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# Nasal screening of healthcare workers for nasal carriage of coagulase positive MRSA and prevalence of nasal colonization with *Staphylococcus aureus*

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**Abstract**
The aim of the study was to screening for coagulase positive methicillin resistant *Staphylococcus aureus* (MRSA) in healthcare workers and prevalence of nasal colonization with *Staphylococcus aureus*. We included 84 healthcare workers' samples (nasal swabs) in the study. Different biochemical tests were done to isolate *Staphylococcus aureus*. Species confirmation for *Staphylococcus aureus* was done using the tube coagulase test and DNase test. Antibiotic susceptibility pattern for MRSA was done by Kirby – Bauer disk diffusion method. Coagulase positive *Staphylococcus aureus* were subjected to oxacillin agar screen method to screen for MRSA. Out of 84 healthcare workers' samples 66(78.6%) were positive for *S.aureus* and 18(21.4%) showed no isolation of *S.aureus.* Out of 66 *S.aureus* isolates, 40(60.6%) was Coagulase positive *Staphylococcus aureus* (CoPS) and 26(39.3%) were Coagulase negative *Staphylococcus aureus* (CoNS). Out of total 40 isolates of Coagulase positive *Staphylococcus aureus* (CoPS), 18(45%) were found to be resistant to methicillin and 22(55%) CoPS isolates were methicillin susceptible *Staphylococcus aureus* (MSSA). The nasal carriage of CoPS in healthcare workers was seen to be 33(82.5%) doctors and 7(17.5%) lab technicians. The nasal carriage of coagulase positive MRSA in healthcare workers were observed to be 15(83.3%) doctors and 3(16.6%) lab technicians. The study showed that out of total specimens collected from healthcare workers, doctors and lab technicians were carrier for MRSA. The highest percentage of nasal carriage of coagulase positive MRSA among healthcare workers was observed to be among the doctors and less percentage in lab technicians. Screening should be made an essential protocol to assess the carrier transmitted drug resistant strains of *Staphylococci* from the community to the hospital settings and hospital settings to community.

**Keywords:** MRSA; MSSA; CoPS; CoNS; Nasal carriage; Antimicrobial drug resistance.

## Introduction

*Staphylococcal* infections cause significant morbidity and mortality in both the community and hospital settings. Treatment of infection caused by *S.aureus* has become more problematic since the development of antimicrobial resistant *Staphylococcus aureus* (MRSA). Since MRSA strain are resistant to all β-lactam antibiotics and the treatment options are limited significantly. The incidence of nosocomial infection caused by MRSA continues to increase worldwide. Infections caused by MRSA strains are associated with longer hospital stay, prolonged antibiotic administration and higher costs than infections caused by methicillin – susceptible *Staphylococcus aureus* (MSSA) strains. The presence of *S.aureus* in the anterior nares of patients may serve as a source of infection to other patients, is known to be a significant risk factor. Within the hospital colonized healthcare workers act as reservoir for the spread of MRSA to uncolonized susceptible patients. Identification of patients and healthcare workers (in outbreak settings) colonized with MRSA, combined with other precautions and taking care of hand hygiene have been helpful in reducing transmission and controlling spread.

MRSA has been implicated in both community acquired and hospital acquired infections. They express heterogenous resistance to methicillin through the penicillin binding protein 2a (PBP2a). This has been found to be the case in community-acquired strains, as opposed to the hospital acquired strains that show homogenous resistance patterns, though both contain the same gene. Some strains are called the epidemic strains, can spread within or between hospitals, and can spread between countries. There is as greater risk now being posed that these methicillin resistant strains could lead to heterogenous glycopeptide resistance that was first reported from Japan in 1997 of intermediate resistance pattern to vancomycin of *Staphylococcus aureus* (VISA). Clinical isolates from invasive infections

can only focus on the severity of the disease but does not give an estimate or prevalence of carriers among the healthy population. This formed the basis for our study and its importance of screening for healthy carriers of MRSA and to study the rate of colonization of CoPs and CoNS among the healthcare workers.

In this study, we investigated the probable carrier rate of the healthcare workers and screened for carriers of MRSA as they could pose a potential risk factor for nosocomial transmission when the same carrier are exposed to the hospital setting during their clinical postings.

