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Research Article



Methicillin Resistant *Staphylococcus aureus* Carriage amongst students of the Medical College

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Abstract

Background: Methicillin - resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital - acquired infections in many countries. Mainly healthcare workers (HCWs) including students are at risk for Methicillin - resistant *Staphylococcus aureus* (MRSA) carriage. Nasal carriage is a major risk factor for *Staphylococcus aureus* infection, especially for MRSA. **Aims:** To screen students for MRSA carriage and to identify risk factors for MRSA colonization. **Methods and Materials:** A total of 220 students were screened for carriage of MRSA. Swabs of both anterior nares and web spaces of the hands and palms were taken, transported and incubated in Tween 80 at 35°C overnight aerobically before inoculation onto Mannitol Salt Agar (MSA). *Staphylococcus aureus* was identified as Gram positive cocci with positive catalase, coagulase test. MRSA were identified at different sites using oxacillin and cefoxitin discs diffusion methods. **Results:** Overall carriage rate was 31(14%) with highest positivity rate of students. All the MRSA isolates were sensitive to all antibiotics. Vancomycin resistant strain was not identified. The commonest site of colonization was the anterior nares. **Conclusions:** Prolonged hospital stay and improper hand washing were found to be the major risk factors for MRSA colonization. Although, it would require screening of larger numbers before arriving at any definite conclusions. Monitoring and eradication of MRSA from students should be considered to prevent continuous spread between students, healthcare facilities and the community.

Keywords: Methicillin - resistant *Staphylococcus aureus* carriage, MRSA carriage, Nasal carriage, *Staphylococcus aureus*, and students.

Introduction

Staphylococcal infections cause significant morbidity and mortality in both community and hospital settings. Treatment of infection caused by *S. aureus* has become more problematic since the development of antimicrobial resistant *Staphylococcus aureus*, Methicillin - Resistant *Staphylococcus aureus* (MRSA) (Wikipedia.com). MRSA has become endemic worldwide within the past two decades (Nour de San *et al.*, 2007) MRSA is often referred as a “Super bug”. Methicillin resistance is not confined to *S. aureus*. During the 1980s, attention was drawn to the high incidence of “Methicillin-resistant *Staphylococcus epidermidis* (MRSE) in nosocomial infections. The MRSA is encoded by *mecA* gene. The main mode of MRSA

transmission is from MRSA-colonized or infected patients to another by direct contact, through touch of health care workers (Kumar *et al.*, 2011). Nasal carriage is a major risk factor for *Staphylococcus aureus* infection, especially for MRSA (Hetty *et al.*, 2003). Anterior nares are the major reservoir of *S. aureus*: 20% of humans are persistently and asymptotically colonized, 60% are intermittently, and 20% are non carriers.

Nosocomial transmissions of MRSA from health care workers to hospitalized patients have been documented, but not in students. Similarly, screening and eradication of MRSA from colonized healthcare workers have been recognized and

recommended as an important part of a comprehensive infection control policy for this organism (Nour de San *et al.*, 2007). With this background that this study was undertaken in our college mainly to screen students for MRSA carriage, and to identify risk factors for MRSA colonization

Materials and Methods

Study duration and population

This study was conducted at Aarupadai Veedu Medical College & Hospital, Puducherry for 6 months period.

Clinical isolates

A total 220 students of 2nd and 3rd year MBBS were screened for MRSA after obtaining informed consent and approval by the institute ethical committee.

Specimen collection

Swabs of both anterior nares of consenting persons were taken with a sterile swab stick moistened with sterile physiological saline and transported in Tween 80 to the Microbiology laboratory unit of the hospital for analysis. Similar swabs of the palms and web spaces of the hands were also taken after at least 30 min of the last hand wash.

The swabs in Tween 80 were incubated at 35°C overnight before being inoculated onto Mannitol Salt Agar (MSA). Inoculated MSA were incubated at 35°C for 18 - 24 hrs. Gram positive cocci isolates which are catalase, coagulase positive using standard techniques, were identified as *Staphylococcus aureus* and were screened for methicillin resistance by modified Kirby - Bauer method using Oxacillin (1 µg) and Cefoxitin (30 µg) disk diffusion methods on Mueller-Hinton Agar (MHA).

Oxacillin disk diffusion method

Oxacillin disk (1 µg) diffusion method was carried out on Mueller Hinton Agar (MHA) supplemented with 2% NaCl to detect MRSA according to CLSI guidelines. The plates were incubated at 35°C and results were recorded after 24 hours of incubation. Isolates were considered resistant when the diameter of inhibition was <10 mm, intermediate resistance

when the diameter was 10 – 12 mm and sensitive when the diameter was >13 mm (Chaudhury and Kumar, 2007; Perl and Golub, 1998).

