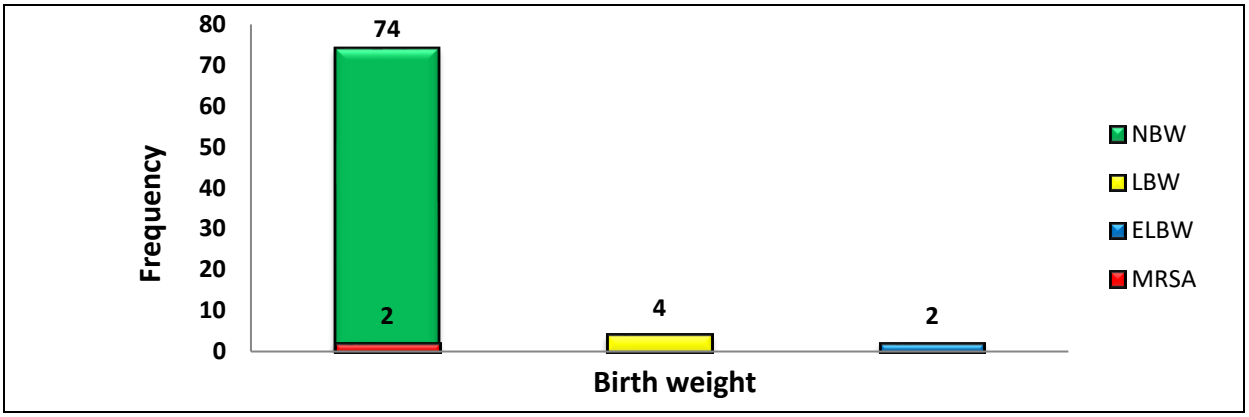

Figure 4: Distribution of MRSA colonization by birth weight.

4 - Mode of delivery

The distribution of neonates according to the mode of delivery is illustrated in Figure 5. Normal Vaginal Delivery (NVD) was the most frequent method, accounting for 46.3% (n=37) of the total births. Caesarean sections (C/S) constituted the remainder of the sample, with 28.7% (n=23) being Urgent C/S and 25.0% (n=20) being elective C/S.

Regarding microbiological results, the two MRSA isolates identified in the neonatal group were recovered from different delivery modes: one case was associated with NVD (subgroup prevalence of 2.7%), and the other case was associated with elective C/S (subgroup prevalence of 5.0%). No MRSA colonization was detected among neonates delivered via Urgent C/S. Statistical analysis using Fisher's Exact Test was performed to evaluate the impact of delivery mode on MRSA acquisition, which yielded no statistically significant association (P = 0.697). This indicates that the mode of delivery did not serve as a significant risk factor for MRSA colonization in this study cohort.

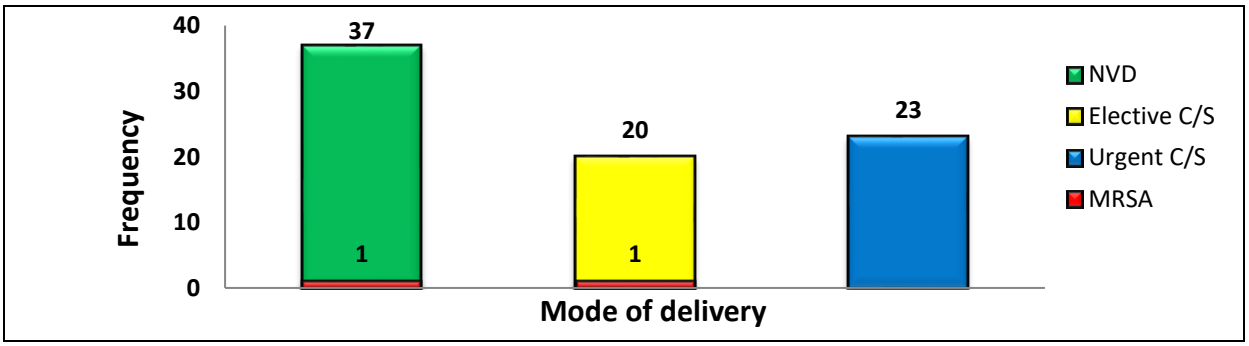

Figure 5: Distribution of MRSA colonization by mode of delivery.

5 - Duration of NICU admission

The length of stay in the NICU for the study population is detailed in Figure 6. The highest frequency of admissions lasted between 1 to 2 days, involving 35.0% (n=28) of the neonates, followed closely by those staying 3 to 4 days (27.5%, n=22). A smaller proportion of the sample required extended hospitalization, with 10.0% (n=8) staying longer than 10 days, while 3.8% (n=3) were admitted for less than 24 hours.

The results indicated no statistically significant association (P > 0.05), suggesting that in this cohort, the length of stay was not a primary determinant for MRSA colonization at the time of sampling.

