

Research Article

# Nasal Carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Healthcare Workers in a Tertiary Care Hospital in New Delhi, India

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## ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen with a high prevalence among healthcare workers (HCWs), posing a risk of transmission to vulnerable patients. Colonized HCWs are often asymptomatic but serve as reservoirs for hospital and community-associated infections. This study aims to determine the nasal carriage rate of MRSA among HCWs at HAHC Hospital, analyze its antimicrobial resistance profile, and detect the presence of *mecA* and *PVL* genes. A cross-sectional study at HAHC Hospital, Jamia Hamdard, New Delhi, India, collected nasal swabs from 250 HCWs and MBBS students, culturing isolates to identify and test *S. aureus* for antimicrobial susceptibility. MRSA screening was performed using the cefoxitin disc diffusion method, and polymerase chain reaction (PCR) was used for *mecA* and *PVL* gene detection. The prevalence of *S. aureus* nasal carriage was 31.6% (79/250), with MRSA accounting for 5.6% (14/250). The MRSA rate among *S. aureus* carriers was 17.7% (14/79). The highest MRSA carriage was observed in nursing staff (16.7%; 5/30), followed by doctors (6.4%), laboratory technicians (5.8%), and MBBS students (3.7%). Housekeeping staff showed no MRSA carriage. MRSA isolates exhibited 100% resistance to levofloxacin and cefoxitin, followed by ciprofloxacin (78.5%), erythromycin (64.2%), clindamycin (57.1%), and gentamicin (42.8%). The *mecA* gene was detected in 13 out of 14 MRSA isolates, while the *PVL* gene was absent. Significant MRSA nasal carriage among nursing staff and final-year MBBS students underscores the urgent need for infection control, regular screening, and delocalization strategies.

**Keywords:** MRSA, Healthcare Workers, Meca, MSSA, B-Lactam Antibiotics.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a formidable challenge in modern medicine due to its role in nosocomial and community-acquired infections. As an opportunistic pathogen, *S. aureus* can colonize various sites of the human body, with the anterior nares being its most common reservoir. A significant portion of the population, approximately 20% as persistent carriers and 30% as intermittent carriers, harbors *S. aureus* in their nasal passages without clinical symptoms [1]. However, this benign colonization can become a conduit for severe infections, especially when associated with methicillin-resistant strains.

MRSA is characterized by its resistance to all  $\beta$ -lactam antibiotics. It is mostly due to the *mecA* gene and rarely due to the *mecC* gene. This gene encodes a penicillin-binding protein (PBP2a), which reduces affinity for  $\beta$ -lactam agents, rendering conventional treatments

ineffective [2]. The *mecA* gene is carried on a mobile genetic element known as the *Staphylococcal cassette chromosome mec* (SCCmec), which facilitates its horizontal transfer across *Staphylococcus* species, contributing to its global spread [3]. *MecC* encodes a divergent penicillin-binding protein, PBP2c, which also reduces affinity for  $\beta$ -lactam antibiotics [García-Álvarez L et al., 2011] [4]. While vancomycin has long been considered the drug of last resort, the emergence of vancomycin-intermediate and resistant strains (VISA and VRSA, respectively) further complicates treatment options and underscores the urgency of effective prevention strategies [5]. Healthcare workers (HCWs) occupy a pivotal role in the transmission of MRSA. Colonization among HCWs often goes undetected due to the absence of clinical symptoms. Studies have shown that colonized individuals can act as reservoirs, facilitating the dissemination of MRSA to patients, co-workers,

and the broader community [6]. The consequences of such transmission are dire: prolonged hospital stays, increased healthcare costs, and elevated morbidity and mortality rates among vulnerable patients [7].

Globally, the prevalence of MRSA among HCWs varies significantly, with developing nations reporting carriage rates ranging from 20% to over 50% in certain hospital units [8]. The burden is particularly high in intensive care units (ICUs), surgical departments, and emergency rooms, areas characterized by high patient turnover, invasive procedures, and intensive antibiotic usage [9]. Within India, reports have indicated MRSA carriage rates ranging from 6% to over 80%, reflecting the heterogeneity in infection control practices, antibiotic stewardship, and healthcare infrastructure [10]. Of particular concern is the increasing convergence of hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). HA-MRSA strains typically harbor SCCmec types I, II, or III and exhibit multidrug resistance, whereas CA-MRSA strains often carry SCCmec types IV or V and express the Panton-Valentine leukocidin (PVL) gene, a potent cytotoxin implicated in necrotizing infections [11].

