

Research Article

Nasal carriage and antimicrobial susceptibility of *Staphylococcus aureus*, with special reference to methicillin resistance, in health care workers in a tertiary care hospital in south India.

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Abstract: Several studies have observed that nasal carriage of MRSA among health care workers, acts as a source of endogenous infection. It also becomes a source for nosocomial and community spread of infections. Nosocomial infections resulting from MRSA are often, resistant to wide spectrum of antibiotics. We conducted a study to determine the prevalence and antibiotic resistance pattern of MRSA nasal carriage, among health care workers of tertiary care hospital of South India. Pre-moistened nasal swabs from health care workers (doctors, nurses, technicians and class IV workers) were obtained. These swabs were plated on 10% sheep blood agar, Mac Conkey agar, mannitol salt agar and Hichrom MeReSa agar. Antibiogram was done by Kirby Bauer disc diffusion method. MRSA were detected by measuring the cefoxitin zone diameter as per CLSI guidelines. The resistance was confirmed by doing the MIC for oxacillin using Hi Comb MIC strip. The MRSA carriers were subjected to decontamination protocol with 2% mupirocin ointment and 2% chlorhexidine bath. After completion of decontamination protocol, the carriers were sampled again. Of the 300 nasal swabs collected, 28 (9.3%) were *Staphylococcus aureus*, of which 4 (1.33%) were MRSA. These isolates were resistant to penicillin (71.4%), and erythromycin (67.9%). All the isolates were sensitive to linezolid and vancomycin. The repeat cultures that were taken after the decontamination protocol, were negative. The prevalence of MRSA among health care workers, in our hospital was 1.33%. This study reiterates the need for early detection and treatment of nasal carriage among health care workers, so as to prevent the spread of MRSA in hospital environment and in the community.

Keywords: healthcare worker, HiMeReSa Media, Hi Comb MIC strip, mannitol salt agar, MRSA, mupirocin, nasal carriage

INTRODUCTION

Staphylococcus aureus is identified as the most common cause of community based and nosocomial infections. This contributes to significant rate of morbidity and mortality. To add to this problem, the strains of *Staphylococcus aureus* show resistance to methicillin (Methicillin resistant *Staphylococcus aureus*). Resistance to methicillin demonstrated by MRSA strains implies resistance to all antibiotics belonging to the beta lactam group [1]. One of the main concerns with MRSA (Methicillin resistant *Staphylococcus aureus*), is the limited number of therapeutic options to treat this infection. The major sources of MRSA in the hospital environment are asymptotically colonized patients and health care workers. Healthcare workers act as links in the transmission of MRSA between patients [2]. Several studies have identified, anterior nares as the ecological niche of *Staphylococcus aureus* [3, 4, 5, 6]. In India, nasal carriage of MRSA among health care workers ranges from 1.8% to 14.28 % [7, 8].

Certain risk factors that have been associated with MRSA carriage are: male gender, stay in surgical or dermatological wards, prior antibiotic treatment, prolonged hospital stay, underlying illness, demographics and usage of catheters [2, 9, 10, 11]. *Staphylococcus aureus* is transmitted to the anterior nares, through contaminated hands and from contaminated environmental surfaces, where it survives for months. In colonized individuals, nasal carriage of *Staphylococcus aureus* acts as a source of endogenous infection and as a source of cross contamination for community spread [5]. Carriage of *Staphylococcus aureus* is considered as a risk factor for the development of infections. Infection with *Staphylococcus aureus* is usually preceded by a period of colonization [12].

Therefore, there is a need for accurate and early detection of MRSA colonization among health care workers. The present study was designed to screen the health care workers for MRSA carriage, to determine their antibiotic susceptibility pattern of the

isolates, to initiate decontamination process in such carriers and to repeat the cultures in such carriers, so as to ascertain that they have been cleared of MRSA nasal carriage.

MATERIALS AND METHODS

A prospective study was conducted among health care workers for duration of 6 months of a tertiary care hospital in south India. Health care workers included in the study were: doctors, nurses, technicians and class IV workers. All health care workers who gave consent to this study were included in the study. Written consent was obtained from the health care workers prior to enrollment in the study. Health care workers with a history of recent nasal surgery, fever, upper respiratory tract infection and on any topical nasal medication were excluded from the study [6]. The study was cleared by the institutional ethical committee.

