ORIGINAL ARTICLE

Frequency and molecular typing of nasal methicillin-resistant *Staphylococcus aureus* in admitted patients at a tertiary care hospital of Pakistan

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent pathogen responsible for infections in both community and healthcare environments. Nasal colonization with MRSA escalates the likelihood of severe infections and sepsis, especially among immunocompromised patients, contributing to morbidity and mortality within healthcare facilities. The aim of this study was to determine the frequency of community acquired MRSA (CA-MRSA) and hospital acquired MRSA (HA-MRSA) isolated from the nasal specimens of the admitted patients and to determine the frequency of PVL gene, SCCmec and spa types among CA-MRSA and HA-MRSA isolates.

Material and Methods: In this prospective study at Shaikh Zayed Hospital, Lahore, 270 patients admitted to medical and surgical wards from 2020 to 2021 underwent nasal MRSA screening from admission to discharge. Standard microbiological tests identified isolates, which were then analyzed for PVL gene detection, SCCmec typing, and spa typing.

Results: Out of 270 admitted patients screened for nasal colonization, 16 (5.9%) were colonized with CA-MRSA, while 13 (4.8%) acquired HA-MRSA. The prevalence of the PVL gene was 93.8% among CA-MRSA and 92.3% among HA-MRSA isolates. Spa typing identified t7358 as the predominant type in both CA-MRSA (75%) and HA-MRSA (100%) isolates. Regarding SCCmec typing, type IV was most prevalent among CA-MRSA (62.6%), while type III-B was most prevalent among HA-MRSA (46.1%) isolates.

Conclusion: The study found a notable frequency of both CA-MRSA and HA-MRSA colonization among admitted patients. MRSA strains showed an elevated prevalence of the PVL gene. Additionally, SCCmec typing was more effective in characterizing CA-MRSA and HA-MRSA than spa typing.

Keywords: Methicillin-resistant *Staphylococcus aureus (*MRSA), PVL gene, SCCmec typing, Spa typing

BACKGROUND

Staphylococcus bacteria are non-motile, round, gram-positive organisms commonly observed as 'grape-like' clusters under a microscope. *Staphylococcus aureus*, the predominant species within this genus, typically inhabits the body as part of its normal flora. The most common site of its colonization is nasal cavity. However, it is also found in axillae, groins, skin, gastrointestinal tract, anus, vagina and vulvae of healthy individuals as commensal.[1] It causes both pyogenic diseases as well as toxin-mediated diseases. It produces pathogenic factors which causes its virulence, allow it to stick to surfaces and enable it to evade the body immune system. Key components of the cell wall include staphylococcal protein A, teichoic acid, lipoteichoic acid, and polysaccharide capsule.[2] *Staphylococcus aureus* produces various pathogenic factors contributing to its virulence, adhesion to surfaces, and evasion of the immune system. Key cell wall components include staphylococcal protein A, teichoic acid, lipoteichoic acid, and a polysaccharide capsule. It also produces several toxins, including enterotoxins, toxic shock syndrome toxin, and leukocidins. Additionally, it produces enzymes like catalase, coagulase, DNAse, hyaluronidase, protease, nuclease, lipase, and staphylokinase.[3]

Staphylococcus aureus strains that resist beta-lactamase-resistant penicillins like methicillin are termed Methicillin-resistant *Staphylococcus aureus*

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This article can be cited as: Aqib Sultan, Mateen Izhar, Adnan Yaseen, Hira Tari**q**. Frequency and molecular typing of nasal methicillin-resistant *Staphylococcus aureus* in admitted patients at a tertiary care hospital of Pakistan. Infect Dis J Pak. 2025; 34(1): 19-26.