Definitive molecular classification of MRSA strains into HA- or CA-MRSA subtypes requires SCCmec typing and spa-typing, which were beyond the scope of the present study; isolates are, therefore, described by epidemiological context of acquisition only. Considering these developments, routine surveillance of MRSA among HCWs becomes imperative.

Nasal carriage screening provides a non-invasive and effective means of identifying asymptomatic carriers, thereby enabling timely decolonization and reducing nosocomial transmission. Moreover, the molecular characterization of isolates, such as PCR detection of *mecA* and PVL genes, offers insights into resistance mechanisms and virulence factors, which are critical for guiding infection control policies [12].

In this study, we aimed to assess the prevalence of MRSA nasal carriage among HCWs at HAHC Hospital, a tertiary care facility in New Delhi. Our objectives included (1) estimating the carriage rate of MRSA among different categories of HCWs and medical students, (2) determining the antimicrobial susceptibility profile of MRSA isolates, and (3) detecting the presence of *mecA* and PVL genes using polymerase chain reaction (PCR). By elucidating the epidemiological and molecular

characteristics of MRSA carriage in our healthcare setting, this research intends to contribute to the development of targeted interventions for infection prevention and control.

## MATERIALS AND METHODS

### Study Area

This cross-sectional prospective study was conducted in the Department of Microbiology, Hamdard Institute of Medical Sciences and Research (HIMSR), and the associated HAHC Hospital, Jamia Hamdard, over a duration of six months. A total of 250 samples were included in the study. The sample size was determined based on an expected MRSA prevalence of 5% among HCWs in comparable Indian tertiary care settings using the formula  $n = Z^2 p(1-p)/d^2$  ( $Z = 1.96$ ,  $p = 0.05$ , precision  $d = 0.03$ ), yielding a minimum sample of 203; 250 participants were enrolled to allow for subgroup stratification [13]. Ethical approval for the study was obtained from the Ethics and Research Committee of Jamia Hamdard University (Approval No.: HIMSR/IEC/004/2022).

**Inclusion Criteria:** Healthcare workers consisted of staff members (doctors, nurses, laboratory technicians, and housekeeping staff). Medical students in their 3rd and 4th year undertaking clinical rotations in hospital wards, outpatient departments, and the emergency unit were included as they have direct patient-care exposure comparable to junior HCWs. 1st and 2nd-year students who had no clinical hospital exposure were included as an internal reference group to evaluate baseline *S. aureus* carriage in the absence of patient contact. Participation was entirely voluntary; written informed consent was obtained from all participants before enrolment, with no academic incentive or penalty associated with participation.

**Exclusion Criteria:** HCWs and students with a history of upper respiratory tract infection, fever, recent nasal surgery, use of nasal medications, or antimicrobial therapy were excluded.

## METHODOLOGY

### Collection of specimens

Nasal swab specimens were collected by using sterile cotton tip swabs, and sterile saline was used to moisten the swabs. Swabs were then carefully rotated four to five times after being inserted 2-3 cm into each anterior nares. The collected nasal swabs were transported to the

laboratory in trypticase soy broth (if a delay in the procedure was expected).

#### Identification of *Staphylococcus aureus* species

Nasal swabs were inoculated onto blood agar (BA) and mannitol salt agar (MSA) plates and incubated aerobically at  $35\pm 2^{\circ}\text{C}$  for 24-48 hrs. *S. aureus* isolates were identified as being gram-positive cocci arranged in clusters with golden yellow beta-hemolytic colonies on blood agar plates, non-motile, catalase-positive, coagulase-positive, bacitracin-resistant, furazolidone-sensitive, and fermenting mannitol [14].

