

Original Research Article

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## Novel Reporting of Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) Strain at a Tertiary Care Centre

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### ABSTRACT

Recently MRSA has become significant problem in hospitals and community. There has been few works on complete phenotyping, SCCmec typing and SCCmecIV subtyping. With this background, this study was conducted to detect the prevalence, susceptibility pattern, association of PVL gene with CA-MRSA strains with SCCmec typing and SCCmecIV subtyping. Study was carried out at a tertiary care centre for duration of 2 years. Samples were collected from patients as CDC-2000. Total 395 samples were collected, MRSA was isolated from 80 samples (80/395; 20.2%). These strains were resistant to commonly used antibiotics but no VRSA isolated. *mecA* and PVL gene were present in 72 (90%) and 48 isolates (60%). The prevalent strain in the community was SCCmecIVc (40%). The sequences were deposited to genebank with accession NO.KF710034 and BANK LT 1664623. The other SCCmec types were not present in the MRSA isolates. Worldwide we were the 7th person and from India we were the 2nd to deposit the strain of similar sequences. There is change in susceptibility pattern. Most of the genetic elements were carried by isolates. In future proper measures to be adopted to reduce the prevalence.

### Keywords

CA-MRSA,  
PVL gene,  
SCCmec typing,  
SCCmecIV  
subtyping.

### Article Info

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## Introduction

*Staphylococcus aureus* has been recognized as an important cause of human disease for more than 100 years (Lowy, 1998) as it carries a wealth of pathogenic determinants, which promote tissue colonization, damage and distant diseases. *S. aureus* has a niche preference for the anterior nares, in adults (Kluytmans *et al.*, 1997; Wertheim *et al.*, 2005) and is shed on healthy skin, including axilla and perineum. Nasal carriage rate varies from 10% to 40% in both the

community and the hospital environment (Que, 2010). Nasal carriage has become a mode of persistence and spread of multiresistant staphylococci, Methicillin Resistant *Staphylococcus aureus* (MRSA). MRSA are resistant to practically all available antibiotics, leading threat to public health in the hospital (for 03 decades) and in the community since the beginning of this century (Que, 2010).

*Staphylococcal chromosome cassette* (SCC) *mec* is a genetic island in MRSA controlling drug resistance, in that *mec* (Ito *et al.*, 2003) element resistance to methicillin and mediates  $\beta$ -lactam resistance. SCC*mec* is DNA piece (15-60 kb) bounded by direct and inverted repeats to facilitate integration at a homologous site. Recombinases *ccrA* and *ccrB* in SCC*mec* are critical genes which can mediate mobilization of the DNA.

SCC*mec* are discriminated on the basis of the structure of their *ccrA-B* and *mecA* complexes (Ito *et al.*, 2001). SCC*mec* I, II, and III are shown to belong to health care associated clones (HA-MRSA). HA-MRSA harbours multiple resistance determinants of relatively large sizes (35 to 60 kb) with difficulty to mobilize. While SCC*mec* types IV, V, and VI are associated with community associated strains (CA-MRSA). SCC*mec* type V was reported for the first time in Australia (Ito *et al.*, 2004). Community acquired SCC*mec* types harbour much smaller loci (about 15 kb) and do not carry multidrug resistant genes. While they appear to be associated with other elements in the same bacterium, including Pantone-Valentine toxin and multiple staphylococcal exotoxins (*set*) genes.

CA-MRSA seems to be less clonal with small SCC*mec* cassettes, so these are easily mobilized. It is now clear that CA-MRSA has not arisen from HA-MRSA that permeated the community but has emerged independently by acquiring its SCC*mec* most likely from CoNS donors. Whether its spread is from the widespread use of  $\beta$ -lactams or because its SCC*mec* and global genetic context provide other advantages to the bacterium is as yet undetermined.

Although originally confined to the hospital environment MRSA has emerged as a community-acquired infection over the last

decade. CA-MRSA is not associated with any risk factors. Predominantly CA-MRSA causes primarily skin and soft tissue infections and sometimes rapidly fatal necrotizing pneumonia, necrotizing fasciitis bone & joint infections.

CDC defined CA-MRSA infection based on

- An outpatient or within 48 hr of hospitalization
- Should lack risk factors as hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient (David *et al.*, 2008; Morrison *et al.*, 2006).
- Definition was used to demonstrate MRSA infections among healthy people.
- Definition was further modified for Active Bacterial Core Surveillance Program for invasive MRSA infections and to exclude the previous isolation of MRSA as a criterion for HA-MRSA.