DOI: https://doi.org/10.61529/idjp.v34i1.329

Receiving date: **26 Jun 2024** Acceptance Date: **18 Feb 2025**
Revision date: **12 Feb 2025** Publication Date: **30 Mar 2025**

(MRSA). MRSA is a leading cause of infections acquired in both hospital and community settings. Treating MRSA infections is challenging due to the limited availability of effective treatment options.[4] Nasal carriage of MRSA is a known risk factor for subsequent infections in admitted patients. It poses a substantial health burden and risk of death, in the hospitalized patients especially with immune-compromised states. MRSA nasal carriage affects patient treatment and reflects the effectiveness of infection control measures in hospital settings.[5] MRSA screening aids in isolating and decolonizing carriers, effectively preventing its spread within hospital settings. MRSA nasal carriage manifesting within 48 hours of admission in patients without recent hospital stays is labeled as community-acquired MRSA (CA-MRSA), while MRSA nasal carriage diagnosed over 48 hours after hospital admission, not present upon admission, is termed hospital-acquired or nosocomial MRSA (HA-MRSA).[6]

MRSA is transmitted from one person to another due to infrequent and ineffective hand washing. Inanimate objects also play a significant role in the transmission of MRSA. In hospitals, staff hands are responsible to propagate MRSA infection from one patient to another due to poor hand hygiene. MRSA can endure dry conditions and is commonly detected on the surfaces of medical equipment when disinfection and sterilization practices are inadequate.[7]

Staphylococcus aureus that produces the Panton-Valentine Leucocidin (PVL) toxin causes skin and soft tissue infections, frequently leading to serious clinical consequences. The PVL gene encodes this toxin, which consists of *LukS-PV* and *LukF-PV* components. These components form a heptamer that attaches to polymorphonuclear leukocyte membranes, creating pores, leading to cell content leakage, cell lysis, and ultimately cell death.[8]

Low-affinity, altered penicillin binding proteins (PBPs) mediate methicillin resistance among staphylococci. Methicillin resistance gene 'mecA' gene encode these altered PBPs. The mecA gene is located on a mobile genetic element called the Staphylococcal-Cassette-Chromosome (SCCmec) element. This element carries two main genetic complexes. The first is the mec gene complex, facilitating methicillin resistance, while the second is the '*cassette chromosome recombinase (CCR) gene complex'*, accountable for its mobility. Various SCCmec types have been identified based on distinct combinations of 'mec' and 'ccr' gene complexes, forming the foundation of SCCmec typing.[9]

Staphylococcal protein A, a significant virulence factor produced by *Staphylococcus aureus* and located in its cell wall, attaches to the Fc region of immunoglobulin G (IgG) at the complement binding site. This binding prevents complement activation, inhibiting the production of C3b and subsequently reducing opsonization and phagocytosis of the organism. The gene encoding Staphylococcal protein A is known as spa, which consists of around 2,150 base pairs. It consists of functionally separate regions: The *Fc-binding region* of immunoglobulin G (IgG), the *X-region*, and the *C-terminus*. The *X-region*, a single locus molecular marker, is utilized in subtyping *Staphylococcus aureus.* Genotypes known as 'spa types' are identified by the highly variable sequences within the *X-region* of the spa gene, facilitating strain classification through spa typing.[10]

Molecular typing of MRSA plays a crucial role in studying its epidemiology, evolutionary course, and genetic diversity. Epidemiological insights gained from molecular typing aid infection control teams in monitoring MRSA spread and implementing targeted infection control measures to mitigate its transmission in healthcare settings.[4] Furthermore, molecular typing of MRSA isolates helps differentiate and characterize strains acquired in the community from those acquired in hospitals. Unfortunately, there has been limited research on the molecular typing of nasal-acquired MRSA in Pakistan. Therefore, the aim of this study was to assess the prevalence of CA-MRSA and HA-MRSA strains isolated from nasal samples of patients admitted to medical and surgical wards, aiming to establish a baseline nosocomial MRSA acquisition rate at a tertiary care hospital in Pakistan. Additionally, it aimed to investigate the prevalence of the PVL gene and identify the predominant SCCmec and spa types among CA-MRSA and HA-MRSA isolates.

