

## Community Acquired MRSA - An Alarming Threat

S. Deepa<sup>1</sup> and Shobha D. Nadagir<sup>2</sup>

<sup>1</sup>Department of Microbiology, Mysore Medical College and Research Institute,  
Mysore - 570 001, India.

<sup>2</sup>Department of Microbiology, KIMS, Hubli, India.

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Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) is an emerging pathogen. There is considerable concern that CA-MRSA may cause infections difficult to treat in the outpatient settings. The present study was undertaken to know the incidence of CA-MRSA and its antibiotic sensitivity pattern. A total of 214 methicillin resistant *Staphylococcus aureus* were detected using oxacillin disk diffusion method. Clindamycin sensitivity was used as surrogate marker for screening CA-MRSA. Clindamycin sensitive MRSA with no obvious history of hospital acquired infection were analyzed further genotypically for molecular characterization. Clindamycin sensitive MRSA were 122(57%). Among these 109 isolates were presumed to be CA-MRSA. Of these 83(76.14%) were positive for *mecA* gene. Forty four (53%) revealed SCCmec type III, 34(40.96%) were SCCmec type III A and 4(4.81%) had SCCmec type IV cassettes. Nineteen isolates showed *pvl* gene. Only 4(4.81%) of clindamycin sensitive MRSA were genotypically proved as CA-MRSA. CA-MRSA were susceptible to erythromycin, gentamicin, ciprofloxacin, co-trimoxazole, tetracycline and vancomycin. There is a need for judicious selection of antimicrobial agents and effective infection control programmes to prevent the spread of community acquired infections.

**Key words:** *Staphylococci*, methicillin resistance, CA-MRSA.

Detection of methicillin resistance in *Staphylococci* is fundamental to modern day nosocomial infection control.<sup>1</sup> These strains have evolved over the last four decades.

The increased incidence of multidrug resistant *Staphylococcus aureus* strains among nosocomial infections has added a challenging dimension to the *S. aureus* problem. These strains are typically labeled as hospital acquired methicillin resistant *S. aureus* (HA-MRSA).<sup>2</sup> Selective pressure due to overuse of antibiotics could have

led to the emergence of MRSA in the community (CA-MRSA).<sup>3</sup> The boundaries between HA-MRSA and CA-MRSA are getting blurred due to the movement of patients and infections between hospitals and community. Also it is becoming difficult to distinguish between HA-MRSA and CA-MRSA on clinical and epidemiological grounds. Much of what we know about these differences came from the studies based on the genotypic characterization of MRSA. It is also known that HA-MRSA and CA-MRSA pose problem in health care facilities due to associated morbidity and health care costs.<sup>4,5</sup>

MRSA detection is very important by the fact that drugs like vancomycin, teicoplanin, pristinamycin which are effective against MRSA are highly toxic and expensive which most poor

\* To whom all correspondence should be addressed.  
Mob.: +91-9686229737;  
E-mail: drdeepa\_intel@yahoo.co.in

patients cannot afford. Also emergence of resistant strains to these drugs play havoc to mankind.

Thus, a study was conducted to identify MRSA isolates and to know the incidence of CA-MRSA in Hubli. An attempt is made to characterize these MRSA isolates by genotyping methods.

## MATERIALS AND METHODS

The present study was conducted at Karnataka Institute of Medical Sciences, Hubli, Karnataka over a period of one year.

Samples were included from patients suffering from skin and soft tissue infections attending Dermatology, Surgery, Orthopedics outpatients and in patients within 48 hours of admission. A detailed history of previous hospital admission within two years, antibiotic intake in previous 6 months, family history of any associated infections and other risk factors for infection were elicited from every patient.

Primary identification of *S. aureus* was done by using Gram stain, catalase, slide and tube coagulase tests and growth on mannitol salt agar.<sup>6</sup>

Antibiotic sensitivity was performed on Muller Hinton agar using Kirby-Bauer disk diffusion method according to CLSI.<sup>7</sup> Disks used were ampicillin (10µg), erythromycin (15µg), gentamicin (10µg), netilmycin (30µg), amikacin (30µg), ciprofloxacin (5µg), tetracycline (3µg), cotrimaxole(25µg). Methicillin resistance was detected using 1µg oxacillin disk on 4% NaCl Mueller Hinton agar and incubated at 35°C. Additional antibiotics for all methicillin resistant strains used were clindamycin (2µg), levofloxacin (5µg), vancomycin(30µg) and rifampin(5µg).

