

## Prevalence and factors associated with the nasal colonization of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* among patients in a tertiary care hospital

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### Abstract

**Introduction:** One of the major risk factors for the nosocomial infection among the hospital population was identified as the nasal carriage of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA). This study was aimed to find the prevalence and factors associated with nasal carriage of *S. aureus* and MRSA among patients in a tertiary care hospital.

**Materials and Method:** A cross sectional study was designed for *S. aureus* and MRSA nasal carriage. History of factors for *S. aureus* and MRSA colonisation was collected using a questionnaire. A total of 750 specimens were streaked directly onto mannitol salt agar. *S. aureus* was identified and antibiotic susceptibility test was done. All MRSA isolates were subjected to polymerase chain reaction (PCR).

**Results:** Among the total specimens collected, *S. aureus* isolated were 115 (15%). Out of this 115 isolates, only 5 were found to be MRSA (4.3%). The overall MRSA nasal carriage rate was only 0.6% (5/750). PCR done for all the MRSA isolates showed positive results for Type III SCC mec gene and negative results for *Panton Valentine leukocidin* gene indicating that they belong to Hospital Acquired MRSA (HA-MRSA). Majority of the patients with MRSA nasal colonisation had no history of hospitalisation, or other factors contributing to colonisation from hospital environment.

**Conclusion:** *S. aureus* nasal carriage rate was 15% and the MRSA nasal carriage rate was only 0.6%. Association was not found between *S. aureus* or MRSA nasal colonization with factors such as previous hospitalisation, antibiotics, infections on skin and soft tissues, use of alcohol and smoking.

**Keywords:** Methicillin-Resistant *Staphylococcus aureus*, Nasal colonisation, *Staphylococcus aureus*, Antibiotics

### Introduction

Nasal carriage of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) were reported as one of the risk factors for the development of nosocomial infections mainly in hospital population.<sup>(1)</sup> MRSA contains one resistance island called Staphylococcal chromosome cassette (SCC) mec, where mec is the genetic element that confers resistance to methicillin.<sup>(2)</sup> The hospital population include not only general but surgical patients, patients admitted to intensive care units (ICU), patients on haemodialysis or continuous peritoneal dialysis, patients with liver cirrhosis and after liver transplantation and HIV-infected patients.<sup>(3)</sup> Most common area of colonisation for MRSA is nostrils. Other areas are axilla and groin.

Healthy younger patients were tend to get community acquired-MRSA infections (CA-MRSA), while that of hospital acquired MRSA (HA-MRSA) strains have been isolated particularly from people in the health care setting.<sup>(4)</sup> Risk factors associated with MRSA carriage include prior antibiotic exposure, prolonged or a previous 1 year hospitalization, surgery, admission to an ICU, HIV-seropositive status, nasal illness and gastrointestinal diseases, living in a nursing home and close proximity to a patient colonized or infected with MRSA.<sup>(5,6)</sup> The risk factors were found to vary in different geographical areas and community.

Routine screening for the nasal carriage of these strains and measures for their eradication among patients will help to reduce the transmission of infections in hospitals. Therefore, this study was aimed to find the prevalence and possible factors associated with *S. aureus* and MRSA nasal colonisation in patients admitted to various surgical wards of a Government Medical College Hospital.

### Materials and Method

**Study design and subjects:** A cross sectional study was conducted in adult patients (12 – 80 yrs of age) who were admitted to the surgical wards of Govt. Medical College Hospital, Thrissur, Kerala, during the period July 2012 to June 2013. Patients admitted in ICU, pay wards and those not willing to participate were excluded. A written consent was taken from the patients for using their information in study and the study procedure was approved by the Institutional Ethics Committee.

**Procedure:** History of factors for MRSA colonisation was collected using a questionnaire. Nasal swab was moistened with sterile saline before collecting the specimen and nasal specimens were collected from patients within 24 hours of admission. The specimens were streaked directly onto mannitol salt agar. After 1–2 days of incubation, all suspected *S. aureus* colonies (yellow colonies with surrounding medium yellow)

were plated onto blood agar. Identification of *S. aureus* from suspicious colonies was based on Gram stain, catalase test, slide coagulase test and tube coagulase test.<sup>(7)</sup> Antibiotic sensitivity testing was also done using commercially available discs by Kirby Bauer method on Muller Hinton agar. The various antibiotic discs used were penicillin (10 units), erythromycin (15 µg), gentamicin (10 µg), cefoxitin (30 µg) and cotrimoxazole (1.25/23.75 µg). The sensitivity was read on the next day as per standards prescribed by Clinical and Laboratory Standards Institute for testing antimicrobial susceptibility.