MATERIAL AND METHODS

In this prospective study, conducted with Institutional Review Board approval, two hundred and seventy patients admitted to the medical and surgical wards of Shaikh Zayed Hospital, Lahore, underwent MRSA nasal

screening from admission until discharge during the years 2020 and 2021 after taking approval from institutional review board SZMC/IRB/0011/19 dated 12th December 2019. All participants provided informed consent prior to their inclusion in the study. Relevant data was recorded using a prescribed data collection form. Samples were processed in the Microbiology laboratory and PCR performed at the molecular biology laboratory of Shaikh Zayed Hospital, Lahore. Patients using local nasal antibiotics or sprays or with a recent history of hospitalization were excluded from the study. Nasal screening of admitted patients was conducted on the day of admission. MRSA isolates detected within 48 hours of admission were classified as community-acquired MRSA. For patients with initially negative cultures, nasal screening was repeated on the 3rd day (after 48 hours) and the 7th day of admission, followed by weekly screenings until discharge. MRSA isolates detected after 48 hours of admission were categorized as hospital-acquired MRSA. Additionally, nasal screening was performed for all patients on their respective days of discharge from the hospital.

For MRSA screening, a sterile commercial swab was gently rotated within the anterior 1.5 cm of the nasal vestibule of each of the patient's nostrils. Swabs were promptly transported to the microbiology laboratory and inoculated on blood agar plates. After overnight incubation at 35 °C, the culture plates were examined. Suspected Staphylococcal colonies were identified using Gram staining, catalase, and tube coagulase tests, with appropriate positive and negative controls. Methicillin-resistance was determined using the cefoxitin disc diffusion test in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines. The sample size of two hundred and seventy was estimated using a 95% confidence level and a 3% margin of error, based on an expected MRSA rate of 7.2% among the admitted cases.[11]

DNA extraction was conducted using a DNA extraction kit from Sacace Biotechnologies following the manufacturer's guidelines. All primers were synthesized by Advance Biosciences.

A specific set of primers was utilized to amplify a 433 base pair fragment that targets the *PVL* gene.[12] The primers *Luk-PV-1 (ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A) and Luk-PV-2 (GCA TCA AGT GTA TTG GAT AGC AAA AGC)* were employed for the detection of PVL genes. The DNA thermocycler was programmed with an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of amplification consisting of denaturation at 94 °C for 30 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was performed at 72 °C for 10 minutes. Subsequently, agarose gel electrophoresis was conducted using a 1% agarose gel containing ethidium bromide, and electrophoresis was carried out at 155-165 V for 20-30 minutes to visualize the amplification product.

MRSA SCCmec typing was conducted using multiplex polymerase chain reaction (PCR), employing primers specifically designed to amplify targeted genes in vitro (13). This multiplex PCR contained nine sets of primers which target SCCmec types I-VI and mecA-the methicillin resistance determinant as an internal control are shown in table 1.

The thermal cycling protocol began with an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of amplification. Each cycle consisted of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 60 seconds. A final extension step at 72 °C for 10 minutes concluded the process. To visualize the amplified products, agarose gel electrophoresis was performed using a 1% agarose gel containing ethidium bromide, followed by electrophoresis at 155-165 V for 20-30 minutes. (Figure-I).

A set of primers (*SPA1113F: TAA AGA CGA TCC TTC GGT GAG C and SPA1514R: CAG CAG TAG TGC CGT TTG CTT*) was used for the detection of spa gene.[14] The thermal cycling protocol began with an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of amplification. Each cycle included denaturation at 94 °C for 30 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 45 seconds. A final extension step at 72 °C for 10 minutes concluded the procedure. Agarose gel electrophoresis was conducted to visualize the amplified product, using a 1% agarose gel containing ethidium bromide, followed by electrophoresis at 155-165 V for 20-30 minutes. PCR products were then purified and sent to Advance Biosciences for gene sequencing. Gene sequences were then entered in DNA gear software (http://w3.ualg.pt/~hshah/DNAGear/) for specific spa types.

RESULTS

A total of 270 admitted patients underwent MRSA nasal screening from admission to discharge. Among them, 149 (55.2%) were female, and 121 (44.8%) were male. Of these patients, 141 (52.2%) were admitted to medical wards, while an almost equal number, 129 (47.8%), were admitted to surgical wards. The majority, 254 (94.1%), of patients belonged to urban areas, with only 16 (5.9%) belonged to rural areas.