Numerous outbreaks of community-based infection affecting diverse groups of professionals, different regions have occurred in the last 10 years. These outbreaks implied dramatic change in the epidemiology of MRSA infections. Risk factors which were common to outbreaks were poor hygienic conditions, close contact, contaminated material, and damaged skin. Limited numbers of MRSA strains were responsible for the outbreaks (USA-300 in US).

MRSA prevalence varied in India depending upon the number of factors. one study which

involved 5-15 yrs age group had prevalence 19% (Ramana *et al.*, 2009). Apart from the diversity in prevalence there was clonal expansion of multi drug resistant strains as detected by Namita D'souza, *et al.*, in Mumbai, India (D'Souza *et al.*, 2010).

Keeping this background, this study was designed with an aim to determine the prevalence and further to carry out molecular characterization of CA-MRSA strains by easy, simple and time cumbersome methods.

### **Materials and Methods**

A cross-sectional study was conducted from July 2011 to July 2013 at department of Microbiology and affiliated tertiary care hospital. Inclusion criterion for the study was followed as per definition of CA-MRSA by CDC-2000. Various clinical samples like swabs from pus, fluids, discharge, anterior nasal site and hand flora were received from various OPDs.

Standard phenotypic methods (Gram stain, colony morphology, catalase test, slide and tube coagulase test, bacitracin resistance, Furazolidine sensitivity, Deoxyribonuclease (DNase) Test, Phosphatase Test, Mannitol Fermentation Test) were used for identification. Afterwards MRSA isolates were identified by using the Cefoxitin (CX-30µg) disk as per CLSI guidelines (Clinical and laboratory standards institute, 2009). All these isolates were tested for their susceptibility to antibiotics active against Gram positive cocci by Kirby Bauer disc diffusion method. However vancomycin resistance was detected by Vancomycin Screen Agar. Molecular characterization was carried out by detection of *mecA* genes, *PVL* toxin, *SCCmec* typing into types I to type V and *SCCmecIV* subtyping into *SCCmecIVa*, b, c and d. DNA extraction was done by using the QIAamp DNA mini

kits from QIAGEN, Germany as per Manufacturer's instructions. DNA extraction was done on the same day after phenotypic identification of MRSA. DNA was stored at -70°C until the PCR. Two sets of multiplex PCRs were carried out separately. In the first PCR (PCR-1) *mecA* and *PVL* gene were detected. In the second PCR (PCR-2) *SCCmec* typing and *SCCmecIV* subtyping was carried out. Standard published primers and thermocycling conditions were followed as per McClure, *et al.*, for PCR-1 and Moussa, *et al.*, for PCR-2. In PCR-1, positive control used for *mecA* gene and *LeukS/F-PV* gene were respectively ATCC MRSA-43300 and ATCC25923. In PCR-2, an internal positive control was used, whose sequences were identified as similar to *SCCmecIVc*. Later on the sequences were deposited to gene bank with accession no KF710034 and BANK LT 1664623. This KF710034 and BANK LT 1664623 were positive control for subsequent PCRs. In both PCRs, ATCC29213 served as negative control.

### **Results and Discussion**

Out of 395 samples *Staphylococcus aureus* and MRSA were isolated from 90 and 80 samples respectively. The prevalence of *Staphylococcus aureus* in the present study was 22.7% and that of MRSA infection was 20.2%. There was change in susceptibility pattern of these MRSA isolates towards regularly used antibiotics. However there was no isolate which was resistant to linezolid and vancomycin.

Sample wise isolation of MRSA is briefed in Table-1. Majority of isolation was from both nasal and hand flora of patients attending OPDs (51.3%). Subsequent sample was pus swabs (26.2%) while the remaining 22.5% involved wound swabs, aural swabs, throat swabs and urine.

Gender was distribution was shown in table-

2. 57.5% of MRSA were from 46 male patients and 42.5% were from 34 female patients. Thus more of male patients were carrying MRSA. The male to female ratio of MRSA carriage was 1.3:1 (M: F). Age distribution in the present study had ranged from 09 months to 77 years of age. Table-3 showed the distribution of MRSA as per age and gender wise. Maximum (35%) isolates were from age group 20-29 years, subsequent age group was above 60 yrs (18.7%). Thus the reproductive age group is affected maximally in female patients than the aged & debilitated one.

