Detection of methicillin resistance in Staphylococci is fundamental to modern day nosocomial infection control. 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). Selective pressure due to overuse of antibiotics could have led to the emergence of MRSA in the community (CA-MRSA). 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. 

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...
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 \textit{S. aureus} was done by using Gram stain, catalase, slide and tube coagulase tests and growth on mannitol salt agar. Antibiotic sensitivity was performed on Muller Hinton agar using Kirby-Bauer disk diffusion method according to CLSI. Disks used were ampicillin (10µg), erythromycin (15µg), gentamicin (10µg), netilmicin (30µg), amikacin (30µg), ciprofloxacin (5µg), tetracycline (3µg), cotrimazaxole(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 was setup to detect \textit{mecA} gene and Staphylococcal Cassette Chromosome \textit{mec} (SCC\textit{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 Panton Valentine Leukocidin (\textit{pvl}) gene.

\textit{SCC\textit{mec}} type I, II, III, IIIA are characteristic of HA-MRSA and \textit{SCC\textit{mec}} types IV, V along with \textit{pvl} gene is characteristic of community- acquired infections. Strains were genotyped and positive strains for the \textit{SCC\textit{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,13 cases were with cellulites,13 cases were with furuncles,7 cases were with necrotizing fascitis 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. 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, \textit{mecA} gene was detected only 83(76.14%) isolates which confirms methicillin resistance. On further typing of \textit{mecA} gene positive strains (\textit{n}=83) majority i.e.53.01% were \textit{SCC\textit{mec}} type III followed by \textit{SCC\textit{mec}} type III A 42.16%. The presence of \textit{SCC\textit{mec}} type III and \textit{SCC\textit{mec}} type III A were confirmatory for HA-MRSA.

\textit{SCC\textit{mec}} type IV was seen only 4.81% and all were positive for \textit{pvl} gene.
Table 1. Antibiotic sensitivity pattern of MRSA strains

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Cd sensitive MRSA n=109</th>
<th>Cd resistant MRSA n=92</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>96.33%</td>
<td>0%</td>
<td>53.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>55.96%</td>
<td>11.95%</td>
<td>07.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>39.44%</td>
<td>08.69%</td>
<td>05.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>88.07%</td>
<td>77.17%</td>
<td>02.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amikacin</td>
<td>89.90%</td>
<td>76.08%</td>
<td>02.61</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50.45%</td>
<td>33.69%</td>
<td>02.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>73.39%</td>
<td>66.30%</td>
<td>01.09</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>70.64%</td>
<td>36.95%</td>
<td>05.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rifampin</td>
<td>100%</td>
<td>89.13%</td>
<td>03.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>meca gene positive isolates</th>
<th>n= 83</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCmec I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SCCmec II</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SCCmec III</td>
<td>44</td>
<td>53.01</td>
</tr>
<tr>
<td>SCCmec III A</td>
<td>35</td>
<td>42.16</td>
</tr>
<tr>
<td>SCCmec IV</td>
<td>04</td>
<td>04.81</td>
</tr>
<tr>
<td>SCCmec V</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pvl gene</td>
<td>04</td>
<td>04.81</td>
</tr>
</tbody>
</table>

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 SCCmec 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.8

Resistance to antibiotics is a major concern world wide and is exemplified by the global spread of MRSA.10

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.11 Clindamycin susceptibility was used as a surrogate marker to screen CA-MRSA.3 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.12, 13 Test conditions like temperature, disk potency, salt concentration, time of incubation play a very important role in detection of methicillin resistance.14 Also studies have shown that 14.4% of isolates have lost meca gene during storage.15

In our study SCCmec type III was identified in 53% isolates and SCCmec 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 SCC mec type III and 31.70% were SCC mec type IIIA.8

SCC mec 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%.16,4 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.17 In Tampa general hospital Florida contrasting results of 43% of isolates were determined to have CA-MRSA presented with significant infection.18

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.19 Even more some studies have reported that pvl gene was detected in methicillin sensitive S. aureus.19 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.19 and pvl positive isolates were mostly methicillin sensitive S. aureus.

Our study shows 4(4.81%) isolates to possess SCC mec types IVand pvl gene. These strains were considered as CA-MRSA. A study in France, has identified pvl gene in all the CA-MRSA isolates.10 Another study has reported 92% of CA-MRSA strains possessed pvl gene.18 A study in England by Holmes etal have reported that PVL is a stable marker for CA-MRSA.19

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 long holds good. Clindamycin susceptibility is not a highly specific marker.5,10 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. 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-trimoxazole, tetracycline and vancomycin.

**ACKNOWLEDGEMENTS**

I thank to IISc, Bangalore for genotyping of my study isolates. I also thank all staff and technicians of KIMS, Hubli who helped me for this study.

**REFERENCES**

DEEPA & NADAGIR: COMMUNITY ACQUIRED MRSA

1207


7. CLSI Performance Standards for Antimicrobial Susceptibility Testing;Seventeenth Informational Supplement. Wayne, PA, USA 2007; M100-S17:27(1).