Out of the 270 patients screened, 29 were found to have MRSA nasal carriage. Among them, 16 (5.9%) were detected with MRSA within 48 hours of admission (CA-MRSA), while 13 (4.8%) acquired MRSA after 48 hours of admission (HA-MRSA). Of those acquiring MRSA during their hospital stay, 10 (3.7%) were detected on the 3rd day of admission, and 3 (1.1%) on the 7th day, with no new cases identified thereafter. All 29 MRSA isolates were subjected to detection of the PVL gene, spa typing, and SCCmec typing. Among the 16 CA-MRSA isolates, 15 (93.8%) were found to be PVL positive, with only 1 (6.2%) isolate testing negative for PVL. Spa typing revealed that the majority, 12 (75%), were type t7358, while 1 (6.2%) was type t030, and 3 (18.8%) were non-typeable. SCCmec typing showed that the majority, 10 (62.6%) isolates, were type IV, followed by 3 (18.8%) type III-B, 1 (6.2%) type III, 1 (6.2%) type I-A, and 1 (6.2%) non-typeable.

Among CA-MRSA isolates, the outcome of MRSA nasal carriage was significantly associated with patient's residential status. The frequency of CA-MRSA was 25% in patients with a rural background, significantly higher than the 4.7% in patients from urban areas, with a p-value of 0.010.

Among the 13 HA-MRSA isolates, 12 (92.3%) were found to be PVL positive, while only 1 (7.7%) was PVL negative. All 13 (100%) isolates were identified as type t7358 by spa typing. SCCmec typing revealed that the majority, 6 (46.1%), were type III-B, followed by 2 (15.4%) type IV, 2 (15.4%) type III, 2 (15.4%) type I-A, and 1 (7.7%) non-typeable.

Table-I: Primers employed for SCCmec typing.

Locus	Primer	Oligonucleotide sequence (5'–3')	Location	Amplicon size (bp)	Specificity (SCCmec type)
A	CIF2 F2 CIF2 R2	*TTCGAGTTGCTGATGAAGAAGG* *ATTTACCACAAGGACTACCAGC*	*18398–18419* *18892–18871*	*495*	I
B	KDP F1 KDP R1	*AATCATCTGCCATTGGTGATGC* *CGAATGAAGTGAAAGAAAGTGG*	*10445–10467* *10728–10707*	*284*	II
C	MECI P2 MECI P3	*ATCAAGACTTGCATTCAGGC* *GCGGTTTCAATTCACTTGTC*	*42428–42447* *42636–42617*	*209*	II, III
D	DCS F2 DCS R1	*CATCCTATGATAGCTTGGTC* *CTAAATCATAGCCATGACCG*	*38011–37992* *37670–37689*	*342*	I, II, IV
E	RIF4 F3 RIF4 R9	*GTGATTGTTCGAGATATGTGG* *CGCTTTATCTGTATCTATCGC*	*45587–45607* *45829–45809*	*243*	III
F	RIF5 F10 RIF5 R13	*TTCTTAAGTACACGCTGAATCG* *GTCACAGTAATTCCATCAATGC*	*59573–59594* *59986–59965*	*414*	III
G	IS431 P4 pUB110 R1	*CAGGTCTCTTCAGATCTACG* *GAGCCATAAACACCAATAGCC*	*49963–49982* *50343–50323*	*381*	
H	IS431 P4 pT181 R1	*CAGGTCTCTTCAGATCTACG* *GAAGAATGGGGAAAGCTTCAC*	*29654–29673* *29976–29956*	*303*	
mec A	MECA P4 MECA P7	*TCCAGATTACAACTTCACCAGG* *CCACTTCATATCTTGTAACG*	*1190–1211* *1351–1332*	*162*	Internal control

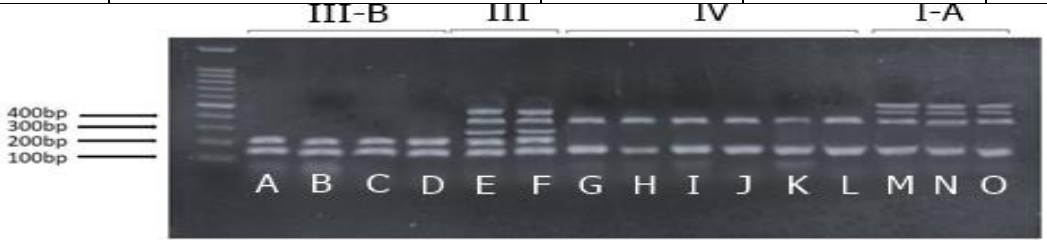

Figure-I: SCCmec types are shown on gel electrophoresis with SCCmec type III-B (lanes A to D), type III (lanes E and F), type IV (lanes G to L), and type I-A (lanes M to O).

