# Distribution of Mupirocin Resistance in Nasal Carriers of Methicillin-Resistant *Staphylococcus aureus* Among ICU Healthcare Workers and Patients in Rasht, Iran

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## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a significant public health concern, contributing to infections in both community settings and clinical environments. Healthcare professionals, in particular, demonstrate elevated rates of MRSA colonization. This research focused on assessing the resistance to mupirocin prevalence amongst nasal MRSA carriers in intensive care unit (ICU) healthcare workers. Nasal swabs were obtained from hospitalized patients and healthcare staff, and *S. aureus* was identified through biochemical and microbiological tests. Antibiograms were conducted on isolated strains, employing a 30 µg cefoxitin disc for MRSA detection, while mupirocin resistance was identified using the disc-diffusion technique (Kirby-Bauer method). The minimum inhibitory concentration (MIC) for mupirocin while the detection of the *mupA* and *mupB* genes was accomplished by polymerase chain reaction (PCR).

Of the 81 *S. aureus* isolates collected from nasal carriers, 20 (24.69%) originated from ICU staff, while 61 (75.31%) were from patients. MRSA constituted 77.7% (63/81) of the isolates overall. High-level resistance to mupirocin was detected in 34.56% (28/81) of isolates when tested with a 200 µg mupirocin disc, with the *mupA* gene detected in the same proportion of isolates. Notably, no low-level mupirocin resistance or *mupB* gene presence was identified in this study. Resistance rates to other antibiotics included rifampin (74.07%), penicillin (87.65%), amikacin (34.56%), gentamicin (56.79%), tetracycline (83.95%), erythromycin (100%), and clindamycin (100%). No resistance was observed for linezolid or Synercid.
The study revealed higher mupirocin resistance among healthcare workers compared to patients, underscoring the need for regular screening of healthcare staff and comprehensive antibiotic resistance profiling to mitigate MRSA transmission within hospital settings.

**Key words:** *Staphylococcus aureus*, Mupirocin, Healthcare workers, MRSA

## 1.Introduction

*Staphylococcus aureus* represents a prominent relevant cause of infections acquired in both public setting and healthcare facilities. Its ability to colonize the skin and nasal passages makes it a significant contributor to various clinical conditions (1). One of the primary difficulties in managing these infections is the increasing prevalence of antibiotic resistance, particularly MRSA. resistance to methicillin is facilitated through the expression of penicillin-binding protein 2a (PBP2a), which reduces the efficacy of β-lactam antibiotics (2, 3). The initial detection of MRSA occurred in the United Kingdom in 1961, and since then, MRSA has emerged as a significant worldwide public health concern. Infections caused by MRSA often result in prolonged hospital stays due to their severity. Transmission primarily occurs through direct contact, with healthcare workers and contaminated medical equipment serving as key vectors. Around 40 to 60 percent infections acquired in healthcare setting are attributed to healthcare workers, who, along with patients carrying MRSA in their nasal passages, pose a risk of spreading the pathogen to other hospitalized individuals, especially in intensive care units (ICUs) (4). Mupirocin, as well branded as pseudomonic acid A or Bactroban, is an essential antibiotic for treating various staphylococcal skin infections. It is minimally absorbed systemically and is excreted primarily via urine. Mupirocin disrupts bacterial protein production by competitively inhibiting the enzyme isoleucyl-tRNA synthetase activity. The U.S. Food and Drug Administration recommends its use as a nasal topical formulation to eradicate *S. aureus* nasal carriage amongst adult patients and healthcare workers (5). Despite mupirocin's critical role in managing *S. aureus* infections, there remains a significant gap in research regarding mupirocin resistance in northern Iran. This study seeks to fill this void by investigating the prevalence of resistance to mupirocin among nasal carriers of *S. aureus*, focusing on healthcare workers and patients across three ICUs in the region.

## 2. Materials and Methods:

### 2.1. Study design and setting

Nasal swab specimens were collected from both ICU-admitted patients and healthcare staff at two academic medical centers (Velayat and Poursina Hospitals) in Rasht, Iran. Prior to sample collection, every contributor was comprehensively briefed on the aims of the study and provided written agreement. The detection of *S. aureus* was carried out through a series of biochemical and microbiological tests, including Gram staining, coagulase and catalase tests, DNase activity assays, and growth as well as fermentation analysis on Mannitol salt agar plates.

