

Prevalence of MRSA Nasal Carriage in Patients Admitted to a Tertiary Care Hospital in Southern India

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ABSTRACT

Introduction: Infections with MRSA, both community and hospital acquired, are well established and the source of infection is often a carrier. There are very few studies showing the magnitude of MRSA nasal colonization among healthy persons from the community. This study was conducted to detect the prevalence of MRSA nasal carriage in patients who did not have any known risk factors associated with HA- MRSA colonization, admitted to a tertiary care centre in Kerala.

Materials and Methods: Nasal swabs were collected from patients within 24 hours of admission. Specimen were inoculated on chromogenic agar (HiCrome MeReSa agar-HiMedia) for MRSA screening. Isolates were then subjected to antibiotic sensitivity tests, SCCmec typing and PVL gene detection.

Results: Out of 683 patients, 16 carried MRSA in their nares (2.3%). Of the 16 strains 13 (81.25 %) strain were SCCmec type III and one belonged to SCCmec type IV (6.25 %). Two strains failed to amplify SCCmec genes. Three strains carried genes for PVL toxin (18.75%).

Conclusion: With a better understanding of the complex epidemiology of MRSA it is increasingly apparent that demarcations between the HA and CA phenotypes are not as clear cut as previously thought. In this study of nasal carriage of MRSA in the community we have demonstrated prevalence consistent with published data. Most isolates however were shown to belong to the type conventionally assigned to HA-MRSA.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) has gained much interest since it is first reported in 1961 and it has become epidemic in many hospitals across the globe. In India the rate of nosocomial infections with MRSA is around 40% [1,2].

MRSA has ceased to be a hospital only pathogen. Over the last two decades they have gained importance as community acquired pathogens. Healthcare (HA) associated and community (CA) associated MRSA strains can be distinguished genetically. Hospital acquired MRSA (HA-MRSA) carries staphylococcal cassette chromosome *mec* (SCCmec) type I, II and III and community acquired *S.aureus* (CA-MRSA) carries SCCmec types IV, V or VI and genes encoding for Pantone-Valentine leukocidin (PVL) [3].

HA-MRSA infections are associated with risk factors like recent hospital stay, living in a long term care facility or carrying an indwelling device or catheter. However, individuals may be colonized with HA-MRSA without these risk factors. CA-MRSA infection and colonization are not associated with the risk factors mentioned above. It is now recognised that CA-MRSA is as important as HA-MRSA in causing hospital outbreaks [4].

The magnitude of nasal colonization of MRSA in personnel who come into close contact with health care facilities is well established. There is however, a paucity of data from healthy individuals in the community. Published data from various centres in India project a prevalence of 1- 5% [5-9].

We present here a study conducted to document the prevalence of MRSA nasal carriage in patients who did not have any known risk factors associated with HA-MRSA colonization, admitted to a tertiary care centre in Kerala. We hypothesised that if at all these patients carry MRSA in their nares, it would be CA-MRSA. We also aimed at finding out the SCCmec type and presence of PVL gene among the MRSA isolates.

Keywords: Nasal colonization, Nasal swabs, Phenotypes

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Microbiology, Government Medical College, Thrissur, Kerala between April 2012 and May 2013. Approval from Institutional Ethics Committee was obtained. Informed consent was taken from patients before swabbing.

The minimum sample size was calculated as 475, anticipating a prevalence rate of 5% based on previous studies and to detect the prevalence with 2% precision. The study subjects were adult patients admitted to surgical, orthopaedics and subspecialty wards within 24 hours of admission.

The following categories of patients were excluded from the study.

1. Patients admitted in Intensive Care Units and pay wards.
2. Patients with any of the following history were excluded from the study:
 - Hospitalisation within past 3 months.
 - History of antibiotic used within past 3 months.
 - Being in close proximity to other ill patients in hospital or household.

Colonization of MRSA is defined as isolation of MRSA in the nasal swabs in the absence of systemic signs of sepsis or overt infection.

Specimens were collected from the anterior nares using cotton swabs moistened with glucose broth. The walls of the vestibules of anterior nares were thoroughly swabbed taking sufficient time. The swabs were inoculated into brain heart infusion broth, incubated for 6-8 hours and subcultured onto chromogenic agar (HiCrome MeReSa agar-HiMedia) and incubated for 48 hours with reading at the end of 48 hours. Suspected colonies from chromogenic agar were confirmed as MRSA by gram stain, growth on mannitol salt agar, catalase and coagulase tests as per standard guidelines.