**Testing of MRSA:** Identification of MRSA was done with both Cefoxitin (30 ug) (DD) and Oxacillin (MIC) [15].

#### Antibiotic Susceptibility Testing

Isolated strains were tested for antibiotic susceptibility using the conventional Kirby-Bauer disc diffusion method on Mueller-Hinton agar. All erythromycin-resistant isolates were subjected to the disc diffusion test to detect inducible clindamycin resistance (iMLSB phenotype), performed as per CLSI 2022 guidelines using erythromycin (15  $\mu\text{g}$ ) and clindamycin (2  $\mu\text{g}$ ) discs placed 15–20 mm apart on Mueller-Hinton agar. The following antimicrobial agents were included: Benzylpenicillin (10 units), Gentamicin (30  $\mu\text{g}$ ), Ciprofloxacin (5  $\mu\text{g}$ ), Linezolid (30  $\mu\text{g}$ ), Erythromycin (15  $\mu\text{g}$ ), Trimethoprim/Sulfamethoxazole (1.25/23.75  $\mu\text{g}$ ), Levofloxacin (5  $\mu\text{g}$ ), Clindamycin (2  $\mu\text{g}$ ), Vancomycin (30  $\mu\text{g}$ ), Cefoxitin (30  $\mu\text{g}$ ), Teicoplanin (30  $\mu\text{g}$ ), Tigecycline (30  $\mu\text{g}$ ), Tetracycline (30  $\mu\text{g}$ ). All antibiotic susceptibility testing was interpreted according to the Clinical and Laboratory Standard Institutes (CLSI) 2022 breakpoint criteria. *S. aureus* (ATCC 25923) was used as the quality control strain for all antimicrobial susceptibility testing in accordance with CLSI 2022 recommendations [16].

#### Genotyping detection of MRSA

DNA was extracted from the test isolates using the phenol chloroform method. Colonies from overnight cultures were inoculated into BHI broth and incubated. The cells were harvested, and the pellet was suspended in EDTA buffer and subjected to phenol chloroform extraction. The DNA was precipitated using ethanol, washed, and dissolved in TE buffer [17]. The amplification reactions were performed in a

total volume of 25  $\mu\text{l}$  containing 3.5  $\mu\text{l}$  of extracted DNA as a template, 1  $\mu\text{l}$  of forward primer, 1  $\mu\text{l}$  of reverse primer, 12.5  $\mu\text{l}$  of 2x master mix, and 7  $\mu\text{l}$  of molecular-grade water. The primer sequence for the *mecA* gene used was as follows: (F: 5'-TCCAGATTACAACCTTCACCAGG-3') and (R: 5'-CCACTTCATATCTTGTAACG-3') (fragment size, 162 bp) [18]. A known *mecA*-positive MRSA isolate served as the positive control, and *S. aureus* (ATCC 25923) (*mecA*-negative) was used as the negative control for all PCR runs. For detection of the Panton-Valentine leukocidin (PVL) gene, the following primer pair was used: luk-PV-F (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3') and luk-PV-R (5'-GCATCAAGTGTATTGGATAGCAAAAGC-3'), yielding an amplicon of 433 bp [19]. PCR cycling conditions were initial denaturation at  $94^{\circ}\text{C}$  for 5 minutes; 30 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 seconds, annealing at  $55^{\circ}\text{C}$  for 30 seconds, and extension at  $72^{\circ}\text{C}$  for 1 minute; followed by a final extension at  $72^{\circ}\text{C}$  for 10 minutes.

The PCR amplifications were performed using the following protocol: initial denaturation ( $94^{\circ}\text{C}$  for 5 minutes), followed by 30 cycles of denaturation ( $95^{\circ}\text{C}$  for 1 minute), annealing ( $54^{\circ}\text{C}$  for 1 minute), and extension ( $72^{\circ}\text{C}$  for 1 minute), with a single final extension for 5 minutes at  $72^{\circ}\text{C}$ . Amplicons obtained from PCR reactions were analyzed by gel electrophoresis in 2% agarose gel stained with ethidium bromide (0.5  $\mu\text{g}/\text{mL}$ ) and finally visualized with ultraviolet light. The amplicon size (bp) of the tested gene was identified and compared to a 100 bp molecular size standard DNA ladder (HiMedia).

#### RESULTS

A total of 250 nasal swab samples were collected from healthcare workers and MBBS students over a period of 6 months. Of these, 118 (47.2%) were from healthcare workers and 132 (52.8%) were from MBBS students. Out of 250 samples, 79 isolates (31.6%) were positive for *Staphylococcus aureus*. 14 isolates (17.7%) were identified as MRSA. The overall nasal carriage rate of MRSA was 5.6% (14/250) among all participants. The mean age was  $25 \pm 10$  years, ranging from 18 to 47 years. The gender distribution included 107 males (42.8%) and 143 females (57.2%). Out of 250 participants, 107 were males, and 143 were females, and *S. aureus* carriage was slightly higher in males (41/107, 38.3%) as compared

to females (38/143, 26.5%). MRSA was found in 8 females (3.2%) and 6 males (2.4%) (Figure 1).