Detection using cefoxitin as a surrogate for oxacillin

The Clinical and Laboratory Standards Institute (CLSI) guidelines (2006) has recommended cefoxitin disc diffusion method for the detection of MRSA. This was performed by using a 30µg cefoxitin disc for all Oxacillin Resistant strains and an inhibition zone diameter of < 19mm was considered as methicillin resistant and > 20 mm was considered as methicillin sensitive (Huang and Platt, 2003 and Chacko *et al.*, 2009).

Disk diffusion test with MH agar

The disk agar diffusion was performed by following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS). Sterile cotton swab was dipped in a suspension, which was adjusted to 0.5 McFarland turbidity. After the swab was streaked uniformly onto MH agar and Antibiotic sensitivity of the MRSA isolates against erythromycin (15 µg), chloramphenicol (30 µg), cotrimoxazole (1.25/23.75 µg), ceftriaxone (30 µg), gentamicin (10 µg), Amoxicillin/clavulanic acid (30 µg) and ciprofloxacin (5 µg) was determined by modified Kirby-Bauer method and incubated at 35°C for 18 - 24 hrs. Antibiotic sensitivity of VRSA strains were not tested against quinupristin/dalfopristin (Synercid) (15 µg) because Isolates with inhibition zone diameter 7 mm around vancomycin disc was absent and not confirmed as Vancomycin Resistant *Staphylococcus aureus* (VRSA). All inoculum on MHA were suspension of pure isolates in physiologic saline with density equivalent to a 0.5 McFarland turbidity standard. *Staphylococcus aureus* ATCC 25923 was used as control. Antibiotic sensitivity testing and result interpretation will be according to CLSI Standards.

Results

A total of 220 medical students, age range 20- 24 years were screened for MRSA Carriage. Among them, 124(56.3%) were Males and 96 (44%) are included in this study (Table -- 1).

Table: 1 Analysis of total samples for MRSA carriage (N=220)

MRSA CARRIAGE	MALES	FEMALES	TOTAL
POSITIVE	12	19	31
NEGATIVE	112	77	189
TOTAL	124	96	220

Table:2 Distribution of MRSA isolates in different sites (N=31)

MRSA CARRIAGE SITES	MALES	FEMALES	TOTAL
NOSE	6	13	19
PALM	3	2	5
WEB SPACE	3	4	7
EITHER NOSE, PALM AND WEB SPACE	0	0	0
TOTAL	12	19	31

Table: 3 Antibiotic sensitivity profiles of MRSA Carriage isolates by disk-diffusion method in different sites (N=31)

S.NO	NAME OF THE ANTIBIOTIC	NOSE (19)	PALM (5)	WEB SPACE (7)
1.	Gentamycin	60%	51%	70%
2.	Chloramphenicol	61.5%	80	60%
3.	Erythromycin	70%	70%	50%
4.	Cotrimoxazole	65%	81%	80%
5.	Ciprofloxacin	80%	72%	40%
6.	Ceftriazone	50%	55%	30%
7.	Amoxyclave	100%	100%	100%
8.	Vancomycin	100%	100%	100%

In all 220, 31 students were Positive for MRSA either in Nose, Palm and Web spaces or in three sites giving a carriage rate of (14%). And in remaining 189 medical students Coagulase negative *Staphylococcus aureus* (CONS) was isolated. The proportion of medical students with nasal carriage was higher 19 (8.6%) than web space 7 (3%) and palm 5 (2%) and no dual carriage and carriage in three sites was observed (Table - 2). Among the 124 of males screened 12 (9.67%) were positive, compared to 96 females 19 (19.79%) of them were positive.

Antibiogram was determined by Kirby Bauer disk diffusion method for all MRSA isolates. The MRSA isolates were sensitive to all antibiotics tested. The

antibiotic sensitivity profiles in the three sites were: - (Table 3). All the MRSA isolates of the three sites Nose, Palm and Web space were sensitive to all antibiotics tested and the isolate resistant to Vancomycin was not identified.

Discussion

Colonization and infection by *S. aureus* are known to be significantly associated with infection among hospitalized patients. In the era of high prevalence of methicillin resistance among *S. aureus*, a relatively high percentage (11 – 19%) of MRSA nasal carriage among hospital patients on admission will increase

the likelihood of MRSA infection during the same episode of hospitalization (Mathanra *et al.*, 2009).

Methicillin resistance among Staphylococci is widespread in India. In a multicentre study from India in 1996, prevalence of MRSA ranged from 27% in Bombay to 47% in Bangalore and 42.5% in Delhi. A study from Eastern India in 2001 revealed 66.6% of *S. epidermidis* and 60% of *S. haemolyticus* strains resistant to oxacillin. The most recent data from India gives a figure of 66% methicillin resistant CoNS among neonatal septicaemic patients; isolated from blood and skin of these patients (Bruno *et al.*, 2005).

Studies on MRSA in intensive care units have also demonstrated that MRSA colonization predisposed to MRSA infection during the same hospitalization (Fadeyi *et al.*, 2010).

