To compare the detection of *S. aureus* carriage in Healthcare Workers of Pediatric Intensive Care Units using Robertson's Cooked Meat medium with and without 10% NaCl

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Summary

Background: about 20% of the world's population has been colonized by *Staphylococcus aureus* in the long term. Nasal carriage of *S. aureus* is an important risk factor for sepsis. In most cases, asymptomatic colonized Healthcare Workers (HCWs) can serve as reservoirs of infection for spreading *S. aureus* strains to susceptible patients.

Aims: detecting *S. aureus* carriage in hospital staff working in Neonatal (NICU) and Pediatric Intensive Care Unit (PICU) in a tertiary care hospital.

Materials and Methods: swabs from the anterior nares and web spaces of both hands of HCWs were processed. Swabs were cultured on Mannitol Salt Agar (MSA) with & without enrichment by RCM with 10% NaCl. After incubating for 48 hours, subculture from RCM was done on MSA. *S. aureus* was identified using standard microbiological techniques. The antimicrobial susceptibility testing was carried out as per Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.

Results: *S. aureus* carriage rate was 18.8%. The carriage was higher in males than in females. It was higher in residents (38.1%) than in nursing staff (5.6%). Higher isolation of *S. aureus* was observed after enrichment with 10% NaCl. Methicillin resistance was as high as 77.8% in *S. aureus* isolates from HCW carriers. All isolates were found sensitive to mupirocin.

Conclusions: to monitor the carriage of *S. aureus* in HCWs, RCM with 10% NaCl should always be used along with MSA. Eighteen point eight percent of HCWs in this study were found to be carriers of *S. aureus*. The study emphasizes the need for regular surveillance of HCWs. Methicillin resistance was very high (77.8%). Healthcare centers are supposed to monitor patients continuously and provide proper treatment.

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Introduction

Staphylococcus aureus is an important pathogenic bacterium and a component of the human microbiome. It resides predominantly in the anterior nares and extra-nasal sites, including the skin, perineum, and pharynx, and less frequently in the gastrointestinal tract and the vagina [11,16]. About 30% of the general population are nasal carriers of the bacterium. Nasal carriage of *S. aureus* is an important risk factor for sepsis [10].

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is recognized as a major nosocomial pathogen [17]. Healthcare Workers (HCWs), who are at the interface between the hospital and the community, may serve as agents of cross-contamination of Hospital-Acquired MRSA (HA-MRSA) and community-acquired MRSA. In most cases, colonized HCWs are generally asymptomatic, but they can serve as reservoirs of infection for spreading MRSA strains to susceptible patients, leading to prolonged hospital stay and increased capital expenditure [15]. *Staphylococcus aureus*-associated nosocomial infection is an important health challenge as isolates

may show multidrug resistance. The spread of MRSA strains can render infection control measures ineffective, especially in resource-limited settings, where testing every HCW may not be practically feasible.

This study was done as a part of the infection control surveillance, and it was carried out with the aims of i) detection of *S. aureus* carriage in hospital staff working in Neonatal (NICU) & Pediatric Intensive Care Unit (PICU) in a tertiary care hospital; ii) to compare detection of *S. aureus* carriage in HCWs of PICUs with & without enrichment with Robertson's Cooked Meat (RCM) medium with 10% NaCl.

Materials and Methods

Study design

A hospital-based cross-sectional study was conducted among HCWs concerned with NICU and PICU at GMC, Nagpur, Maharashtra. A total of 48 HCWs were recruited with their consent.