Among the 250 participants, nurses had the highest MRSA carriage rate at 16.6% (5/30), followed by doctors (6.4%), lab technicians (5.8%), and MBBS students (3.7%). Housekeeping staff showed no MRSA colonization. Based on their areas of work, the

highest rate of MRSA carriers was found in HCWs of the emergency department, 21.4% (3/14). Among MBBS students, those in their 1st and 2nd year showed no carriage of MRSA, whereas 3rd and 4th year students were identified as MRSA carriers.

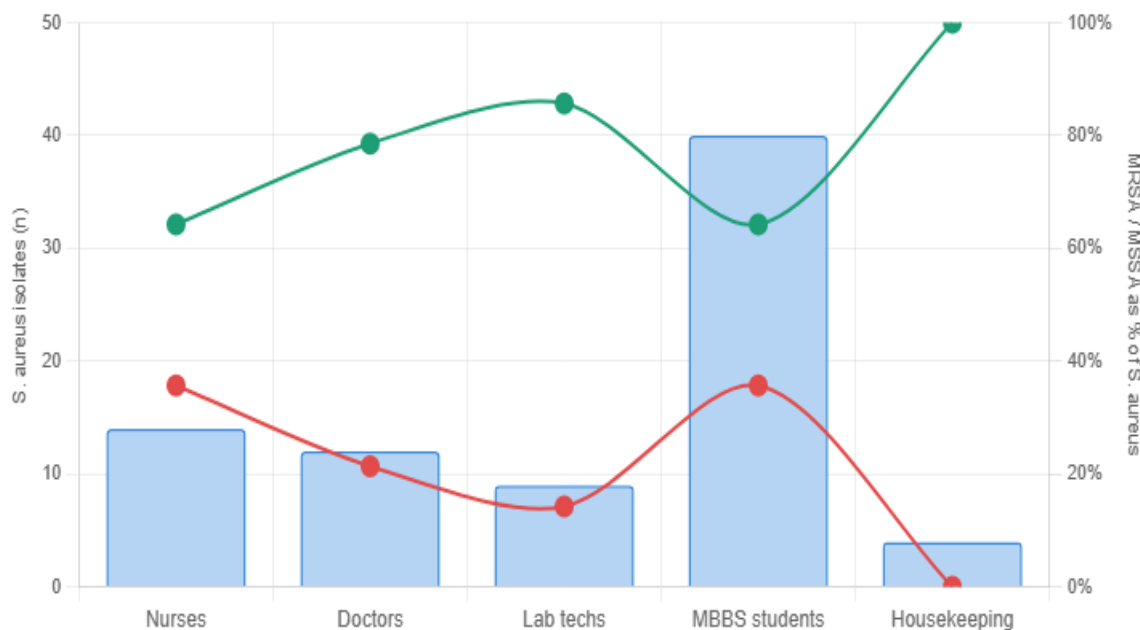


Fig. 1: MRSA Distribution among Different Occupational Groups

Antibiotic susceptibility testing for 79 *Staphylococcus aureus* isolates was performed using the Kirby-Bauer disc diffusion method with 13 antimicrobial agents. Among these, linezolid and vancomycin demonstrated the highest efficacy, with 100% sensitivity observed in all isolates. This was followed by tigecycline (96.2%), teicoplanin (94.9%), and tetracycline (84.8%). Moderate sensitivity was noted for cefoxitin (82.2%), gentamicin (77.2%), and trimethoprim/sulfamethoxazole (59.4%). Both clindamycin and erythromycin showed similar sensitivity rates of 55.6%. Lower sensitivity was observed for ciprofloxacin (31.6%) and levofloxacin (29.1%), while benzylpenicillin exhibited the least effectiveness, with only 7.5% of isolates showing susceptibility. These results highlight the continued effectiveness of glycopeptides and oxazolidinones against *S. aureus*, whereas fluoroquinolones and  $\beta$ -lactams showed markedly reduced activity.