Samples were collected from the anterior nares of health care workers, using pre-moistened swabs. The swabs were inoculated on 10% sheep blood agar medium, Mac Conkey agar, mannitol salt agar and HiChrom agar (HiMeReSa Media, Himedia, Mumbai). After 24 hours of aerobic incubation at 37°C, yellow colonies on mannitol salt agar and blue coloured colonies on Hi chrome agar (Figure 1) were identified as Methicillin resistant *Staphylococcus aureus*. The other standard microbiological tests which were put up included: colony morphology, pigment production, Gram staining, catalase test, tube coagulase test and fermentation of mannitol.



Fig-1: Green coloured colonies on HichromMeReSa indicate Methicillin resistant *Staphylococcus aureus*

For antibiotic susceptibility testing, Kirby Bauer disc diffusion method was followed. The following antibiotic discs were put for antibiotic sensitivity testing on Mueller Hinton agar plate: penicillin (10 units), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), trimethoprim sulphamethoxazole (1.25/23.75 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin

(10 µg), linezolid (30 µg) and vancomycin (30 µg). Quality control was done using ATCC strain *Staphylococcus aureus* 25923 [12]. Zone diameters were interpreted as sensitive, intermediate, and resistant as per CLSI guidelines [13]. CLSI has recommended ceftazidime disc diffusion method for the detection of MRSA. A 0.5 Mac Farland standard suspension of the isolate is prepared and lawn culture is done on a Mueller Hinton agar plate. A ceftazidime disc (30 µg) is placed on this plate, and incubated at 37°C for 18 hours. The zone diameter is measured. An inhibition zone of ≤ 21 mm is considered as methicillin resistant, and an inhibition zone of ≥ 22 mm is considered as methicillin sensitive [13].

MIC for oxacillin was tested using Hi Comb MIC strip (Himedia, Mumbai) as shown in Figure 2. The interpretive criteria for oxacillin MIC are: MIC $2 \leq \mu\text{g}$ is susceptible and MIC $\geq 4 \mu\text{g}$ is resistant [11,13].



Fig-2: Detection of oxacillin resistance using Hicomb MIC strip. The MIC of the above strain is 16

Statistical Analysis

Computerised Excel spread sheet (Microsoft Excel 2009) was used to enter the data. Percentage description of data was given (SPSS version 20, software will be used.)

RESULTS

A total of 300 nasal swabs were obtained from health care workers.

Table-1: Sex distribution among the health care workers who were sampled

Sex	Number(n=300)	Percentage %
Male	89	29.7
Female	211	70.3
Total	300	100

There were 89 males (29.7%) and 211 females (70.3%), with a male to female ratio of 3:7.

Table-2: Age distribution among the health care workers who were sampled

Age group	Total number (n = 300)	Percentage %
11- 20 years	35	11.7
21-30 years	143	47.7
31-40 years	68	22.7
41-50 years	22	7.3
>50 years	32	10.6
Total	300	100

Majority (143/300, 47.7%) of the health care workers were in the age group of 21-30 years.

Table-3: Distribution of health care workers who were sampled

Category	Number(n=300)	Percentage %
Class IV workers	103	34.3
Doctors	78	26
Technicians	71	23.7
Nurses	48	16
Total	300	100

Among the health care workers (n=300), majority of them were Class IV workers (103, 34.3%). The remaining samples were taken from doctors (78, 26%), technicians (71, 23.7%) and nurses (48, 16%). Knowledge about health care associated infections among the health care workers was good.

Of the 300 nasal swabs taken, the organisms isolated were Coagulase negative Staphylococcus 173(57.67%), followed by Micrococci 79(26.33%). The yield of *Staphylococcus aureus* was 28(9.33%) among the collected samples. There was no growth in 10 samples (3.33%). Among the 28 *Staphylococcus aureus* isolates, 4 strains were found to be MRSA, and the remaining 24 strains were MSSA. The overall positivity of MRSA in this study is 1.33% (4/300), as shown in Fig-3.