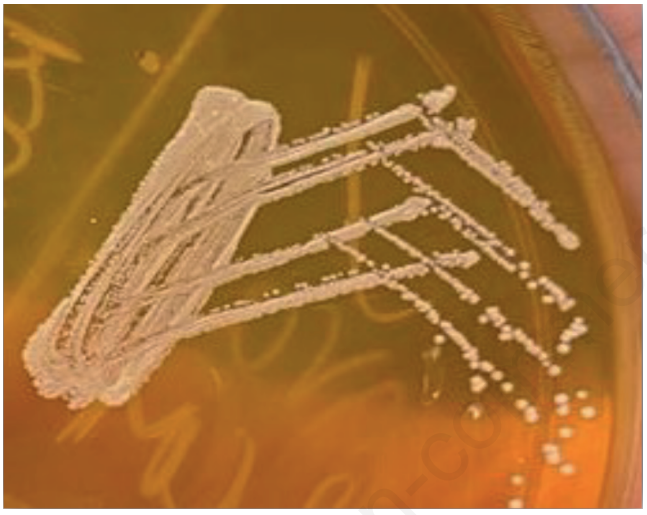

Figure 1. Nasal swab culture on Mannitol Salt Agar.

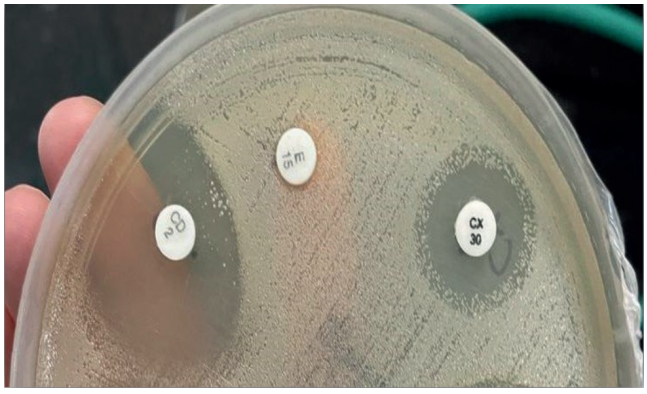

Figure 2. Inducible Clindamycin Resistance.

Sample collection

Nasal samples were collected with a sterile cotton wool swab moistened with normal saline. Both nostrils were sampled with the same cotton wool swab (one at a time) by gently rotating against the inner surface of the anterior nares. Two swabs were collected, soaked in 0.3 ml normal saline, sent to the Microbiology department, and processed immediately [14]. Swabs were also collected from the web spaces of both hands. Nasal and interdigital swabs from the same individual were stored in a single tube with normal saline.

Laboratory processing

Nasal swabs were inoculated onto Mannitol Salt Agar (MSA) plates. The plates were incubated aerobically for 18-24 hours at 37°C. Swabs were also transferred to RCM with and without 10% NaCl (after initial inoculation on MSA) and incubated at 37°C. After 72 hours, MSA was again inoculated with swabs from RCM.

S. aureus was initially screened based on the presence of yellow-colored colonies and yellow discoloration around the colonies on MSA, which were then subcultured on Nutrient Agar (NA) (Figure 1). The isolates from NA were further identified by Gram staining and catalase test reaction and confirmed phenotypically by coagulase test as per standard procedures [2].

S. aureus isolates were subjected to antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was carried out by using Kirby–Bauer's disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. The following standard antibiotic disks were used: penicillin G (10 U), trimethoprim-sulfamethoxazole (1.25/23.75 µg), gentamycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), doxycycline (30 µg), linezolid (30 µg), and mupirocin (5 µg). MRSA detection was done using cefoxitin disks (30 µg). In addition, for the isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for Inducible Clindamycin Resistance (ICR) using D-zone test [12] was done (Figure 2).

Results

The study observed varying rates of *S. aureus* isolation based on different enrichment (Table 1). Without RCM enrichment, *S. aureus* was isolated in only 4 (8.33%) swabs, which increased to 12.5% after using RCM and 18.8% after enrichment with RCM containing 10% NaCl.

The carriage rate of *S. aureus* and MRSA in HCWs of NICU and PICU is shown in Table 2. It shows that in NICU, there was carriage of *S. aureus* in 14.8% HCWs, and all were MRSA. Meanwhile, in the PICU, the carriage rate was noted to be 23.8%. Thus, out of 48 HCWs screened, 9 (18.8%) showed *S. aureus* carriage, of which around 78% were MRSA.