All MRSA isolates exhibited complete resistance (100%) to levofloxacin and cefoxitin. High levels of resistance were also observed against ciprofloxacin (78.5%), erythromycin (64.2%),

clindamycin (57.1%), and gentamicin (42.8%). In contrast, lower resistance rates were noted for teicoplanin (14.2%), tetracycline (21.4%), and trimethoprim/sulfamethoxazole (28.5%). Notably, no disc diffusion-based resistance was detected against tigecycline and linezolid, indicating these agents remain highly effective against MRSA isolates. Vancomycin disc zones did not fall below the resistance threshold; formal susceptibility confirmation by MIC determination is recommended, given the known limitations of glycopeptide disc diffusion (Figure 2).

The MSSA isolates showed 100% sensitivity to cefoxitin, tigecycline, linezolid, vancomycin, and teicoplanin. 64.5% of strains showed resistance against ciprofloxacin, whereas in MRSA it was as high as 78.5%. Similarly, in levofloxacin, MSSA was only 64.5% resistant. Overall, the antibiotic resistance in MSSA isolates was much lesser compared to MRSA. The high fluoroquinolone resistance rates observed in MSSA isolates (64.5% for both ciprofloxacin and levofloxacin) likely reflect the pattern of empirical broad-spectrum fluoroquinolone prescribing for respiratory and

urinary tract infections in this tertiary care setting, a well-established driver of selection pressure. MSSA fluoroquinolone resistance exceeding 50% has been documented in

Comparable Indian hospital settings [20], supporting the plausibility of this finding (Figure 3).