The cefoxitin resistant isolates were considered as methicillin resistant and subjected to molecular typing by polymerase chain reaction at Christian Medical College, Vellore, Tamil- Nadu, India. SCCmec typing and *Panton Valentine leukocidin (PVL)* gene detection were done according to procedure guidelines by method described by Oliveira et al. and Lina et al. respectively.<sup>(8,9)</sup>

**SCC mec typing:** A multiplex PCR was carried out for the SCCmec typing. DNA template was prepared initially. Briefly a thick suspension was made from the fresh culture of the test and control strains in 500µl of saline. The suspension was centrifuged at 8000 rpm for 5 minutes. After discarding the supernatant, cells were suspended in 50µl lysostaphin and incubated for 10 minutes at 37°C. Proteinase K (50µl of 100 µg/ml) and Tris buffer (150 µl of 0.1 moles/L, pH 7.5) were added and further incubated at 37 °C for 10 minutes. The cellular debris were removed after heating at 100°C for 10 minutes followed by centrifugation at 8000 rpm for 5 minutes.

Master mix, 23µl (2.5µl of PCR buffer, 2.5µl of MgCl<sub>2</sub>, 1µl of dNTPs, 4.5µl of MiliQ H<sub>2</sub>O, 6 sets of forward and the reverse primers and 0.5µl of *Taq* Polymerase) mixed with 2µl of the extracted DNA was amplified in a Thermal Cycler 9700 (Applied Biosystems, Norwalk, USA) using the following program. The denaturation step at 94 °C for 2 minutes (for initial) followed by 30 cycles of DNA amplification. This was done by denaturation at 94°C for 30 seconds, primer annealing at 53°C for 30 seconds and primer extension at 72°C for 1 minute. The final reaction product was held at 72°C for 4 minutes and the products were stored at 4°C. The products were

analyzed using agarose gel (1.5%) electrophoresis using TBE buffer, stained with ethidium bromide and detected using UV light.

**Panton Valentine Leukocidin detection:** The *PVL* gene detection was carried out by primers of *PVL* gene flanking area using the procedure described above. The strain was detected for *PVL* gene using the 433 bp DNA marker.

## Results

Nasal specimens were collected from 750 patients. Among the total specimens collected, *S. aureus* isolated were 115 (15%). Out of those 115 isolates, only 5 were found as MRSA (4.3%). The overall MRSA nasal carriage rate was only 0.6% (5/750). Among the *S. aureus* isolates, 45% were from male and 55% were from female patients (Fig. 1). Twenty seven percent of isolates were from patients of age group 31 to 40 years. Among the patients from whom *S. aureus* was isolated, 67% (77/115) had no history and remaining 33% had history of hospitalisation. Among the 33% (38/115), 16% were hospitalised within 3 months, 17% were hospitalised more than 4 months ago. Furthermore, most of them (30/38) had a duration of <15 days of hospital stay. Patients with *Staphylococcal* nasal colonisation, 31% had minor/major surgical procedures done within 1 year and 21% had received antibiotics during last three months. Chronic skin infection including recurrent pyoderma was found in 10%. Among the total patients, 13% had a habit of nose picking, 3% used snuff, 12% were smokers, 13% had chronic illness like diabetes or liver disease (Table 1).