HCWs were categorized into two age groups: below 40 years and above 40 years. Nasal carriage was observed in groups below 40 years (Table 3). Occupationally, residents constituted the largest fraction, followed by nurses. Screening for *S. aureus* carriage showed it was highest in residents, followed by nurses. The categorization of the volunteers depending on the antibiotic treatment received in the past three months is also shown in Table 3.

Table 4 compares antibiotic susceptibility of carrier strains of *S. aureus* from NICU and PICU. Table 5 compares non-β-lactam antibiotic susceptibility of Methicillin-Sensitive *S. aureus* (MSSA) and MRSA.

Table 1. *S. aureus* isolation on MSA without RCM enrichment and with RCM containing and not containing 10% NaCl (n=48).

On MSA without RCM enrichment (%)	With RCM enrichment without 10% NaCl (%)	With RCM containing 10% NaCl enrichment (%)
4 (8.33)	6 (12.5%)	9 (18.8)

MSA, Mannitol Salt Agar; RCM, Robertson's Cooked Meat.

Table 2. Carriage of *S. aureus* and MRSA in HCW of NICU & PICU.

Organism	NICU (%) (n=27)	PICU (%) (n=21)	Total (%) (n=48)
S. aureus	4 (14.8)	5 (23.8)	9 (18.8)
MRSA	4 (14.8)	3 (14.3)	7 (14.6)

MRSA, Methicillin-Resistant *Staphylococcus aureus*; NICU, Neonatal Intensive Care Unit; PICU, Pediatric Intensive Care Unit.

Table 3. Demographic data of HCWs with carriage of *S. aureus* and MRSA.

Characteristic (n=48)	*S. aureus* (%)	MRSA (%)
Age		
<40 years (n=40)	9 (22.5)	7 (17.5)
≥40 years (n=8)	0 (0)	0 (0)
Profession		
Faculty (n=1)	0 (0)	0 (0)
Residents (n=23)	8 (34.7)	6 (26.1)
Nurse (n=19)	1 (5.3)	1 (5.3)
Attendant (n=5)	0 (0)	0 (0)
Antibiotic treatment in the past 3 months		
Yes (n=21)	5 (23.8)	3 (14.3)
No (n=27)	4 (14.8)	4 (14.8)

HCW, Healthcare Workers; MRSA, Methicillin-Resistant *Staphylococcus aureus*.

Table 4. Drug resistance in *S. aureus* carrier strains from NICU & PICU. Note: inducible clindamycin resistance was detected in 3 isolates from NICU and 2 from PICU.

Drugs	NICU strains (%) n=4	PICU strains (%) n=5	Total (%)
Penicillin	4 (100)	5 (100)	9 (100)
Cefoxitin	4 (100)	3 (60)	7 (78)
Erythromycin	3 (75)	2 (40)	5 (56)
Clindamycin	3 (75)	2 (40)	5 (56)
Cotrimoxazole	0 (0)	0 (0)	0 (0)
Doxycycline	1 (25)	1 (20)	2 (22)
Linezolid	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)
Gentamicin	1 (25)	1 (20)	2 (22)
Mupirocin	0 (0)	0 (0)	0 (0)

NICU, Neonatal Intensive Care Unit; PICU, Pediatric Intensive Care Unit.

Table 5. Drug resistance pattern in MRSA and MSSA isolates.

Drugs	MRSA (%) (n=7)	MSSA (%) (n=2)	Total (%) (n=9)
Penicillin	7 (100)	2 (100)	9 (100)
Erythromycin	4 (57)	1 (50)	5 (56)
Clindamycin	4 (57)	1 (50)	5 (56)
Cotrimoxazole	0 (0)	0 (0)	0 (0)
Doxycycline	2 (29)	0 (0)	2 (22)
Linezolid	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)
Gentamicin	1 (14)	1 (50)	2 (22)
Mupirocin	0 (0)	0 (0)	0 (0)

MRSA, Methicillin-Resistant *Staphylococcus aureus*; MSSA, Methicillin-Sensitive *Staphylococcus aureus*.