### 2.2. Phenotypic identification of MRSA and mupirocin resistant *S. aureus*

To identify MRSA isolates, a 30 µg cefoxitin disc (Mast Group, Ltd, U.K.) was employed as a reliable surrogate marker for methicillin resistance detection. Additionally, mupirocin resistance was assessed using discs with concentrations of 5 µg and 200 µg (Mast Group, Ltd, U.K.), with isolates cultured on Mueller-Hinton agar (Merck, Germany) following the Kirby-Bauer disk diffusion method. After a 24-hour incubation at 37°C, results were interpreted based on the guidelines established in the Clinical and Laboratory Standards Institute (CLSI, 2024) reference tables.

### 2.3. Determination of minimal inhibitory concentration (MIC)

The established protocols for E-test strips (AB Biodisk, Solna, Sweden) was used for measuring mupirocin MIC. Isolates were categorized as susceptible when demonstrating MIC values ≤4 mg/L. Mupirocin resistance was further divided into two categories: low-level resistance (MIC range: 8–256 mg/L) and high-level resistance (MIC ≥512 mg/L). The rederence strain *S. aureus* ATCC 29213 was utilized for quality assurance, and all findings were evaluated according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) breakpoints.

### 2.4. Antimicrobial Susceptibility Testing

The antibiotic resistance patterns of the isolates was evaluated through the standardized Kirby-Bauer disc diffusion method, with antibiotic discs procured from Mast Company (United Kingdom). The susceptibility of all MRSA isolates was tested against rifampin (AP; 10 µg), Synercid (quinupristin-dalfopristin) (K; 30 µg), clindamycin (CD; 2 µg), erythromycin (E; 15 µg), linezolid (LZD; 30 µg), penicillin (PG; 10 µg), amikacin (AK; 30 µg), gentamicin (GM; 10 µg), tetracycline (T; 30 µg), and cefoxitin (30 µg). Testing was conducted on Mueller-Hinton agar in accordance with the protocolesset forth by the CLSI. The standard strain *S. aureus* ATCC 25923 was incorporated in each testing cycle to ensure accuracy and reliability of the results.

### 2.5. MRSA and mupirocin-resistant *S. aureus*

The polymerase chain reaction (PCR) amplification mixture contained 12 µL of PCR master mix, 10 pmol of each primer (specific sequences listed in Table 1), and 50–200 ng of template DNA obtained through extraction. Sterile double-distilled water was incorporated to attain the final reaction volume of 25 µL. The thermal cycling protocol comprised an initial denaturation phase at 94°C (10 minutes), followed by 35 amplification cycles (94°C for 1 minute denaturation, 45°C for 1 minute primer annealing, and 72°C for 75 seconds extension), concluding with a terminal extension step at 72°C for 10 minutes.

Additionally, the *mecA* gene, along with the *mupA* and *mupB* genes, was amplified to identify MRSA and mupirocin-resistant *S. aureus* strains, respectively (Table 1). The amplification conditions for these genes were similar to those described above, except for the annealing temperatures: 55°C for *mecA* and 60°C for both *mupA* and *mupB*. The PCR amplicons were examined using electrophoretic technique at 100V using 1.5% agarose gel and visualized under a UV transilluminator.

**Table 1:** Oligonucleotide primer sequences and specifications employed in molecular analyses

| Target | Primer | Sequence (5′ → 3′) | Product size (bp) | Reference |
|---|---|---|---|---|
| *mecA* | F | TGGCTATCGTGTCACAATCG | 304 | (6) |
| | R | CTGGAACTTGTTGAGCAGAG | | |
| *mupA* | F | TATATTATGCGATGGAAGGTTGG | 457 | (6) |
| | R | AATAAAATCAGCTGGAAAGTGTTG | | |
| *mupB* | F | CTAGAAGTCGATTTTGGAGTAG | 674 | (6) |
| | R | AGTGTCTAAAATGATAAGACGATC | | |

### 2.6. Statistics

Based on sample size and data distribution, SPSS™ version 26.0 (IBM Corp, USA) by applying either Chi-square or Fisher's exact tests, was used for statistical analyses. Statistical significance was defined as a p-value less than 0.05.