Commercially available discs were used for antibiotic sensitivity testing by Kirby Bauer method on Muller Hinton agar according to performance standards of Clinical and Laboratory Standards Institute (CLSI) [10]. The antibiotics tested were Penicillin (10 U), Erythromycin (15µg), Gentamycin (10µg), Cephalexin (30µg), Cefoxitin (30µg) and Cotrimoxazole (1.25-23.75µg). Zone sizes were interpreted according to CLSI guidelines.

The strains were further subjected to PCR at Christian Medical College, Vellore to

- Confirm the presence of *mecA* gene
- Type the *SCCmec*
- Detect the presence of PVL gene

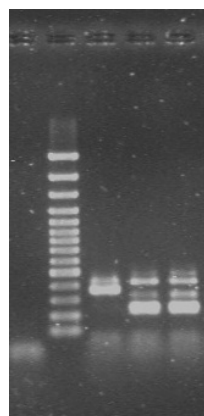
PCR for the detection of *SCCmec* and PVL gene was done according to procedure guidelines by Oliveira DC et al., and Lina G et al., respectively [11,12].

RESULTS

A total of 683 patients fulfilled the criteria to be included in the study. Out of 683 patients, 16 carried MRSA in their nares (2.3%).

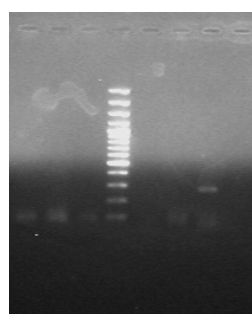
They were resistant to all the antibiotics tested viz Penicillin (10 units), Erythromycin (15µg), Gentamycin (10µg), Cephalexin (30 µg), Cefoxitin (30 µg) and Cotrimoxazole (1.25-23.75 µg).

[Table/Fig-1,2] show PCR control runs and interpretations for *SCCmec* and PVL genes respectively.



| S.No | Strain ID | Interpretation |
|------|--------------------------|----------------|
| 1 | M.H2O (Negative control) | Satisfactory |
| 2 | Ladder 100bp | Satisfactory |
| 3 | Control strain 1 | Type-III A |
| 4 | Control strain 2 | Type-III |
| 5 | Control strain 3 | Type-III |

[Table/Fig-1]: Control run and interpretation of *SCCmec* gene typing.



| S.No | Strain ID | Interpretation |
|------|--------------------------|-----------------------|
| 1 | 9D13 | Negative (old primer) |
| 2 | GRE (Control) | Negative (old primer) |
| 3 | 1D30 | Negative (old primer) |
| 4 | Ladder | 100bp |
| 5 | M.H2O (Negative control) | Negative |
| 6 | Test strain (1D30) | Negative |
| 7 | GRE | PVL Positive 433bp |

[Table/Fig-2]: Control run and interpretation of PVL gene PCR.

Of the 16 isolates 13 (81.25 %) strains were *SCCmec* type III and one belonged to *SCCmec* type IV (6.25%). Two strains failed to amplify the *SCCmec* genes. Three strains carried genes for PVL toxin (18.75%) [Table/Fig-3].

DISCUSSION

We studied the rates of MRSA colonization of anterior nares in adults within 24 hours of admission to a tertiary health care hospital in Southern India.

We chose to swab the anterior nares as they are the natural niches of *S.aureus* and are the most consistent areas from which the organism has been isolated [13,14]. Rectum, perineum, axilla and

| Test strain | SCCmec gene | PVL gene | Test Strain | SCCmec gene | PVL gene |
|---------------|-------------|----------|----------------|-------------|-------------------------------|
| Test strain 1 | Type-III | Negative | Test strain 9 | Type-III A | Negative |
| Test strain 2 | Type-III | Negative | Test strain 10 | Type-III B | Negative |
| Test strain 3 | Type-III | Negative | Test strain 11 | Type -III B | Positive(433bp) (433bp) |
| Test strain 4 | Type-III | Negative | Test strain 12 | Type -III | Positive(433bp) |
| Test strain 5 | Type-III | Negative | Test strain 13 | Negative | Negative |
| Test strain 6 | Type-III | Negative | Test strain 14 | Type -IV | Negative |
| Test strain 7 | Type -III | Negative | Test strain 15 | Negative | Negative |
| Test strain 8 | Type -III | Negative | Test strain 16 | Type -III | Positive(433bp) (((433bp)) |

[Table/Fig-3]: Results of *SCCmec* typing and PVL gene PCR.

device insertion sites are the other favoured sites for colonization. However these sites were excluded anticipating that fewer patients would likely consent for participation.