Discussion

It is observed that *S. aureus* isolates increase with RCM enrichment with and without 10% NaCl (Table 1). RCM enrichment and RCM with added NaCl discourage the growth of other organisms (except for enterococci) and selectively recover staphylococci.

The study found a 18.8% carriage rate for *S. aureus* and 14.6% for MRSA (Table 2). It aligns with the global nasal *S. aureus* carriage rate of 12-30% [9]. Among the nine *S. aureus* carriers, a higher proportion (23.8%) was isolated from the PICU compared to the NICU, which was found to be 14.8%. The entirety of NICU carriers exhibited MRSA presence, contrasting with only 14.3% of PICU carriers displaying methicillin resistance, thus underscoring a greater prevalence of MRSA carriage in the NICU setting.

Males exhibited a higher carriage rate than females (4:3 ratio), suggesting a male preponderance, despite more females being recruited (Table 3). This aligns with the findings by Baroja *et al.* [3]. Higher carriage in those below 40 years of age may be attributed to the active engagement of younger HCWs with patients. The majority of carriers were residents and nurses, conceivably attributable to their protracted interaction with patients. This finding is also supported by Boncompain *et al.* [4].

In contrast to studies from Western Nepal and Tanzania [8,9], where nurses were major carriers, residents and nurses were the primary carriers in this study. Carriers who did not take antibiotics in the past three months were all methicillin-resistant, indicating passive transmission of resistant strains (Table 3).

Resistance patterns showed maximum resistance to penicillin, methicillin, erythromycin, and clindamycin, with no resistance to vancomycin, linezolid, and mupirocin, as depicted in Table 4. This suggests the effectiveness of mupirocin for decolonizing *S. aureus*. The absence of resistance could be ascribed to the infrequent use of these antibiotics in hospital settings.

Analyzing antibiotic resistance in MRSA and MSSA strains, MRSA isolates exhibited higher resistance to erythromycin, clindamycin, and doxycycline than MSSA isolates. Inducible clindamycin resistance was observed in 4 out of 7 MRSA and 1 out of 2 MSSA cases (Table 5).

In a comparative analysis of *S. aureus* and MRSA prevalence across different geographical locations, several studies have provided insights into the varying rates of these bacterial infections. In Mysore, Deepashree *et al*. reported *S. aureus* prevalence of 25.5% and MRSA rate of 6.5% [6]. In Western Nepal, Khanal *et al.* found a slightly lower *S. aureus* prevalence of 15.7%, with an MRSA rate of 3.4% [9]. The study conducted in North Ethiopia by Gebreyesus *et al.* presented data on MRSA exclusively, indicating a prevalence of 20.3% [7]. Agarwal *et al*. conducted research in Uttar Pradesh, revealing a high prevalence of *S. aureus* at 48% and an MRSA rate of 14% [1]. Pourramezan *et al*. studied at Tehran, Iran, and reported *S. aureus* prevalence of 22.5% [13].

The present study focused on both *S. aureus* and MRSA carriage, with observed rates of 18.8% and 14.5%, respectively. These findings underscore the geographical variability in the prevalence of *S. aureus* and MRSA, highlighting the importance of region-specific considerations in understanding and addressing this microbial carriage.

The colonization of hand web spaces indirectly reflects the frequency of handwashing practices among HCWs. Cooper and colleagues suggest a significant reduction in ward-level prevalence and colonized patient days of *S. aureus* when hand hygiene compliance increases from zero to 20% [5].

Conclusions

The high prevalence of MRSA in HCWs underscores the likelihood of transmitting highly resistant strains to susceptible patients. Consequently, the study proves the importance of regular surveillance of HCWs and advocates for implementing hospital infection control practices across all healthcare settings.

The study highlights the necessity for routine MRSA education and screening strains and mitigate health risks, particularly for newborns and severely ill pediatric patients.

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