OPD wise distribution of MRSA isolates is given in Table-4. Highest MRSA isolates (28.5%) were from surgical OPD. Details of antimicrobial susceptibility pattern are showed in Table-5. There was change in the susceptibility pattern of all MRSA isolates to the commonly used antibiotics. All these isolates (100%) were resistant to penicillin while 48.8% were resistant to cotrimoxazole. There was no isolate which was resistant to Linezolid and vancomycin. No VRSA was detected out of the MRSA isolates.

### **Molecular characterization of MRSA**

90% of *mecA* gene, 60% of *PVL* gene was detected in PCR-1. In PCR-2 the prevalent strain in the regional community was SCC*mecIV* with subtype SCC*mecIVc* detected in 40% of MRSA isolates. The sequences were deposited to genebank with accession NO.KF710034 and BANK LT 1664623. The other SCC*mec* types were not present in the MRSA isolates. The details are shown in fig-1 & fig-2 for PCR-1 & PCR-2 respectively.

There is variation in prevalence of MRSA as confirmed by various studies. The fact of concern is the rise in prevalence of MRSA as shown by NNIS survey with the increase

in hospital prevalence rate from 2.4% in 1975 to 29% in 1991 (Panlilio *et al.*, 1992). In the present study, MRSA prevalence was 20.2% which corroborated other studies. Ashok pathak *et al.*, carried out the study on nasal carriage of MRSA in healthy preschool children in Ujjain, India. They had shown the prevalence rate of MRSA was 16.3%. High percentage of MRSA isolation from the present study might be because of the more number of nasal swab samples compared to other samples.

The median age for adults infected with CA-MRSA varied from 20 to 47 years. The maximum numbers of patients were from age group of 20 yrs to 59 yrs. Though MRSA does not show predilection for any particular gender, total percentages of MRSA isolation in the present study, from male and female were respectively 57.5% and 42.5%. There was higher incidence of MRSA in male population compared to female population. These similar findings, higher incidence of MRSA infection among males has also been reported by Chua *et al.*, which correlated with our findings.

CA-MRSA is an emerging problem in the obstetric population with most common presentation as skin or soft tissue infection involving multiple sites. Recurrent skin abscesses during pregnancy should raise prompt investigation for MRSA (Laibl *et al.*, 2005). In the present study also there was 53% involvement of women patients attending obstetric OPD. These findings raised suspicion of CA-MRSA as an emerging infection in obstetric populations in our setting.

All the MRSA isolates were 100% resistant to penicillin and 100% sensitive to Linezolid; similar results were reported by Kaleem *et al.*, In the present study no VRSA was isolated, while all these MRSA isolates had shown increase in resistance

(30-48.8%) with the other antibiotics. Similar type of sensitivity was demonstrated by INSAR group of India and Kaleem *et al.*, In the past CA-MRSA strains were not

multidrug resistant, but in the present study most of the isolates were multi drug resistant, a matter of concern.

**Table.1** Sample wise distribution of MRSA (n = 80)

Nature of specimen	Number of specimens	Percentage (%) of isolation from sample
Nasal swab	28	35
Pus swab	21	26.2
Hand swab	13	16.3
wound swab	06	7.5
Aural swab	05	6.2
Throat swab	05	6.2
Urine	02	2.6
Total	80	100

**Table.2** Gender wise distribution of MRSA (n = 80)

Sample	Male	Female	Total Percentage (%) of isolation from both male and female
Nasal swab	10	18	35
Pus swab	15	06	26.2
Hand swab	08	05	16.3
wound swab	05	01	7.5
Aural swab	04	01	6.3
Throat swab	03	02	6.3
Urine	01	01	2.4
Total	46	34	100

**Table.3** Age and Gender wise distribution of MRSA (n=80)

Age in Years	Male (Percentage)	Female (Percentage)	Total	Total Percentage (%) of isolation
<9	07(8.7)	02(2.5)	09	11.2
10-19	04(5)	04(5)	08	10
20-29	10(12.5)	18(22.5)	28	35
30-39	07(8.7)	03(3.8)	10	12.5
40-49	03(3.8)	02(2.5)	05	6.3
50-59	03(3.8)	02(2.5)	05	6.3
>60	12	03	15	18.7
Total	46	34	80	100