## 3. Results

Among the 81 *S. aureus* isolates obtained from the nasal carriage of healthcare workers and patients, 20 (24.69%) were sourced from ICU staff, while 61 (75.31%) were derived from patients. Additionally, 25 of the 81 isolates (30.86%) were collected from Velayat Hospital (burn hospital), and 56 of the 81 (69.14%) were obtained from Poursina Hospital. The overall prevalence of MRSA isolates was 77.7% (63 out of 81). Among the 20 isolates collected from ICU staff, 90% (18 isolates) were identified as MRSA, and 10% (2 isolates) were methicillin-sensitive *S. aureus* (MSSA). The results from the disc diffusion method were consistent with the PCR amplification of the *mecA* gene.

The antibacterial susceptibility tests revealed that 34.56% (28 out of 81 isolates) of the strains exhibited high-level mupirocin resistance, as determined using a 200 µg mupirocin disc. Among these mupirocin-resistant *S. aureus* isolates, 64.28% (18 out of 28 isolates) were collected from patients, and 35.72% (10 out of 28 isolates) were collected from healthcare staff. According to CLSI guidelines, a 200 µg mupirocin disc is used to detect isolates with high-level mupirocin resistance.

### 3.1. Detection of MupA and Mupirocin Resistance

The *mupA* gene responsible for mediating high-level resistance to mupirocin, was detected in 34.56% (28 out of 81) of the mupirocin-resistant *S. aureus* isolates. High-level mupirocin resistance was assessed using 200 µg discs, whereas 5 µg discs were used to detect low-level resistance. Notably, neither low-level mupirocin-resistant isolates nor the *mupB* gene were identified in this study. The *mupB* gene is typically used in conjunction with other targeted primers to identify high-level mupirocin resistance.

Among the 81 isolates analyzed, 34.56% (28 isolates) exhibited a MIC of mupirocin ≥512 µg/mL, categorizing them as high-level mupirocin-resistant. Conversely, no isolates demonstrated low-level mupirocin resistance.

### 3.2. Antibiotic Susceptibility Profile

All isolates demonstrated susceptibility to linezolid and Synercid. In contrast, all isolates exhibited resistance to erythromycin and clindamycin. The susceptibility rates for other antibiotics were as follows: rifampin (74.07%, 60/81), penicillin (87.65%, 71/81), amikacin (34.56%, 28/81), gentamicin (56.79%, 46/81), and tetracycline (83.95%, 68/81).

## 4. Discussion

*Staphylococcus aureus* represents a highly pathogenic microorganism capable of causing diverse clinical manifestations ranging from localized cutaneous infections to life-threatening systemic conditions such as joint infections, heart valve inflammation, bone infections, and bloodstream infections. This bacterium commonly colonizes the skin and passages (2, 3) particularly among healthcare workers, where it serves as a significant reservoir for infection transmission to patients, colleagues, and medical equipment (7). In this study, 24.69% (20 staff members) and 75.31% (61 patients) of participants were identified as nasal carriers of *S.*

*aureus*. These rates surpass those reported in previous studies by Muhammad Kashif Salman et al. (24%), Chen et al. (19.3%), and Boncompain et al. (30%) (8-10). The prevalence of nasal carriage among healthcare workers and patients varies considerably across regions with differing public health infrastructures. In alignment with this study's findings (61 out of 81 isolates), research by Conceição et al. (2013) in Portugal and Weterings et al. (2019) in the Netherlands also reported higher nasal carriage rates among staff compared to patients (11, 12). However, additiona research involving expanded sample populations and extended follow-up periods are essential for more definitive conclusions.

MRSA is a significant reason of infections in high-risk populations and is classified into healthcare-acquired (HA-MRSA) and community-acquired (CA-MRSA) strains (2, 3). Mupirocin remains an effective antibiotic for eradicating MRSA in carriers and managing infections of the skin and underlying soft tissues, highlighting its importance in infection control strategies (5).

In this study, we employed both phenotypic and molecular methods to identify mupirocin resistance among MRSA isolates obtained from the nasal carriage of healthcare workers and patients. Analysis revealed a MRSA colonization prevalence of 77.7% (63/81) within the studied population. A meta-analysis by Dadashi et al. (2018) reported a comparable frequency of MRSA infections in Iran, although at a lower rate of 43.0% (13). The disparity in MRSA prevalence may be attributed to variations in the isolates source, participant demographics, and the specific hospital settings involved.