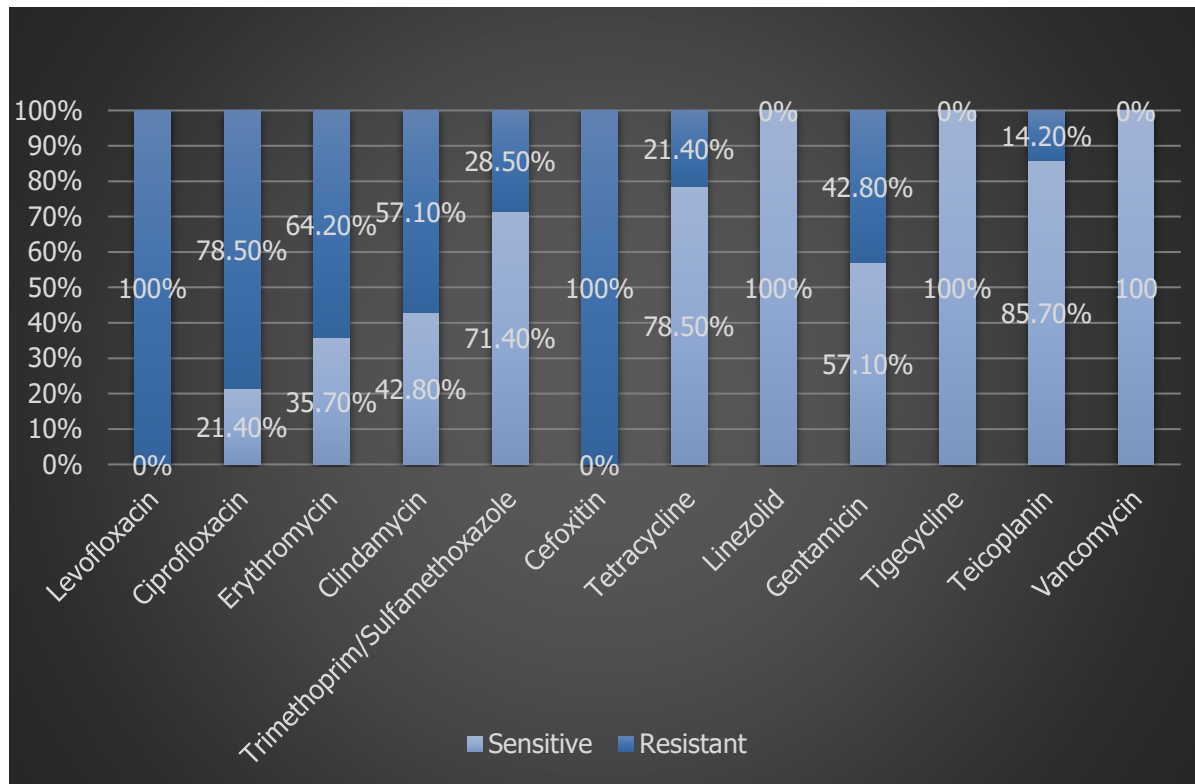


Fig. 2: AST Pattern in MRSA Isolates

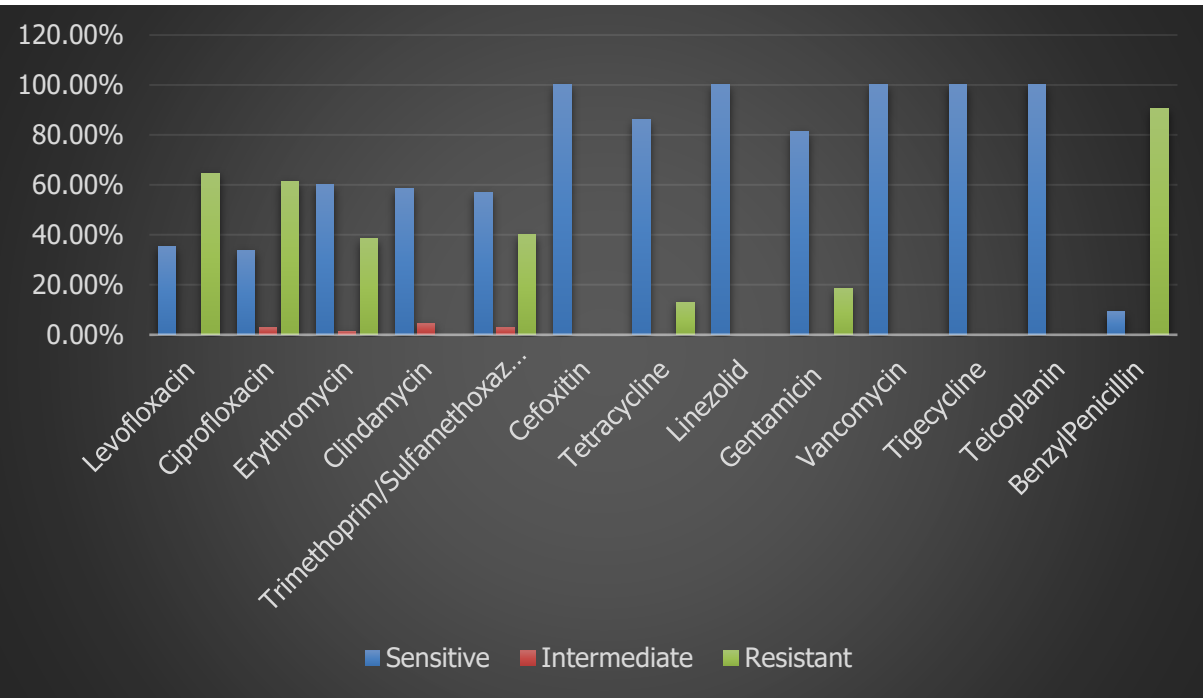


Fig. 3: AST Pattern in MSSA Isolates

Among the 14 MRSA isolates, all were resistant to cefoxitin and oxacillin by phenotypic testing. PCR confirmed the *mecA* gene in 13 out of 14

isolates (92.9%). The one discordant isolate, which was cefoxitin-resistant but *mecA*-negative, may represent carriage of the

alternative *mec* gene, hyperproduction of beta-lactamase conferring borderline oxacillin resistance, or a technical PCR artefact. None of

the isolates was positive for the *mecC* gene by conventional PCR screening (Figure 4).