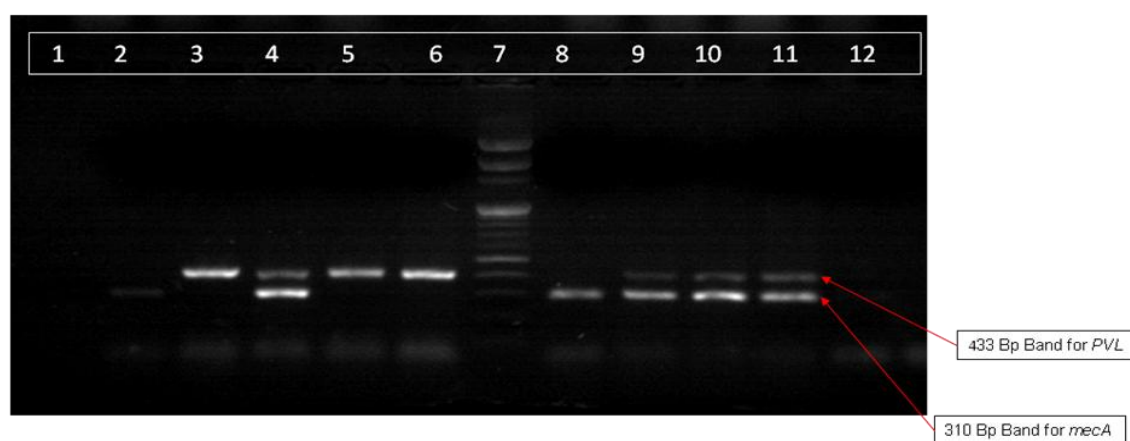
**Table.4** OPD and Gender wise isolation of MRSA (n=80)

OPD	Male (percentage)	Female (percentage)	Total (Male+Female)	Total Percentage (%) of isolation from OPDs
Medicine	14(17.5)	04(5)	18	22.5
Surgery	17(21.3)	06(7.5)	23	28.8
Obstetrics	00(0)	18(22.5)	18	22.5
Paediatrics	07(8.8)	04(5)	11	13.8
Eye	03(3.8)	01(1.2)	4	5
ENT	05(6.2)	01(1.2)	6	7.4
Total	46(57.5)	34(42.5)	80	100

**Table.5** Antimicrobial susceptibility pattern of MRSA isolates (n= 80)

Drug ( $\mu$ g)	Sensitive (Percentage)	Intermediate (Percentage)	Resistant (Percentage)
Penicillin (10units)	0(0)	0(0)	80(100)
Erythromycin (15 $\mu$ g)	28(35)	19(23.7)	33(41.3)
Clindamycin (2 $\mu$ g)	39(48.8)	17(21.2)	24(30)
Ciprofloxacin (5 $\mu$ g)	28(35)	15(18.7)	37(46.3)
Cotrimoxazole (1.25/23.75 $\mu$ g)	18(22.5)	23(28.7)	39(48.8)
Gentamicin (10 $\mu$ g)	32(40)	20(25)	28(35)
Linezolid (30 $\mu$ g)	80(100)	0(0)	0(0)

**Molecular characterization of MRSA by PCR-1**



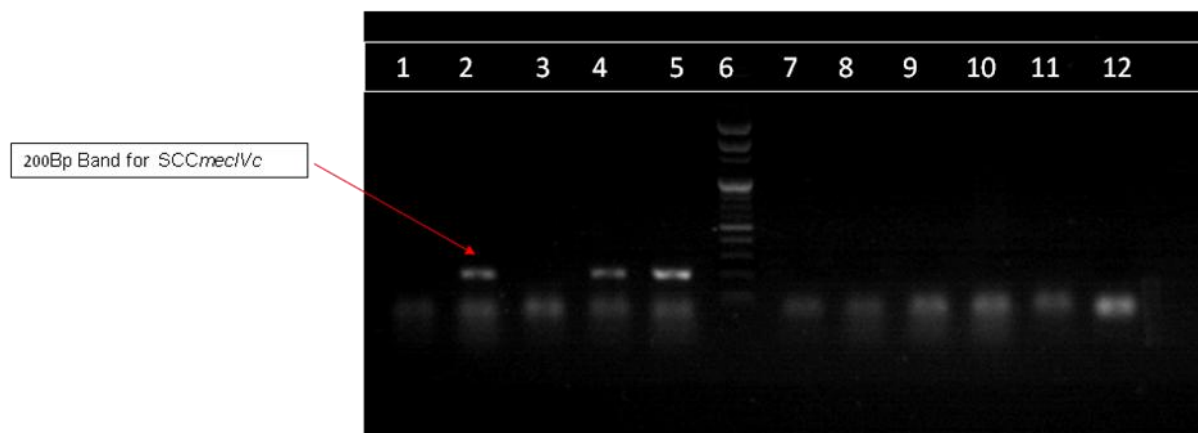
**Fig.1** Showed gel electrophoresis of first PCR (PCR-1) products. Showing band for *mecA* at 310 BP, in sample no 2, 4, 8, 9, 10 and 11. Showing band for *PVL* at 433BP in sample no 3, 4, 5, 6, 9, 10 and 11.