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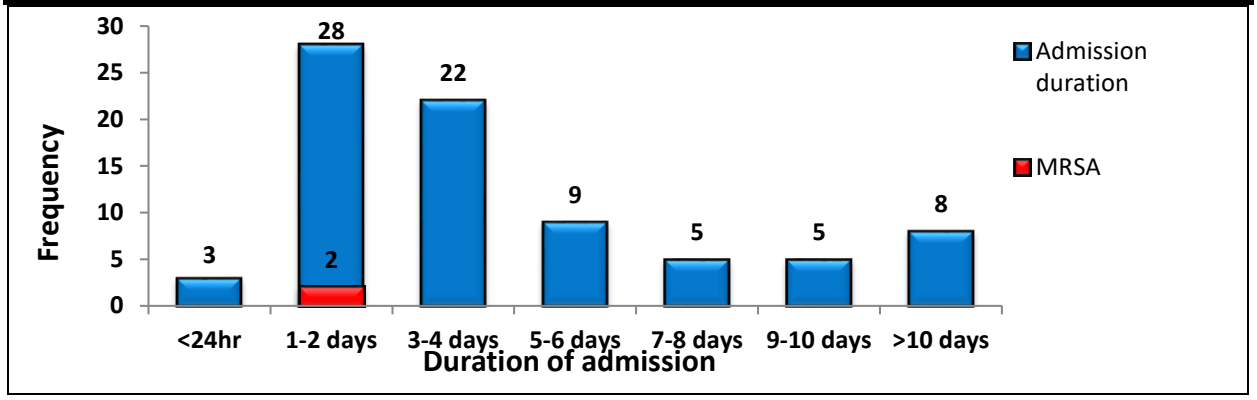

Figure 6: Distribution of MRSA colonization by duration of admission.

NICU environmental screening results

The microbiological profile of the NICU environment was assessed through the screening of various equipment and surfaces, as detailed in Table 3. A single isolate of MRSA was recovered from the suction machines, representing an environmental prevalence of 2.7% (n=1). Among the samples that showed bacterial presence, Gram-negative organisms were the most frequent, with *Acinetobacter baumannii* being isolated from incubators, ambubag masks, and trolley surfaces, accounting for 7.9% (n=3) of the total environmental samples. Other isolated organisms included *Staphylococcus haemolyticus* found on incubators and *Staphylococcus epidermidis* detected on NICU glass partitions, each representing 2.7% (n=1) of the samples.

Table (3): Distribution of isolated microorganisms from environmental surfaces.

Place of Swab	no growth	*staphylococcus epidermidis*	*staphylococcus haemolyticus*	MRSA	*acinetobacter baumannii*	Total
Incubators	10	0	1	0	1	12
Monitors	5	0	0	0	0	5
M.V	4	0	0	0	0	4
Infusion pumps	4	0	0	0	0	4
Suction machines	3	0	0	1	0	4
NICU glass partitions	2	1	0	0	0	3
Ambubags masks	1	0	0	0	1	2
Trolleys surfaces	1	0	0	0	1	2
Phototherapy machines	2	0	0	0	0	2
Total	32	1	1	1	3	38
Percent	84.3%	2.7%	2.7%	2.7%	7.9%	100%

Medical staff screening results

The microbiological screening of HCWs in the NICU provided critical data on potential colonization reservoirs among staff members, as illustrated in figure (7). Out of the 22 staff swabs collected, 68.2% (n=15) exhibited positive microbial growth, while 31.9% (n=7) remained sterile. The predominant isolate identified among the staff was *Staphylococcus epidermidis*, which was found in 41.0% (n=9) of the samples. Other Gram-positive isolates included *Staphylococcus haemolyticus* and *Staphylococcus capitis*, one isolate for each (4.5%, n=1). Gram-negative colonization was notably low, represented by a single isolate of *Klebsiella pneumoniae* (4.5%, n=1). Additionally, one case of *Candida albicans* (4.5%, n=1) was recorded. Most significantly, MRSA was identified in 9.0% (n=2) of the healthcare staff, which represents a higher subgroup prevalence compared to the neonatal (2.5%) and environmental (2.7%) samples.

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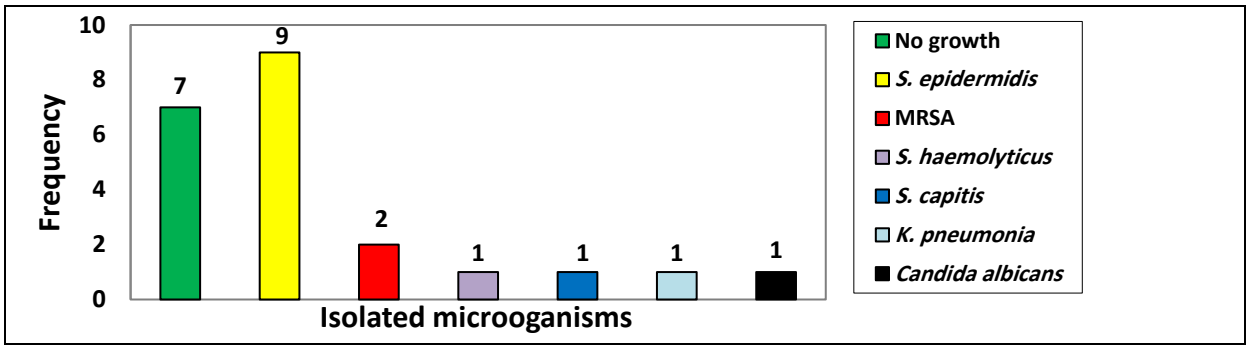

Figure 7: Distribution of isolated microorganisms from HCW nasal swabs.