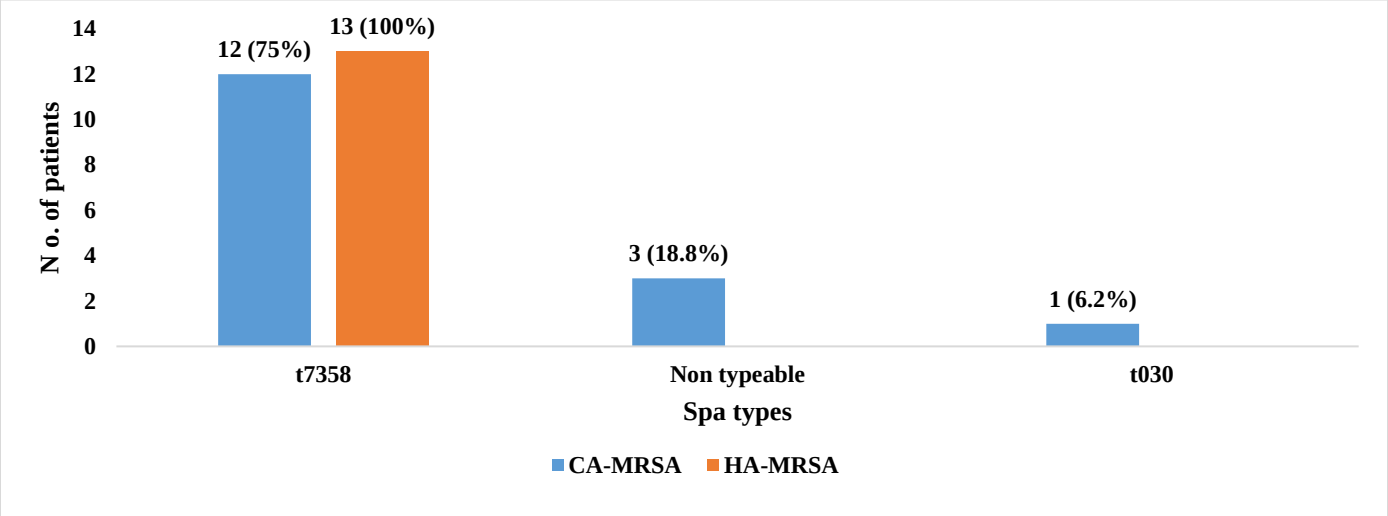

Figure-II: Distribution of various spa types among 16 CA-MRSA and 13 HA-MRSA isolates, highlighting t7358 as the predominant type in both categories.