**Materials and Methods**

The study was designed to investigate the carrier rate of the healthcare workers for recovery and concomitant identification of MRSA isolates directly from specimens taken from the anterior nares. Healthcare workers including hospital staff and doctors working in surgical intensive care unit, pediatric ward, HDU, OT-ICU and staff of microbiology department at J. N. Medical College, AMU, Aligarh, who are at a great risk for MRSA carriage consisted of the study group. From each subject, one swab from anterior nares were collected. Sterile cotton wool swabs moistened with mannitol salt broth were used to collect the specimens.

*Media preparation*

Nasal swabs were processed within 2 hours of collection and primary plating was done on blood agar, MSA without oxacillin and MSA containing oxacillin. After inoculation, plates were incubated at 35ºC in oxygen and read after 24 and 48 hours. The colony suggestive of MRSA was confirmed as *Staphylococcus aureus* by tube coagulase and DNase test. Methicillin resistance was confirmed with cefoxitin susceptibility testing according to CLSI method and determination of MIC of oxacillin (≥4 µg/ml) was done by agar dilution method.

Mannitol salt agar (MSA) containing Muller-Hinton agar, 1% mannitol, 6.5% sodium chloride and phenol red as indicator were prepared in the laboratory. This media were used to screen *Staphylococcus aureus* in the specimens. In this medium phenol red was incorporated to detect the mannitol fermenting colonies of *Staphylococcus aureus* which appeared as yellow colored colonies.

Mannitol salt agar with oxacillin (MSAO) containing Muller-Hinton agar, 1% mannitol, 6.5% sodium chloride, phenol red as indicator and 4mg/L of oxacillin was used to screen methicillin resistant *Staphylococcus aureus* in the specimens. Any bacterial growth after 24 hours and 48 hours at 35ºC was indication of resistance to methicillin.

After 20 hours of incubation, plates were examined for *Staphylococcus aureus* as follows. MSA and MSAO, all yellow colored colonies were subcultured for further identification. On sheep blood agar, all white, yellow or golden yellow colonies with or without hemolysis were further characterized to rule out *Staphylococcus aureus*. A slide coagulase test was done on all suspicious colonies. If culture was negative for *Staphylococcus aureus*, plates were re-incubated for an additional 24 hours.

*Susceptibility testing*

MICs were determined by the reference agar dilution method as per NCCLS guidelines. Two-fold dilutions were tested (ranges determined by antibiotic) using Mueller-Hinton agar. Agar used for oxacillin was supplemented with 4% NaCl. MICs were read after incubating plates at 37ºC for 20-24 hours. Oxacillin and cefoxitin disk diffusion screening was performed using a 1µg oxacillin disk and 30 µg cefoxitin disk respectively on 18-24 hour growth cultures inoculated to Mueller-Hinton agar and incubated for 24 hours at 35ºC. Interpretation was according to NCCLS guidelines.

**Results**

A total of 84 specimens collected from healthcare workers were inoculated on sheep blood agar, mannitol salt agar with oxacillin (MSAO) and CHROMagar for MRSA colonization. This consisted of 45 nasal swabs and 39 finger web swabs.

**Table 1: Isolation of *S.aureus* from healthcare workers on sheep blood agar.**

| Source | | Total no. of Specimens | Growth of *S.aureus* | |
|---|---|---|---|---|
| | | | Negative (%) | Positive (%) |
| Nasal | Doctors | 60 | 13 (21.7) | 47 (78.3) |
| | Lab technicians | 24 | 05 (20.8) | 19 (79.2) |

| | | | |
|---|---|---|---|
| Total | 84 | 18 (21.4) | 66 (78.6) |

On sheep blood agar plate, 66 (78.57%) specimens were positive for *S.aureus.* Out of total, 47 *S.aureus* isolates were recovered from the doctors and 19 isolates were recovered from the lab technicians' swab specimens.