Antimicrobial susceptibility profile of MRSA isolates

The antimicrobial susceptibility patterns of the five confirmed MRSA isolates were evaluated against a broad panel of antibiotics. All five isolates (100%) demonstrated complete susceptibility to a wide range of critical therapeutic agents, including Daptomycin, Teicoplanin, Vancomycin, Linezolid, and Tigecycline. Furthermore, high-level effectiveness (100%) was observed for Rifampin, Nitrofurantoin, and fluoroquinolones such as Ciprofloxacin, Levofloxacin, and Moxifloxacin. Conversely, absolute resistance (100%, n=5) was recorded for all beta-lactam antibiotics tested, including Penicillin G, Ampicillin, Oxacillin, Cefoxitin, and Cefotaxime, confirming the multidrug-resistant nature of these strains. Intermediate levels of resistance were noted for other antimicrobial classes. Gentamicin showed the highest resistance rate among non-beta-lactams at 60% (n=3), followed by Clindamycin and Erythromycin, which both exhibited a 40% (n=2) resistance frequency. Lower resistance rates of 20% (n=1) were identified for Ceftaroline, Tetracycline, and Trimethoprim-sulphamethoxazole. These results indicate that while the MRSA isolates remain highly sensitive to glycopeptides and newer generation antibiotics, they exhibit significant co-resistance to several commonly used clinical agents, necessitating a tailored approach to neonatal empiric therapy.

Discussion

Distribution of MRSA in the NICU of ZMC

The findings of this study reveal an overall MRSA prevalence of 3.6% (5/140) within the NICU at ZMC. When disaggregated, the data show a colonization rate of 2.5% (2/80) among neonates, 2.6% (1/38) in the NICU environment, and a notably higher rate of 9.0% (2/22) among medical staff. The neonatal colonization rate of 2.5% is significantly lower than the global cumulative incidence of 7.2% reported in recent meta-analyses (Esemu et al., 2024). Similarly, studies conducted in developed countries such as the United States, the United Kingdom, and parts of Europe have reported colonization rates ranging from 3.9% to 8.4% (Wu et al., 2023). On the other hand, it is higher than the 0.8% reported in a cohort from Shenzhen, China, illustrating how MRSA prevalence varies with regional epidemiology, infection control practices, and patient characteristics (Lin et al., 2018). Furthermore, this result is nearly identical to the pooled global prevalence of 3.4% reported for asymptomatic MRSA colonization in young children (Yang et al., 2024), placing Zliten within the lower percentile of the international MRSA burden for NICUs. The relatively low prevalence at ZMC may be attributed to successful adherence to hand hygiene protocols or a smaller patient-to-nurse ratio during the study period, which limits horizontal transmission.

Interestingly, the environmental prevalence (2.6%) is also lower than that reported in other Libyan tertiary care centers, such as Benghazi, where environmental contamination in ICUs reached 9.5% (Al-Abdli and Baiu, 2016). This suggests that the current surface disinfection protocols at ZMC are relatively effective, or that this study was limited to the NICU, unlike other local studies that involved multiple departments and general hospital settings.

Healthcare workers as potential MRSA carriers in the NICU

MRSA was identified in 9.0% of staff members, representing a higher prevalence than that observed among neonates (2.5%) and environmental samples (2.7%). Although the difference between staff and neonatal colonization rates did not reach statistical significance (P = 0.198), this finding remains clinically important. According to Beaumont et al. (2024), healthcare workers can serve as transient or persistent reservoirs for MRSA, contributing to horizontal transmission even in the absence of statistically significant differences. Consequently, the presence of MRSA among staff highlights the importance of continuous surveillance, strict

adherence to infection prevention protocols, and periodic screening programs to reduce transmission risk within NICU settings.

Antimicrobial susceptibility patterns and stewardship implications of MRSA

The susceptibility profile of the MRSA isolates in this study suggests that several core anti-MRSA agents remain reliably active, supporting their continued role as preferred options for managing serious infections in high-risk settings such as the NICU. This preserved activity is consistent with contemporary reviews by Turner et al. (2019), which describe the sustained clinical utility of glycopeptides and other advanced agents against hospital-associated MRSA. While global concerns exist regarding gradual minimum inhibitory concentration (MIC) shifts and emerging resistance, the efficacy observed in this study cohort provides reassurance for current empirical and targeted therapy protocols.

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