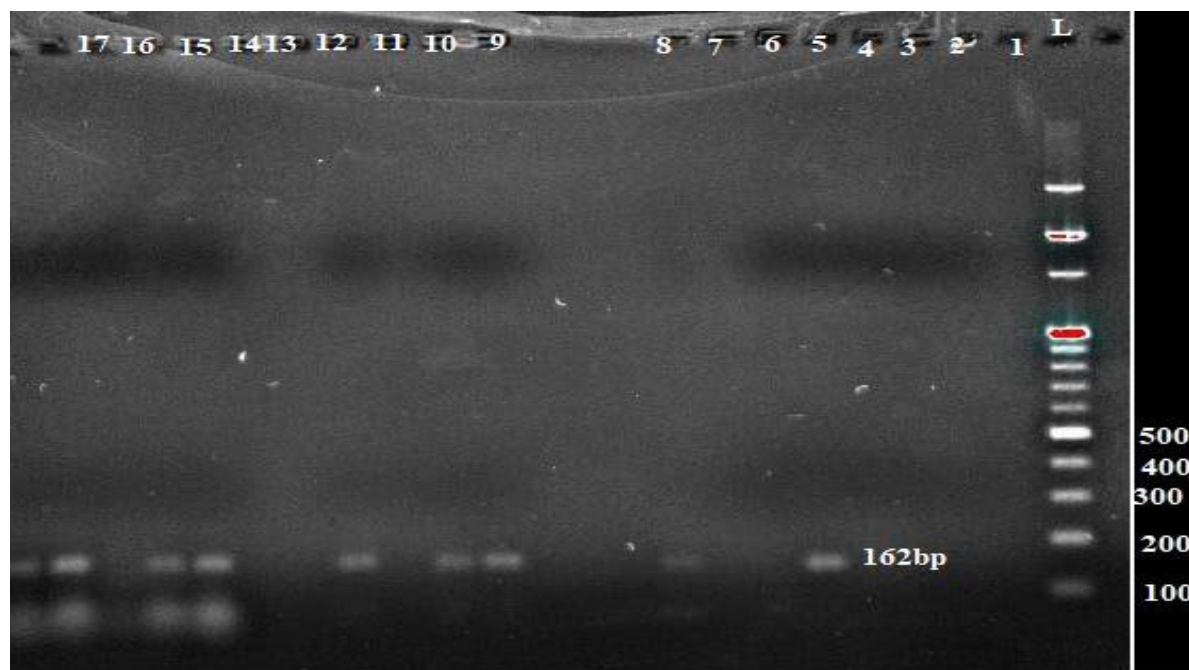


Fig. 4: Meca Gene Detection

## DISCUSSION

*Staphylococcus aureus* has emerged as a major pathogen in both hospital and community settings, responsible for a wide spectrum of diseases ranging from minor skin and soft tissue infections to severe systemic illnesses that can lead to significant morbidity and mortality. The growing incidence of methicillin resistance among *S. aureus* strains has further complicated treatment, as resistance to methicillin typically indicates resistance to all  $\beta$ -lactam antibiotics, including cephalosporins and monobactams, critical agents in the treatment of staphylococcal infections. Despite the advent of more effective antimicrobial agents and the implementation of enhanced infection control measures, such as improved hand hygiene, methicillin-resistant *S. aureus* (MRSA) continues to challenge healthcare systems globally. Hospital-acquired MRSA (HA-MRSA) infections present not only therapeutic difficulties but also impose a substantial burden on infection control resources. Understanding the antibiotic resistance profile of MRSA is essential to guide effective therapeutic strategies, minimize hospital stays and costs, and curb the spread of resistant organisms. Such insights ultimately contribute to reducing both patient morbidity and mortality. The present cross-sectional study was conducted in the microbiology laboratory of HAHC Hospital,

Jamia Hamdard, New Delhi, to determine the prevalence of nasal MRSA carriage among healthcare workers (HCWs) and medical students. Both phenotypic methods (including cefoxitin disc diffusion) and molecular methods (PCR for *mecA* genes) were used for detection, along with antimicrobial susceptibility profiling. Out of 250 nasal swab samples collected, *S. aureus* was isolated from 79 (31.6%) samples. The nasal carriage rate of MRSA was found to be 5.6%, closely aligning with studies from similar settings by Nisha Giri et al. in Nepal (5.2%) [21], Vaidya Rutvi et al. in Gujarat (6%) [22], and Yukti Sharma et al. in Delhi (5%) [23].

The carriage of MRSA among HCWs in our study may be attributed to suboptimal infection control practices and the overuse of antibiotics. Notably, none of the carriers reported underlying conditions like diabetes or hypertension, suggesting occupational exposure as a probable factor. Our finding of 31.6% *S. aureus* carriage among HCWs is consistent with the results of Nabil Abdullah El Aila et al. (31%) [24] and Sonal Saxena et al. (29.4%) [25]. Gender-wise distribution revealed a higher prevalence of *S. aureus* nasal carriage among males, which is consistent with findings by Rongpharpi et al. in Assam [26]. However, MRSA carriage was found to be higher in females, a pattern also observed by