**Table 2: Percentage isolations of *Staphylococci* from the anterior nares of healthcare workers.**

| Organism type | | Healthcare workers | |
|---|---|---|---|
| | | Doctors (n=60) | Lab technicians (n=24) |
| Total *Staph.* Isolated | | 47 | 19 |
| CoPS | Total | 33 | 07 |
| | CoPS % | 70.2 | 36.8 |
| | MRSA | 15 | 03 |
| | MSSA | 18 | 04 |
| CoNS | Total | 14 | 12 |
| | CoNS % | 29.8 | 63.2 |

Of the 84 specimens screened, 66 isolates were *Staphylococcus aureus*. With the help of biochemical tests, 40 were identified as CoPS, and 26 were CoNS (Table-2). MIC (oxacillin) and the oxacillin agar screen obtained 18 isolates were MRSA from CoPS. 66 specimens showed nasal colonization with *Staphylococcus aureus*. *Staphylococci* were isolated from 47 (71.2%) specimens taken from doctors and 19 (28.8%) from lab technicians. Of the 47 specimens from doctors, 33 (70.2%) were CoPS, and from a total of 19 *S.aureus* isolates from lab technicians, 7 (36.8%) were CoPS. 18 MRSA isolates were isolated from 33 CoPS isolates from healthcare workers, the highest rate of nasal carriage for CoPS MRSA were seen among the doctors and less rate showed by lab technicians.

**Table 3: Nasal colonization with CoPS MRSA in anterior nares of healthcare workers.**

| Organism type | | No. isolated | Total percentage (%) |
|---|---|---|---|
| *Staphylococcus aureus* | | 66 | 78.5 |
| CoPS | MRSA | 18 | 27.2 |
| | MSSA | 22 | 33.3 |

## Discussion

Methicillin-resistant *Staphylococcus aureus* has during the last three decades, evolved as one of the most important causes of hospital infections worldwide. The data obtained from this study revealed that there were reservoirs or carriers of MRSA in healthcare workers. Out of the 84 samples collected from healthcare workers, 66 yielded *Staphylococcus aureus*, while 18 specimens showed no growth of *Staphylococcal* colonies, but showed growth of Gram negative bacteria from the nasal swab collected from the anterior nares. There have been interesting publications regarding the association of *Staphylococcal* nasal carriage with glucocorticoid receptor gene polymorphisms, which states that genotype dependent variation leading to glucocorticoid insensitivity may cause

immune enhancement precipitating autoimmune disorders, while protecting from *S.aureus* colonization. This is just descriptive, which warrants for further investigation into the field and the organism that was isolated in this case were gram-negative bacteria that were not subjected to further analysis, as it did not fall within the scope of the project. The factors that distinguish between a carrier and a non-carrier are still unknown. Enhanced adhesion of *Staphylococcus aureus*, to cell associated and cell free secretions, along with induction of reduced mucociliary activity, could well explain the nasal colonization by *Staphylococcus aureus*.

Further, 47.6% of healthcare workers screened showed nasal carriage of CoPS, while 30.9% had nasal colonization with CoNS. this goes to prove that one of the ecological niche for the colonization of *Staphylococci* is the anterior nares, as most of the nasal specimens yielded staphylococcal growth on culture. The greater amount of bacteremia cases recorded, have been due to *Staphylococcus aureus* of endogenous origin, since they originate from colonies of nasal mucosa. Most invasive infections are assumed to originate from nasal carriage. Hence, it is imperative that nasal carriage due to *S.aureus* strains should be prevented in order to stem the rate of infection, and in preventing the transmission of resistant strains of the organism.

Although nasal carriage of *S.aureus* is harmless in healthy individuals, they can become carriers who could pose the risk of spreading infections to the community at large, and since the section of individuals under this study were healthcare workers, their interaction and exposure to hospital environment could cause major risks in transmitting to hospital patients and spreading nosocomial infections.

Screening for resistant strains of *Staphylococci* in healthcare workers should be adopted as a protocol in medical colleges, in order to curb the spread of drug resistant *Staphylococci* from the hospital to community. This will also help in monitoring the healthcare workers population who might pose a risk to patients and hospital personnel and the community at large.

**Conclusion**

The study showed that out of total specimens collected from healthcare workers, doctors and lab technicians were carrier for MRSA. The highest percentage of nasal carriage of Coagulase positive MRSA among healthcare workers was observed to be among the doctors and less percentage in lab technicians. Screening should be made an essential protocol to assess the carrier transmitted drug resistant strains of *Staphylococci* from the community to the hospital settings and hospital settings to community.

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