**Table 1: Factors in patients with *Staphylococcus aureus* nasal colonisation**

Hospitalised patients	33%
Duration of hospital stay	1 – 5 days
Surgical procedures done within 1 year	31%
Received antibiotics during last 3 months	21%
Chronic skin infection	10%
Habit of nose picking	13%
Use of snuff	3%
Smokers	12%
Chronic illness	13%

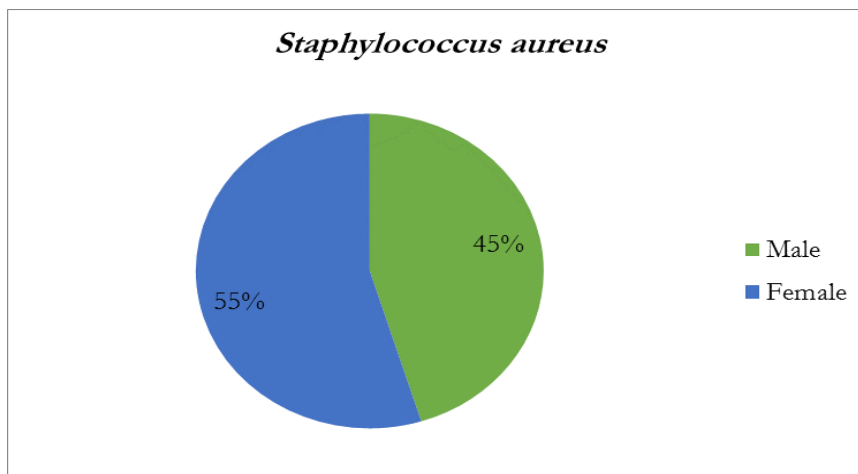


Fig. 1: Distribution of gender with *Staphylococcus aureus* nasal colonization

Susceptibility pattern of *S. aureus* isolates showed that 92% were resistant to Penicillin, 69% resistant to Erythromycin, 16% resistant to Gentamicin, 29% to Cotrimoxazole and 4% to Cefoxitin / Methicillin (Fig. 2).

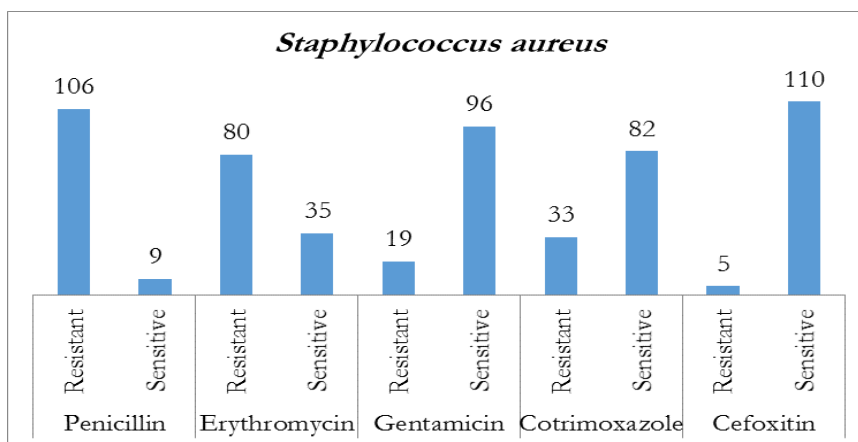


Fig. 2: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates

Out of the MRSA isolates, all were resistant to Penicillin. Resistance to Erythromycin was 40%, where as resistance to Gentamicin and Cotrimoxazole were 80 and 20%, respectively (Fig. 3). Cefoxitin results can be applied to other penicillinase stable penicillins like cloxacillin, dicloxacillin, flucloxacillin, methicillin and nafcillin according to CLSI.

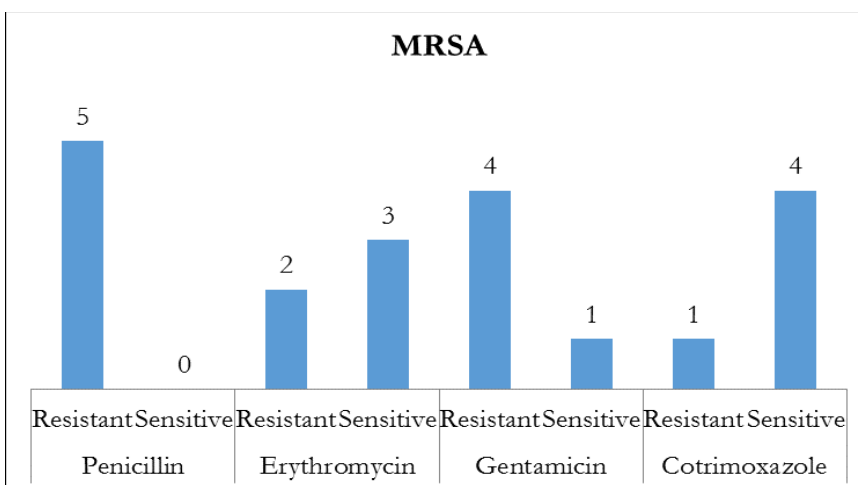
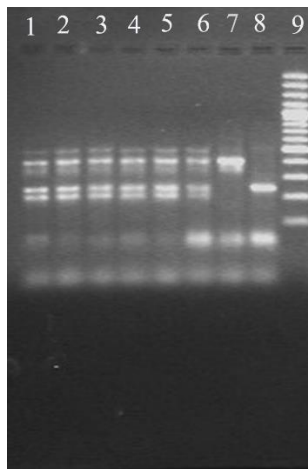