Mahde Assafi et al. [27]. Differences in skin physiology, hormonal profiles, and personal hygiene practices between males and females may account for this disparity. Among various professional groups, nurses exhibited the highest MRSA carriage rate. This observation corroborates previous studies by Nabil Abdullah El Aila et al. [24], which also reported elevated MRSA rates among nursing staff, likely due to their frequent and prolonged patient interactions. Among medical students, third-year students exhibited the highest MRSA carriage rate. In terms of departmental distribution, emergency and surgical wards had the highest MRSA carriage among HCWs. This reflects findings by Mahde Assafi et al. [27], where overcrowding, high patient turnover, and frequent physical contact may enhance MRSA transmission risks. Antibiotic susceptibility testing of all *S. aureus* isolates revealed 100% sensitivity to linezolid and vancomycin, results that are in concordance with those reported by Hamideh Richi Sharabiani et al. [29]. MRSA isolates exhibited high resistance to levofloxacin and ciprofloxacin, mirroring findings by Neeta D. Gade et al. [30], and showed lower resistance to teicoplanin (14.2%), consistent with the 96% sensitivity reported by Belete et al., 2025. [31]. PCR analysis confirmed the presence of the *mecA* gene in 13 out of 14 (92.9%) MRSA isolates.

#### Limitations of the study

This study has several limitations that should be considered when interpreting the findings. First, as a single-center cross-sectional study conducted at one tertiary care hospital, the results may not be generalizable to other healthcare settings or geographic regions. Second, the cross-sectional design precludes any causal inference regarding the direction of MRSA acquisition or the effect of specific risk factors on carriage rates. Third, SCCmec typing was not performed, which prevents formal molecular classification of isolates into HA-MRSA or CA-MRSA subtypes; isolates are described by epidemiological context only. Fourth, glycopeptide (vancomycin and teicoplanin) susceptibility was assessed by disc diffusion, which is not endorsed by EUCAST for *S. aureus* due to reduced sensitivity in detecting heteroresistance; MIC-based confirmation (broth microdilution) is recommended in future work. Fifth, follow-up nasal swabbing to confirm decolonization of MRSA-positive carriers was not conducted within this study, limiting insight into decolonization outcomes. Sixth, the study design did not permit

year students demonstrated significant *S. aureus* carriage rates, possibly due to shared accommodations or increased exposure in clinical settings. Similarly, fourth-year students exhibited the highest MRSA carriage rates, aligning with findings by Feyissa Efa et al., who reported higher MRSA prevalence among senior clinical students and interns [28]. Increased clinical exposure and time spent in patient-care areas likely contribute to these observations.

multivariate logistic regression to adjust for potential confounders (e.g., duration of hospital employment, antibiotic exposure history), which should be incorporated in future studies. Finally, some occupational subgroups (e.g., housekeeping staff,  $n = 10$ ) had small sample sizes, limiting the statistical power of subgroup comparisons.

#### CONCLUSION

In this study, the overall nasal carriage rate of *Staphylococcus aureus* was found to be 31.6%, with MRSA accounting for 5.6% of the total 250 healthcare workers and medical students screened. Among medical students specifically, MRSA prevalence was 3.7%, with the highest rates observed in fourth-year students, followed by third-year students. This trend may be attributed to increased clinical exposure and frequent presence in hospital wards and outpatient clinics, where the risk of encountering MRSA is higher. Gender-based analysis revealed a slightly higher prevalence of *S. aureus* colonization among males, whereas MRSA carriage was more common in females. Department-wise distribution showed that MRSA and *S. aureus* colonization was particularly notable among emergency department nurses, likely due to their frequent and prolonged patient interactions in high-risk clinical environments. Antimicrobial susceptibility profiling of MRSA isolates demonstrated significant resistance to multiple commonly used antibiotics. However, no resistance was observed against linezolid and tigecycline, suggesting these agents remain highly effective and may serve as viable options for both treatment and decolonization strategies. The cefoxitin disc diffusion test showed strong concordance with *mecA* gene detection by PCR, indicating its reliability as a phenotypic alternative in identifying MRSA, especially in settings with limited molecular diagnostic capabilities. MRSA infection in health care workers can be controlled by regular surveillance and decolonization. Additionally, an

awareness campaign for the HCWs about universal techniques of hand washing and hygiene can minimize risks of transmission of hospital-acquired MRSA. The low rate of nasal carriage of MRSA among HCWs reflects that the infection control practices and training programs in the HAHC hospital were quite effective.

**Authors' contributions:** Arooj and Shyamasree Nandy were responsible for the conception and design of the study. Arooj was responsible for data collection and interpretation. Uzma and Vansh write, review, and edit the manuscript. All authors read and approved the final manuscript.

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