Isolation rates of *S.aureus* and MRSA from our routine clinical specimens in our laboratory has been approximately 8% and 4% respectively. In the present study, prevalence of MRSA colonization was found to be 2.3%. These patients did not have history of recent hospital admission or other risk factors associated with MRSA colonization. The nasal swabs were collected within 24 hours of admission to exclude colonization of nares resulting from hospitalization.

The prevalence of MRSA colonization is comparable to other published data from India. In a study conducted by Pathak et al., among healthy preschool children in Ujjain showed prevalence of MRSA nasal colonization as 1.02% [5]. Nasal carriage rates of MRSA were found to be 1.83% and 3.89% from similar studies conducted at Kashmir and Chandigarh respectively [6,7]. Colonization rates of MRSA outside hospital environments have been documented to be low across geographic areas [15-18].

All 16 isolates in our study were found to be resistant to all antibiotics tested, a finding distinct from other published Indian studies [6,7]. The potential of these colonizers to act as sources for hospital infection outbreaks with multidrug resistant strains is a cause for concern.

We hypothesized that most of the isolates would belong to *SCCmec* type IV, V or VI. Interestingly in our study the majority (13/16) of isolates were found to carry *SCCmec* type III and only three had PVL gene. In a study conducted in Taiwan on 6057 children between 2 -5 years in a community setting, it was found that 18% of MRSA carriers had *SCCmec* type II and III in their genomes and PVL gene was absent [19]. In a population based study conducted in Brazil to detect nasal colonization of MRSA, it was found that all MRSA isolates belonged to *SCCmec* type IV and none of them had PVL gene [20]. It has been recognised that presence of PVL alone does not predict the community origin of MRSA colonization [21]. However, the large population based NHANES study showed a specific association of *SCCmec* type IV with PVL gene. 50.7% of MRSA were *SCCmec* type IV and 49.3% *SCCmec* type II in this study [22]. In India, *SCCmec* type III is the most prevalent strain in hospital setting and there is insufficient data regarding *SCCmec* types prevalent in the community [23].

The distinction between CA-MRSA and HA-MRSA isolates has been found to have blurred recently. Increasing cross transmission of HA and CA-MRSA between hospitals and communities has rendered the epidemiology of MRSA complex. Centres for Disease Control and Prevention investigators have proposed a category of MRSA infections, "health care-associated, community-onset" MRSA (HACO-MRSA) infection which includes cases that would be HA-MRSA infections by history of health care exposure but have onset in the community [24]. The colonizing strains that we isolated were likely to be acquired from the community, but showed a pattern suggestive of origin in the hospital.

LIMITATION

We have identified some limitations in our study. Sites other than anterior nares were not swabbed and this may have resulted in underestimation of MRSA colonization. Nasal colonization of *S.aureus* and MRSA is dynamic in nature and hence it is possible to have 'intermittent carriage states'. Cross sectional design of this study makes predictions of changes in colonization with time impossible. Epidemiological typing was not done; hence clonal origin of the organism could not be established.

CONCLUSION

With a better understanding of the complex epidemiology of MRSA it is increasingly apparent that demarcations between the HA and CA phenotypes are not as clear cut as previously thought. In this study of nasal carriage of MRSA in the community we have demonstrated prevalence consistent with published data. Most isolates however were shown to belong to the type conventionally assigned to HA-MRSA. Further studies are required to characterise MRSA types in the community and more importantly, to identify whether a true redistribution of the HA-MRSA strains is occurring.

ACKNOWLEDGEMENT

This study was funded by State Board for Medical Research, Kerala.

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FINANCIAL OR OTHER COMPETING INTERESTS: As Declared above .

Date of Submission: Dec 08, 2015

Date of Peer Review: Dec 31, 2015

Date of Acceptance: Jan 13, 2016

Date of Publishing: Feb 01, 2016