Based on inclusion criteria, 109 clindamycin sensitive and oxacillin resistant isolates were presumed to be CA-MRSA and were further analyzed genotypically. Of these 63(57.79%) were isolated from outpatients and 46(42.20%) were isolated from inpatients within 48 hours of admission.

Genotyping of these strains were done by using multiplex PCR in Indian Institute of Science, Bangalore (IISc). All of 109 isolates were positive for Deoxyribonuclease test. Chromosomal DNA extraction was done Multiplex PCR<sup>8</sup> was setup to detect *mecA* gene and Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types I, II,

III, IV, V cassettes. PCR products were stored at 4°C for 18-24 hours before running on gel electrophoresis. A separate PCR was setup for Pantone Valentine Leukocidin (*pvl*) gene.

SCC*mec* type I, II, III, IIIA are characteristic of HA-MRSA and SCC*mec* types IV, V along with *pvl* gene is characteristic of community-acquired infections.<sup>9</sup> Strains were genotyped and positive strains for the SCC*mec* cassettes were categorized into HA-MRSA and CA-MRSA accordingly.

## RESULTS

A total of 214 isolates of MRSA were isolated during the study period. The incidence of MRSA in our study was found to be 45.82 % Distribution of 214 MRSA isolates in different samples: majority i.e. 52.33% were from pus samples, 32.71% patients had primary skin infections [30 cases were associated with boils, 15 cases were with cellulites, 13 cases were with furuncles, 7 cases were with necrotizing fasciitis and 5 cases had open wounds]. 21.02% patients had abscesses, 14.95% patients had wound infections, 10.74% patients had bone infections, 6.54% patients had external ear infections, 3.73% patients had incision site infections, 2.33% patients presented with infected sebaceous cyst and 1.86% patients were admitted for road traffic accidents with skin abrasions. Only 13(6.97%) blood samples yielded MRSA, among them 8 had septicemia and 5 patients had bronchopneumonia with septicemia.

Clindamycin disk sensitivity was used as surrogate marker for screening CA-MRSA.<sup>3</sup>

A total of 122 (57%) isolates were clindamycin sensitive. As per inclusion criteria only 109 clindamycin sensitive isolates were presumed to be community acquired strains.

Out of 109 strains phenotypically detected MRSA, *mecA* gene was detected only 83(76.14%) isolates which confirms methicillin resistance. On further typing of *mecA* gene positive strains (n=83) majority i.e.53.01% were SCC*mec* type III followed by SCC*mec* type III A 42.16%. The presence of SCC*mec* type III and SCC*mec* type III A were confirmatory for HA-MRSA.

SCC*mec* type IV was seen only 4.81% and all were positive for *pvl* gene.

**Table 1.** Antibiotic sensitivity pattern of MRSA strains

Antibiotics	Cd sensitive MRSA n=109 %	Cd resistant MRSA n=92 %	Z value	P value
Erythromycin	96.33	0	53.49	<0.0001
Tetracycline	55.96	11.95	07.54	<0.001
Gentamicin	39.44	08.69	05.56	<0.001
Netilmycin	88.07	77.17	02.03	<0.05
Amikacin	89.90	76.08	02.61	<0.01
Ciprofloxacin	50.45	33.69	02.44	<0.05
Levofloxacin	73.39	66.30	01.09	>0.05
Cotrimaxazole	70.64	36.95	05.06	<0.01
Rifampin	100	89.13	03.35	<0.001
Vancomycin	100	100	0	>0.05

Cd-clindamycin

Statistical analysis done by Chi -Square test using SPSS software.

P value<0.05 was considered as significant.

There is significant difference in the susceptibility pattern of clindamycin sensitive and clindamycin resistant MRSA for all antibiotics except levofloxacin.

**Table 2.** Showing the results of multiplex PCR

<i>mecA</i> gene positive isolates	n= 83	%
SCC <i>mec</i> I	0	0
SCC <i>mec</i> II	0	0
SCC <i>mec</i> III	44	53.01
SCC <i>mec</i> III A	35	42.16
SCC <i>mec</i> IV	04	04.81
SCC <i>mec</i> V	0	0
<i>pvl</i> gene	04	04.81

Totally 19(17.43%) isolates out of 109 isolates showed the presence of *pvl* gene. Of these only 4 isolates were *mecA* gene positive. Presence of *pvl* gene is a marker for community acquired infections. Presence of SCC*mec* types IV, and *pvl* gene are confirmatory to CA-MRSA.