Resistance to Mupirocin amongst MRSA isolated from nasal carriers was observed to be elevated in patients relative to healthcare workers. A conducted study by Dardi Charan Kaur et al. (2014) examined 38 *S. aureus* strains isolated from healthcare workers in a tertiary care rural hospital, of which 20 were identified as MRSA. Their analysis of resistance levels of mupirocin, using 5 µg discs for low-level resistance and 200 µg discs for high-level resistance, revealed that only two isolates were mupirocin-resistant (14).

The higher prevalence of resistance to mupirocin amongst healthcare workers might be related to their limited awareness of hand hygiene, contact precautions, and appropriate infection control measures. Mupirocin is commonly employed as a therapeutic agent for diverse cutaneous infections caused by Staphylococcus species. In this investigation, the resistance rate to mupirocin was observed to be 34.56% (15), which aligns approximately with the 40% documented by Shahsavan et al. However, significant variability in mupirocin resistance rates has been observed across different studies (13, 16, 17).

Unfortunately, the mupirocin resistance rate in this study was relatively high, likely as a result of the improper application of mupirocin in treating skin infections. The uncontrolled application of mupirocin has been linked to the development of resistance against it, which presents a significant concern in hospitals, particularly in ICUs. In this study, the rate of mupirocin-resistant *S. aureus* among MRSA isolates from ICUs was found to be 34.56%. Notably, the results obtained through the disc diffusion method were consistent with those derived from molecular techniques. In contrast, Kavitha et al. (2019) reported no mupirocin resistance in ICUs; however, their study did not employ molecular methods (18). In line with

our findings, Rashidi Nezhad et al. documented a high-level mupirocin resistance rate of 41.4% among hospitalized patients in ICUs in Tehran, Iran (19). Furthermore, Abolfazl Khandan et al. (2018) documented the presence of nasal colonization by *S. aureus* in both ICU personnel and patients, which was effectively eradicated using mupirocin ointment (20). According to CLSI guidelines, the established method for distinguishing between low-level and high-level mupirocin-resistant strains involves determining the MIC and detecting the *mupA* gene via PCR (21).

Despite the established methods, some studies have used the disc diffusion technique to differentiate between low-level (5 µg discs) and high-level mupirocin resistance (200 µg discs) among *S. aureus* isolates (13, 16, 17).

The rising challenge of antibiotic resistance in bacterial infections is significantly increasing mortality rates, prolonging hospital stays, and driving up healthcare costs, thereby imposing a substantial financial strain on national health systems. Methicillin-resistant *S. aureus* (MRSA) infections, particularly in intensive care units, further complicate the efforts of healthcare providers, affecting both staff and patients (3, 22-24). Over the past few years, identifying genes responsible for antibiotic resistance genes in *S. aureus* has been reported across various regions of Iran (25-27). This trend aligns with global concerns, as antimicrobial resistance has been shown to result in treatment failures, increased resource utilization, and higher healthcare expenditures. For example, studies estimate that infections due to antibiotic-resistant cost the U.S. healthcare system more than $2 billion annually and contribute to over $4.6 billion in costs for treating multidrug-resistant pathogens. The economic and clinical impacts underscore the critical imperative to enhance infection prevention protocols and responsible antibiotic use to combat this escalating threat.

The discrepancies observed across different studies may be result from variations in infection control practices and treatment approaches adopted across different geographical regions (28). Given that the current study found higher rates of mupirocin resistance among healthcare workers compared to patients, it suggests that mupirocin resistance poses a significant threat in hospital environments. Therefore, routin monitoring of healthcare personnel combined with continuous evaluation of antibiotic resistance trends is vital to avert the spread of MRSA within hospitals. Ultimately, our findings indicate that linezolid and quinupristin-dalfopristin (Synercid) could serve as effective alternatives for treating *S. aureus* infections.

**Ethics**

All ethical guidelines were thoroughly observed during the development of this manuscript.

**Authors contribution**

Acquisition of data, assessment and elucidation of data: **H.B.**
Drafting of the manuscript: **P.A.P.**
Critical revision of the manuscript for important intellectual content: **A.M.**

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## Data availability

Every piece of data produced or examined in the course of this research is fully contained within this article.


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## Conflict of interest

The authors affirm that there are no conflicts of interest to report.

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