This greatly raises concerns about community infections caused by health care - associated strains of MRSA. Community - acquired MRSA (CA-MRSA) is potentially a new emerging pathogen with most strains susceptible to many antimicrobials except for β -lactam antibiotics.

In our study a total of 220 students' age range between 20 - 24 years were screened for MRSA carriage. Among them 124 (56.36%) were males and 96 (44%) were females. Among 220 medical students screened, 31 were positive for MRSA either in nose, palm and web spaces or in three sites giving a carriage rate of 14%. And in remaining 189 students, Coagulase negative *Staphylococcus aureus* (CONS) was isolated. The proportion of health care workers with nasal carriage was higher 19 (8.6%) than web space 7 (3%) and palm 5 (2%) and no dual carriage and carriage in three sites was observed. Among the 124 of males screened 12 (9.67%) were positive, compared to 96 females 19 (19.79%) of them were positive.

Huang and Platt, 2003 compared cefoxitin disc diffusion test with oxacillin agar screening and detection of *mecA* gene by PCR. Fifty strains of *S. aureus* + isolated from clinical samples were isolated by routine antibiotic susceptibility testing. Out of the 50 isolates, 28 were found to be methicillin resistant by oxacillin disc diffusion test, 30 were resistant by oxacillin screen agar method, and 32 were resistant with cefoxitin disc diffusion. For these

32 isolates, *mecA* gene was positive. In these study, results of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene. Thus, this test can be an alternative to PCR for detection of MRSA in resource constraint settings.

From this study, it was clear that prolonged hospital stay was a risk factor for methicillin resistance whereas stay for more than 25 days was found to be a highly significant risk factor. Dermatophytosis and Herpes simplex virus infection were other risk factors for nasal carriage of *S. aureus*. Mathanra *et al.* 2009 screened 200 subjects (90 inpatients, 53 outpatients, and 57 HCWs) for carriage of Methicillin resistant *Staphylococci aureus* (MRSA) at different sites using oxacillin blood agar and Mannitol salt agar with oxacillin. Overall carriage rate was 8.5%, with the highest rate in inpatients (15.6%) while the lowest in HCWs (1.8%) in these patients the commonest site of colonization of MRSA was the anterior nares.

In this studies, MRSA carriage was particularly high among the Doctors (65.2%) and Nurses (64.7%) (Nour de San *et al.*, 2007). In our present study, the *S. aureus* colonization rate (14%) in students was more when compared to (8.5%) in Pondicherry, but less when compared to (28.1%) in Taiwan (Mathanra *et al.*, 2009), (27.3%) in the American Indian population and 52.5%, in the study done in Nigeria health care settings. In one study from Saudi Arabia, an overall nasal carriage of 38.0% was observed, and the carriage rate varied in different age groups. In an earlier study in Nigeria, subjects from health care-related facilities Nasal carriage (38.9%) was higher than hand (25.3%) and Doctors (22.7%) were the predominant carriers when compared with Nurses (16.7%).

Our study also showed a higher MRSA carriage rate, in nasal carriage and was higher 19 (8.6%) than web space 7 (3%) and palm 5 (2%). This indicates the necessity for further emphasis on hand hygiene for students especially before going home, similar to recommendations currently given to patients and their families, to avoid transmission of MRSA to family members. These findings also point to the importance of eradicating MRSA colonization in students and their family members, and their family contacts. Since MRSA infection is a potentially severe event, early diagnosis is essential. Prevention is thus important and

relies on hygienic measures and strict protocols based on aseptic manipulation by students. In nasal carriers of MRSA, eradication of bacteria by a topical antimicrobial ointment has been associated with a significant reduction of the incidence of bacteraemias. Locally applied or synthetic antibiotics are most commonly used to treat MRSA. Nasal ointments or sprays and oral antibiotics have variable efficacy and their use frequently results in antimicrobial resistance among *S. aureus* strains. Of the commonly used agents, Mupirocin (Pseudomonic acid) ointment has been shown to be 97% effective in reducing *S. aureus* nasal carriage (Fadeyi *et al.*, 2010).

Indiscriminate use of antibiotics may lead to resistance to all the other groups of antibiotics. A very few MRSA resistant to Vancomycin and /or Teicoplanin have been found in the USA. Though our study found 100% sensitivity to Vancomycin, improper and indiscriminate use may in near future lead to resistance to these drugs all over the world. So use of appropriate antibiotic treatment in order to avoid the spread of multidrug resistant strains is recommended.

Conclusion

Students of the medical college do not appear to be a major source of MRSA carriage, although it would require screening of larger numbers before arriving at any definite conclusions. In corroboration with the above studies, the present study also showed that the Cefoxitin 30µg disc method appears to be an acceptable alternative for rapid identification of MRSA. However, further confirmatory study can be done by detection of *mecA* by PCR method, which is considered as the gold standard. There is need for the development, adoption and enforcement of appropriate control policies with many points of critical care like our own where there are no existing or effective MRSA control programs.

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