## DISCUSSION

*S. aureus* has shown a disconcerting propensity to develop resistance to antimicrobials and has become a challenge for infection control programme and clinicians.<sup>8</sup>

Resistance to antibiotics is a major

concern world wide and is exemplified by the global spread of MRSA.<sup>10</sup>

Patients were considered to harbor CA-MRSA if they had no contact with healthcare facilities within past two years, no antibiotic intake in previous 6 months and samples collected from outpatients or inpatients within 48 hours of admission.<sup>11</sup> Clindamycin susceptibility was used as a surrogate marker to screen CA-MRSA.<sup>3</sup> Based on the above criteria 109 MRSA isolates were included in our study and were further characterized by genotypic methods. These strains were isolated from 42.20% inpatients within 48 hours & 57.79% from outpatients. Most of the out patients had skin ailments, otherwise were healthy individuals.

In our study out of 109 isolates phenotypically showing resistance to methicillin, only 83(76.14%) isolates showed the presence of *mecA* gene. There are reports stating only 81-95% of phenotypically identified MRSA possessed *mecA* gene.<sup>12,13</sup> Test conditions like temperature, disk potency, salt concentration, time of incubation play a very important role in detection of methicillin resistance.<sup>14</sup> Also studies have shown that 14.4% of isolates have lost *mecA* gene during storage.<sup>15</sup>

In our study SCC*mec* type III was identified in 53% isolates and SCC*mec* type IIIA in

42.16 %. These isolates were confirmed to have hospital acquired MRSA infection. In a cross sectional study conducted at Bangalore 59.75% were SCCmec type III and 31.70% were SCCmec type IIIA.<sup>8</sup>

SCCmec type IV which is a marker for CA-MRSA was found in only 4.81% of the isolates. Several authors have reported the prevalence of CA-MRSA ranging from <1% to 36%.<sup>16,4</sup> A low prevalence of CA-MRSA <1% has been reported from all parts of the world. But in Chicago CA-MRSA accounted for up to 22% of MRSA isolates.<sup>17</sup> In Tampa general hospital Florida contrasting results of 43% of isolates were determined to have CA-MRSA presented with significant infection.<sup>18</sup>

No formal link between *pvl* gene and *mecA* gene has been reported but the combination of *pvl* gene and *mecA* gene is the feature of CA-MRSA infection.<sup>10</sup> Even more some studies have reported that *pvl* gene was detected in methicillin sensitive *S. aureus*.<sup>19</sup> In our study a total of 19 isolates showed *pvl* gene, of which only 4 (4.81%) isolates were positive for *pvl* gene and *mecA* gene. Other 15 strains were negative for *mecA* gene which was depicting methicillin susceptibility. Many authors have reported 11% of *pvl* gene in *S. aureus*.<sup>19</sup> and *pvl* positive isolates were mostly methicillin sensitive *S. aureus*.

Our study shows 4(4.81%) isolates to possess SCCmec types IV and *pvl* gene. These strains were considered as CA-MRSA. A study in France, has identified *pvl* gene in all the CA-MRSA isolates.<sup>10</sup> Another study has reported 92% of CA-MRSA strains possessed *pvl* gene.<sup>18</sup> A study in England by Holmes et al have reported that PVL is a stable marker for CA-MRSA.<sup>19</sup>

In our study only 3.66% of 109 clindamycin sensitive MRSA were confirmed to have CA-MRSA. Clindamycin susceptibility which was said to be the marker of community acquired infection, no longer holds good. Clindamycin susceptibility is not a highly specific marker.<sup>3,10</sup> In a study at University hospital in Central United States on 161 clindamycin susceptible MRSA, only 24.69% were community acquired and molecular typing showed only 2.46% of CA-MRSA.<sup>20</sup> Clindamycin is not a routinely prescribed drug for minor ailments in our set up. This might be the reason for higher incidence of clindamycin susceptibility in our study.

More surveillance studies are required to evaluate the extent of dissemination of CA-MRSA in different setting.

## CONCLUSION

CA-MRSA has emerged as a potentially invasive pathogen. Clindamycin susceptibility no longer holds good as a marker for community acquired infections. All these four isolates were sensitive to erythromycin, gentamycin, ciprofloxacin, co-trimazole, tetracycline and vancomycin.