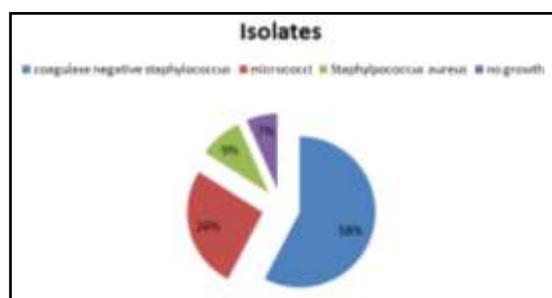


Fig-3: Various organisms isolated from nasal swabs

Antibiotic sensitivity pattern of the 28 *Staphylococcus aureus* isolated is shown in Figure 4. Of the 28 *Staphylococcus aureus* isolated, 71.4% were resistant to penicillin, 67.9% to erythromycin, 57.1% were resistant to clindamycin, and 17.9% to

trimethoprim/sulphamethoxazole, 10.7% resistance to cefoxitin, chloramphenicol, and ciprofloxacin. 7.1% resistance was found to doxycycline and gentamicin. All isolates were sensitive to linezolid and vancomycin were also shown in Figure 4.

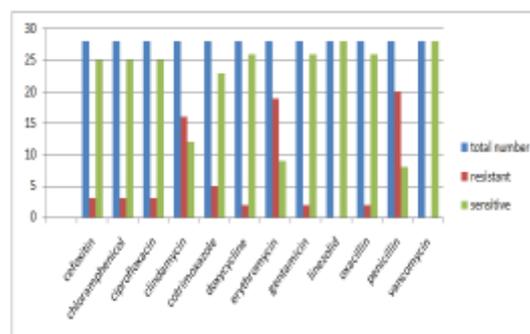


Fig-4: Antibiotic sensitivity pattern of the isolated Staphylococcus aureus strains

Table-4: Number and percentage of MRSA carriers

Gender	Number screened	No. of MRSA carriers	Percentage of MRSA carriers (%)
Female	211	01	0.47
Male	89	03	3.37

Of the 89 males screened, 3(3.37%) were found to be carriers of MRSA. Of the 211 females screened, 1(0.47%) was found to be a MRSA carrier.

The various wards, from which Methicillin sensitive *Staphylococcus aureus* (MSSA) and Methicillin resistant *Staphylococcus aureus* (MRSA) were isolated, is shown in the Figure 5. Two MRSA carriers were from pharmacy and one MRSA carrier each, from Psychiatry and Obstetrics and Gynaecology, were isolated (Figure 5).

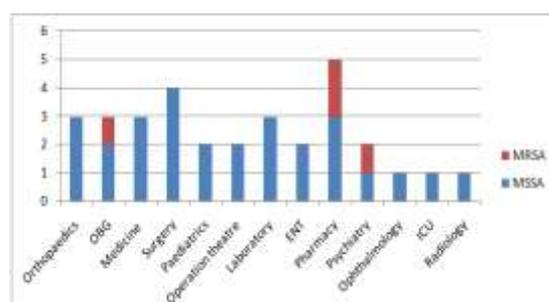


Fig-5: The various wards in which Staphylococcus aureus and MRSA were isolated

Table-5: Professional category, gender and department wise distribution of MRSA carriers

Category	Gender	Department	No. of MRSA carriers
Class IV workers	Female	OBG	01
Pharmacists	Male	Pharmacy	02
Nurses	Male	Psychiatry	01

In relation to the professional category, 2 male pharmacists, 1 female attender in OBG department and 1 male nurse from Psychiatry, were found to be MRSA carriers.

DISCUSSION

MRSA is a pathogen that can result in a wide spectrum of infections ranging from simple wound infection to life threatening illnesses [3]. Infection in patients, hospital personnel and inanimate hospital environment serve as potential reservoirs of infection [5]. MRSA strain first appeared in UK and Denmark in 1960 soon after introduction of Methicillin [11, 14]. MRSA is due to the acquisition of *mec A* gene which is carried on the SCC *mec* element.

Several growth conditions influence the phenotypic expression of methicillin resistance, these include: temperature and osmolarity of the medium. Heteroresistant strains of *Staphylococcus aureus* may become fully resistant, and may get selected in those patients receiving beta-lactam antibiotics, which in future leads to therapeutic failure. Therefore, from a clinical point of view, these should be considered fully resistant [1]. MRSA strains worldwide belong to five clonal complexes. They are CC5, CC8, CC22, CC30 and CC45. In India, the major clone is CC8-MRSA-III [10].