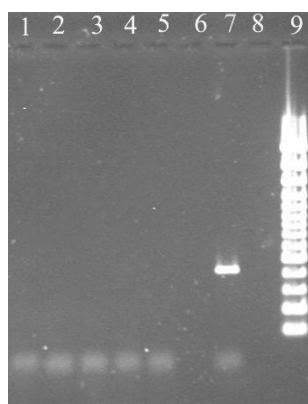
Fig. 3: Antibiotic susceptibility pattern of MRSA isolates

Out of 5 MRSA isolates, one isolate was completely resistant to the tested drugs. This patient had a surgery, spent 5 days in surgical ICU and had a total hospital stay of 10 days duration, during which he got antibiotics. Four patients had no history of hospitalization within the last one year.

**Molecular typing:** PCR done for all the MRSA isolates showed Type III SCCmec gene (Fig. 4) and negative results for PVI gene (Fig. 5).



**Fig. 4:** SCCmec typing by polymerase chain reaction. Lanes 1-5: Test strains 1-5; Lane 6-8: control strains 1-3 and Lane 9: 100bp DNA ladder



**Fig. 5:** Detection of PVI gene by polymerase chain reaction. Lanes 1-5: Test strains 1-5; Lane 6 and 8: Blank; Lane 7: positive control PVI (433 bp) and Lane 9: 100 bp DNA ladder

## Discussion

*S. aureus* nasal carriage rate in this study was 15%. The MRSA nasal carriage rate was only 0.6%. Chatterjee et al. demonstrated the colonization rates of MRSA as 0 to 9.2% in Indian community.<sup>(10)</sup> In this study, we found very low prevalence of *S. aureus* and MRSA nasal colonization probably because of proper hand washing and personal hygiene. People in this geographical area usually take bath twice a day and keep their hands clean in between their work and after meals.

In our study, 67% of *S. aureus* were isolated from patients who did not have any history of hospitalization in the past one year. Furthermore, 80% of MRSA isolates also had no history of hospitalization. There are many studies showing that even healthy individuals without prior exposure to a health care centre can get nasal colonization of *S. aureus* and even MRSA.<sup>(10,11)</sup>

A clear delineation was not yet been concluded between CA-MRSA and HA-MRSA strains.<sup>(4)</sup> Some HA-MRSA strains can cause community onset MRSA infections. Probably due to increasingly common management of HA-MRSA infections at home, the isolates of HA-MRSA found to be circulated mainly in the adults of the community.<sup>(4)</sup> Moreover, the study was conducted in a tertiary health care centre where patients are referred from other hospitals for expert management. The carriage state of MRSA might be acquired by spending long time, like nursing an MRSA colonized patient in a peripheral hospital. In this study, no association was found between *S. aureus* and MRSA nasal colonization with factors like use of antibiotics, skin and soft tissue infections, smoking and alcohol.

## Conclusion

*S. aureus* nasal carriage rate was 15% and the MRSA nasal carriage rate was only 0.6%. Factors such as previous hospitalisation, duration of hospital stay, use of antibiotics, personal habits and chronic skin infections were not associated with *Staphylococcus aureus* and MRSA nasal colonisation. Short duration with fewer samples was the main limitations of this study. Hence, a multicenter study with more sample size is warranted for the MRSA detection.

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