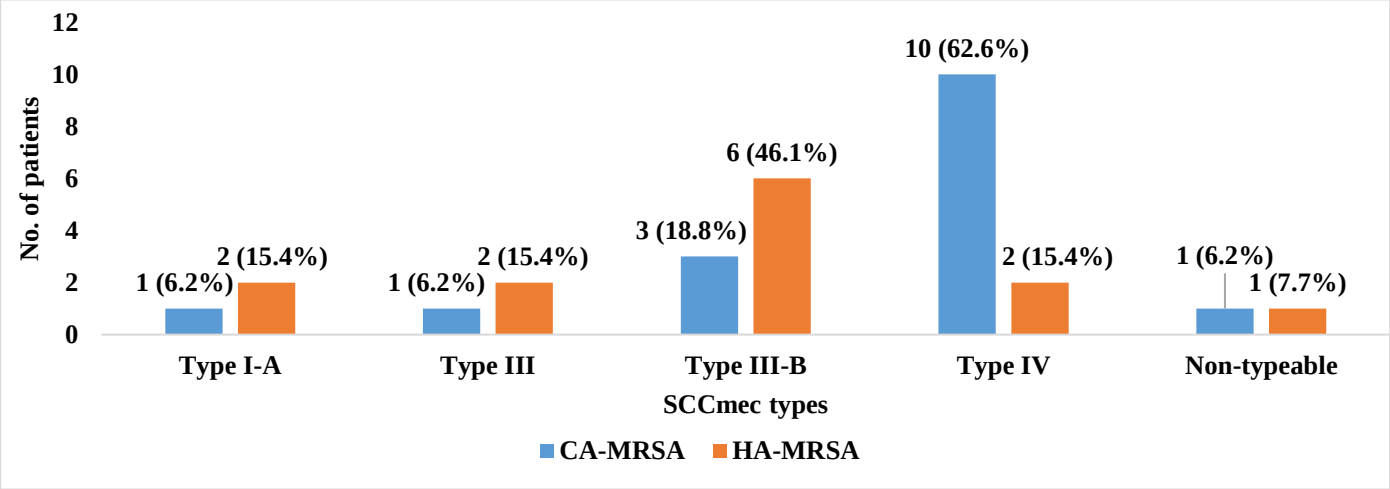

Figure-III: Distribution of SCCmec types among 16 CA-MRSA and 13 HA-MRSA isolates, with type IV predominant among CA-MRSA and type III-B predominant among HA-MRSA.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* has become a significant contributor to infections in healthcare and community settings in recent decades. Its ease of transmission, particularly due to inadequate hand hygiene, complicates control efforts. In healthcare environments, staff hands play a significant role in propagating MRSA from one patient to another, underscoring its role as a significant pathogen associated with hospital-acquired infections. Nasal carriage of MRSA is widely recognized as a critical risk factor for subsequent severe infections in hospitalized patients, resulting in considerable morbidity and mortality.[15]

The study found that 16 (5.9%) of patients exhibited MRSA nasal carriage upon admission, categorizing them as community-acquired MRSA (CA-MRSA), whereas 13 (4.8%) acquired MRSA after 48 hours of admission, indicating hospital-acquired MRSA (HA-MRSA). These findings highlight a greater prevalence of CA-MRSA compared to HA-MRSA among the study cohort, potentially due to factors like crowded community living, poor hand hygiene, and antibiotic misuse. Among those who acquired MRSA during their hospital stay, 10 (3.7%) were identified by the third day, and 3 (1.1%) by the seventh day. Notably, no new cases of MRSA carriage identified beyond the seventh day, suggesting that the length of hospitalization didn't significantly influence HA-MRSA positivity, with the

majority acquiring MRSA within the initial three days of admission.

In another study conducted in Glasgow, Scotland, it was found that 9% of patients were colonized with MRSA upon admission (CA-MRSA), while 7% acquired MRSA colonization during their hospitalization (HA-MRSA).[16] In another study carried out in rehabilitation centers in Germany, it was observed that 1.2% of patients were colonized with nasal MRSA upon admission (CA-MRSA), while 0.3% acquired MRSA colonization during their stay (HA-MRSA) (17). These results of these studies are consistent with those of the current study.

As per the present study, patients from rural backgrounds exhibited a higher prevalence of CA-MRSA (25%) compared to those with an urban background (4.7%). In contrast to a study conducted in Lahore, which reported a higher prevalence of CA-MRSA among urban subjects (22.98%) compared to rural ones (11.11%), this current study reveals differing findings (18). This discrepancy may be associated with factors such as increasing misuse of antibiotics, overcrowded living conditions, ineffective hand hygiene practices and poorer socioeconomic status in rural areas as compared to urban areas.