The gold standard for detection of MRSA is PCR/PBP2a, which detects the *mecA* gene. This is expensive and may not be available in all health care settings. It is economical to resort to cefoxitin disc diffusion to detect MRSA. Several studies have also reported that when compared to the oxacillin disc diffusion, the results of cefoxitin disc diffusion correlate better with the presence of *mecA* gene [15]. The resistance in MRSA can be confirmed using MIC (Hicomb MIC).

According to Mathanraj *et al.*, the rate of colonization of MRSA was 1.8% among health care workers, which is similar to our finding of 1.33% MRSA carrier rate [2]. Colonization of MRSA was found to be 37.5% among health care workers, according to the study done by Bala K *et al.*, in North India [14]. This is high compared to our study, where the rate of colonization is 1.33%. Vinodhkumaradithya A *et al.*, found 13% of the nasal swabs from surgical staff grew *Staphylococcus aureus*, out of which 15.4% were MRSA carriers [6].

Mathanraj *et al.*, in his study found that, males were more common carriers than the females. They also suggested that further evaluation regarding male preponderance of carriage needs to be done, with the possible role of hormones [2]. The increase, in male preponderance was also noted by Bidya *et al.*, [4]. In our study also, there was a male preponderance which was noted. Out of 89 males screened, 3(3.37 %) males

were MRSA carriers. 211 females were screened, of which, 1(0.47 %) female was a MRSA carrier.

In relation to the professional category, 2 male pharmacists, 1 female attender in OBG department and 1 male nurse from Psychiatry, were found to be MRSA carriers. None of the doctors, who participated in this study, were MRSA carriers. This correlates with the study conducted by Kaur DC, in which doctors had the lowest prevalence of colonization [8].

Penicillin resistance, in our study was found to be 71.4%. A resistance of 100% found by Bala K *et al.*, and Goyal R *et al.*, [14, 7]. In our study, resistance to erythromycin was 67.9%, which is comparable to the study conducted by Bala K *et al.*, who observed 66.6% resistance to erythromycin. [14]. In our study, resistance to ciprofloxacin was found to be 10.7% and, resistance to gentamicin and doxycycline was, 7.6%. In the study conducted by Vinodhkumaradithya *et al.*, they found a 23% resistance to ciprofloxacin, and a 7.1% resistance to gentamicin and doxycycline [6]. No resistance was observed to linezolid and vancomycin. This co-relates with several studies conducted by Pathak *et al.*, [5], Bala K *et al.*, [14] and Rongpharpi [16].

All the MRSA carriers detected by our study were subjected to decontamination protocol. Decontamination protocol consisted of application of 2% mupirocin ointment to the nostrils, twice daily and 2% chlorhexidine shampoo, once daily for a week [9, 16]. Two sets of culture were performed, at least 72 hours apart. These cultures need to be negative, after completion of treatment, for the health care worker to be considered decolonized [16]. In our study, the MRSA carriers, after completion of decontamination protocol were subjected to repeat cultures. These repeat cultures came negative.

It is important to note that, there is a fourfold increase in the risk of infection with nasal MRSA colonization, than with MSSA colonization. Healthcare workers, who acquire MRSA, transmit these multidrug resistant strains to their family members, who eventually spread such strains into the community [4].

Maintaining hand hygiene is the most important step in the prevention of spread of MRSA [17]. It has been observed that handwashing is the leading intervention, for limiting the spread of nosocomial infections [18]. The rate of MRSA carriage among health care workers in our hospital was 1.33%, which is low when compared to other Indian studies. This rate can increase, if proper surveillance and control measures are not in place. Therefore, it is important to have preventive measures and surveillance methods in place, in all health care settings.

CONCLUSION

In conclusion, we like to say that, simple preventive measures like hand washing, using sterile mask and gown and avoiding touching one's nose during work, should be reinforced in all health care settings. This study reiterates the need for periodic surveillance, early and accurate detection, and treatment of MRSA carriers. This should be accompanied with appropriate hospital infection control measures, to prevent the nasal carriage of MRSA in hospital health care workers.

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