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## REFERENCES

1. Chawla K, Shetty Anoop K, Rao Sugandhi P. Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Directly from Clinical Specimens by Multiplex Polymerase Chain Reaction (PCR) and Conventional Culture Methods – A Comparative Study. *Indian Journal for the Practicing Doctor*: 5(1): (2008-03 - 2008-04):1-4.
2. Said-Salim B, Mathema B, Braughton K et al. Differential distribution & expression of Panton valentine leukocidin gene among Community acquired Methicillin resistant *Staphylococcus aureus*. *J Clin microbiol*, 2005; 43(7): 3373-3379.
3. Krishna BVS, Patil A, Chandrasekhar MR. Community acquired methicillin resistant *Staphylococcus aureus* infections in South Indian city. *South east Asian J Trop med public health*. 2004; 35(2):371-374.
4. Cassandra D, Farr BM, Calfee DP. Community acquired Methicillin resistant *Staphylococcus aureus*, A Meta analysis of prevalence & risk factors *Clin Infect*. 2003; 36:131-139.
5. Chaix C, Durand-Zaleski I, Alberti C, Brun-Buisson C. Control of endemic Methicillin resistant *Staphylococcus aureus*; a cost benefit analysis in a intensive care unit. *Antimicrob agents*. 1999; 282:1745-1751.
6. Koneman E. *Staphylococci & related Gram positive cocci* in Winn W Jr, Allen S, Janda

- W, Koneman E, Procop G & Woods editors – Koneman's Color atlas & text book of diagnostic microbiology 6th edn. Edinburg: Lippincott Williams & Wilkins: 2006: 649-655.
7. CLSI Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Wayne, PA, USA 2007; M100-S17:27(1).
  8. Arakere G, Nadig S, Swedberg G et al. Genotyping of Methicillin resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, *South India. J Clin Microbiol.* 2005; **43**(7):3198-3202.
  9. Ribeiro A, Cicero Dias, Silva-Carvalho MC, Berqua L et al. First Report of Infection with Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in South America. *J Clin Microbiol.* 2005; **43**(4):1985-1988.
  10. Dufour P, Gillet Y, Bes M et al. Community-Acquired Methicillin-Resistant *Staphylococcus aureus* infections in France. Emergence of single clone that produces Panton valentine leukocidin. *J Clin Infect Disease.* 2002; **35**: 819-824.
  11. Harbath S, Fancors P, Schrenger J et al. Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Switzerland *Infect Med* 2000; **17**(4):289-298.
  12. Wielders CLC, Fluit AC, Brisse S et al. MecA gene is widely distributed in *Staphylococcus aureus* population. *J Clin Microbiol.* 2002; **40**(11): 3970-3975.
  13. Bishop EJ, Grabsch EA, Ballard SA et al. Concurrent analysis of nose & groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual specimen PCR and routine culture assays for detection of colonization by MRSA. *J Clin Microbiol.* 2006; **44**(8): 2904-2908.
  14. Skow R, Smith R, Larsen AR et al. Phenotypic detection of Methicillin-Resistant in *Staphylococcus aureus* by disk diffusion testing and Etest on MHA. *J Clin Microbiol.* 2006; **44**(12): 4395-4399.
  15. Griethusyen AV, Inge Van Loo, Belkum AV et al. Loss of mecA gene during storage of Methicillin-Resistant *Staphylococcus aureus* strains. *J Clin Microbiol.* 2005; **43**(3):1361-1365.
  16. Kiliac A, Li H, Staratton WC et al. Antimicrobial susceptibility patterns & Staphylococcal cassette chromosome mec types as well Pantonvalentine leukocidin occurrence among Methicillin-Resistant *Staphylococcus aureus* isolates from children & adults in Middle Tennessee. *J Clin Microbiol.* 2006; **44**(12):4436-4440.
  17. Pricla A, Trinande, Pacheco RL et al. Prevalence of Scc mec type IV in nosocomial blood stream isolates of Methicillin-Resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2005; **43**(7):3435-3437.
  18. Moroney MM, Heller LC, Arbuckle J et al. Staphylococcal cassette chromosome mec & Pantonvalentine leukocidin characterization of MRSA clones. *J Clin Microbiol.* 2007; **45**(3): 1019-1021.
  19. Holmes A, Ganner M, McGuane et al. *Staphylococcus aureus* isolates carrying Panton valentine leukocidin genes in England & Wales: frequency, characterisation & association with clinical disease. *J Clin Microbiol.* 2005; **43**(5): 2384-2390.
  20. Sunanthararm N, Hacek D, Peterson LR. Low prevalence of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in adults at university hospital in the Central United States. *J Clin Microbiol.* 2001; **39**:1669-1671.