In the present study, it was observed that 93.8% of CA-MRSA isolates and 92.3% of HA-MRSA isolates tested positive for the PVL gene. In contrast, a study from Nepal reported a higher prevalence of the PVL gene among CA-MRSA isolates (90.4%), with a much lower detection rate in HA-MRSA isolates (7.1%).[19] Another study conducted in Lahore also revealed a significantly higher prevalence of the PVL gene in CA-MRSA isolates (61.76%) compared to HA-MRSA isolates (21.81%).[20] Similarly, a study conducted in the United States found that 77% of CA-MRSA isolates harbored the PVL gene, whereas it was detected in only 4% of HA-MRSA isolates.[21] Hence, earlier studies consistently show a prevalent presence of the PVL gene among CA-MRSA isolates, aligning with the current study's findings. However, the current study unexpectedly reveals a higher incidence of the PVL gene among HA-MRSA isolates compared to previous research, which raises concerns. This difference could potentially be associated with inappropriate antibiotic use and deficiencies in infection control measures within hospital environments. According to recent research, a significant proportion of CA-MRSA isolates, 62.6%, were identified as SCCmec type IV. Similarly, a study in Japan reported that 71.7% of CA-MRSA isolates belonged to this type.[22] In Iran, outpatient isolates also showed SCCmec type IV as the most prevalent, accounting for 26% of cases.[23] These findings align closely with those of the current study.

Based on the current study, the predominant SCCmec type among HA-MRSA isolates was III-B, accounting for 46.1%, followed by type IV at 15.4% and type III at 15.4%. In a study from Rawalpindi, SCCmec type III was reported as the most prevalent among HA-MRSA isolates (77.27%), followed by type IA (31.81%).[24] Similarly, research conducted in Iran identified SCCmec type III as the most prevalent among inpatient isolates, comprising 32% of cases, with type IV following closely at 26%.[23]

In the present study, spa type t7358 emerged as the predominant type among both CA-MRSA and HA-MRSA isolates. Among CA-MRSA isolates, 75% were identified as type t7358, 6.2% as type t030, and 18.8% were non-typeable. All HA-MRSA isolates were classified as type t7358. In contrast, a study in Iran found that spa type t030 was the most prevalent among both HA-MRSA (50%) and CA-MRSA (45.61%) clinical isolates.[25] In another study conducted in Iran, spa typing of MRSA isolates obtained from clinical samples indicated that spa type t030 was the predominant type observed in both hospitalized patients and those treated as outpatients.[23] In a separate study conducted in Saudi Arabia, spa typing of MRSA clinical isolates obtained from both clinical samples and carrier colonization sites revealed that spa type t044 was the predominant type, comprising 30.18% of the isolates.[10]

Globally, the increasing prevalence of MRSA infections presents a significant challenge, potentially leading to treatment failure with current empirical therapies. Regrettably, efforts to control its transmission in both community and healthcare environments have been unsuccessful. To control its spread, it is imperative to adhere to infection prevention measures, including effective and frequent handwashing, the use of gloves and sanitizers, surface disinfection, sterilization of surgical equipment, and the isolation and decolonization of MRSA-positive individuals. Furthermore, it's essential to use antibiotics judiciously.[26]

The current study is limited by its single-center design, which may restrict the generalizability of its findings. The association of risk factors with MRSA colonization was not studied as a part of this study. Additionally, the study did not involve isolating or decolonizing MRSA nasal carriers, nor did it monitor their clinical status as part of the investigation.

CONCLUSION

Nasal screening indicated a rise in colonization by MRSA in both community and hospital settings. The study found a higher occurrence of PVL positivity among both CA-MRSA and HA-MRSA isolates. SCCmec typing proved more effective than spa typing in distinguishing between community and hospital-acquired MRSA. Interestingly, the duration of hospital stay did not significantly impact HA-MRSA positivity.

RECOMMENDATIONS

It is proposed to implement universal MRSA nasal surveillance for all patients upon admission and after 48 hours of their hospital stay routinely. Given the high prevalence of the PVL gene among MRSA in our setting, testing for PVL gene detection in all MRSA isolates may not be necessary; they should be considered PVL positive unless proven otherwise. SCCmec typing is recommended for distinguishing and characterizing CA-MRSA and HA-MRSA strains.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Aqib Sultan: Final approval, agreement to be accountable for all aspects of the work

Mateen Izhar: Final approval, agreement to be accountable for all aspects of the work

Adnan Yaseen: Final approval, agreement to be accountable for all aspects of the work

Hira Tariq: final approval, agreement to be accountable for all aspects of the work

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