11 – Positive control (*mecA* gene–ATCCMRSA43300, *PVL* gene- ATCC25923)

12 – Negative Control (ATCC MSSA 29213)

7– Molecular marker (100bp)

### Molecular characterization of MRSA by PCR-2



**Fig.2** showed gel electrophoresis of second PCR (PCR-2) products. Showing band for SCCmecIVc at 200 BP, in sample no 4 and 5.

- 2 – Internal positive control (Gene Bank accession no- KF710034 and BANK LT 1664623)
- 1 – Negative Control (ATCC MSSA 29213)
- 6 – Molecular marker (100bp)

Though Linezolid and vancomycin are reserve drugs but there constant use in future may develop resistance in MRSA.

All the 80 MRSA isolates were subjected to two types of multiplex PCRs. The initial multiplex PCR was done to identify MRSA carrying *mecA* and *PVL* gene and in the second multiplex PCR, *SCCmec* typing and *SCCmecIV* subtyping was carried out. In the present study *mecA* was isolated from 72 (90%) MRSA isolates. The discrimination between the phenotypic and genotypic methods might be because of loss of *mecA* gene on storage.

Similar type of discrimination was shown by Arjanne van Griethuysen or Olowe. *PVL*, a necrotizing cytotoxin is considered as specific for the human and rabbit polymorphonuclear cells (Loffler *et al.*, 2010) also an important marker for pathogenic CA-MRSA infections. In developing countries like India (Nagarajan *et al.*, 2010) there is high incidence of *PVL*

gene in CA-MRSA. However there have been reports of absence of *PVL* gene in CA-MRSA. In a study conducted by Nagarajan Abimanyu *et al.*, had shown 40% of MRSA carried the *PVL* gene. In a study conducted at Mumbai by Namita D'suza *et al.*, had shown the 60% positivity of *PVL* gene in MRSA isolates. In the present study also 60% of MRSA isolates carried the *PVL* gene which corroborated with the above study findings.

32 MRSA isolates (40%) were of *SCCmecIVc* type. Further sequences were deposited in gene bank with accession no- KF710034 and BANK LT 1664623. In this study we found the prevalent strain in the community was multidrug resistant of type *SCCmecIVc*. Namita D'suza *et al.*, performed MLST of the MRSA strains and found the prevalent strain of *SCCmecIVc*.

The highlights of this study included compiled, simple and easy format for the identification, susceptibility testing by using

both phenotypic and genotypic methods for CA-MRSA. The novel features were reporting of multidrug resistant CA-MRSA in communities. For the first time from the local community, we had reported the prevalent circulating strain of SCC*mecIVc* subtype.

In conclusion, there is a constant rise in MRSA associated with change in epidemiological parameters. There is an ongoing change in prevalence, resistance profile and epidemiology of CA-MRSA strains. Though CA-MRSA carriers are asymptomatic but may act as potent source for spread in the community. Risk factors are not prominently seen with CA-MRSA compared to HA-MRSA.

In India the carriage rate is high because of close community, improper hygiene and poor socioeconomic strata. As the asymptomatic carriers spread the infection along with the toxin facilitates increase in the invasive diseases. There is increasing trend of colonisation in pregnant women apart from the neonates and adults. Initially CA-MRSA were resistant to few antibiotics but in the present study most of the isolates were multidrug resistant. The prevalent strain in the local community is of type SCC*mecIVc*. Namita D'suza *et al.*, had signified the association of SCC*mecIVc* with the influenza strain.

Same episodes may occur in the local community (study place). Early identification of carriers and effective antibiotic policy, hand hygiene and decolonisation of the carrier patients are foremost required to prevent the spread in community.

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