Frequency and Diversity of Staphylococcal Cassette Chromosome mec Elements in nasally Carried Methicillin Resistant Staphylococcus Aureus of Healthcare Workers

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Abstract: Objective: To determine the frequency and SCCmec type of nasally carried MRSA in HCWs of a tertiary care hospital.

Materials and Methods: Nasal swabs were collected from three hundred and eighty healthcare workers working in various clinical wards of Lahore General Hospital, Lahore. Identification of Staphylococcus aureus was done by observing colony morphology and mannitol fermentation on mannitol salt agar, Gram stain, catalase test and DNase test. The phenotypic resistance to methicillin was determined using Cefoxitin disk 30 µg according to CLSI guidelines. All the isolates showing Cefoxitin resistance were confirmed for the presence of mecA gene and typed for SCCmec I, II, III, IV (a, b, c, d) and V by PCR. For quality control and for the confirmation of the results, DNA sequencing was done for random isolates for all the SCCmec types recovered in the present study.

Results: Out of 380 nasal samples, 89 (23.42%) cultures yielded the growth of S. aureus out of which 31 (34.83%) were MRSA. The overall frequency of MRSA among all the HCWs was 8.2%. Overall 47 SCCmec elements were found in total 29 MRSA isolates. Out of 29 MRSA isolates, 13 (44.82%) were hospital acquired, 7 (24.13%) were community acquired and 9 (31.03%) isolates had SCCmec types of both hospital acquired and community acquired origins.

Conclusion: The colonized healthcare workers harboring MRSA are being acting as mixing bowls of different SCCmec genes. Our study emphasizes the need for the formulation of regular nasal decolonization policies for effective infection control within our healthcare setups.

Keywords: MRSA; HA-MRSA; CA-MRSA; SCCmec

Received: Januar 26, 2018; Accepted: February 24, 2018; Published: April 18, 2018

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Colonization is an important step in the pathogenesis of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Both patients and healthcare workers (HCWs), colonized by MRSA, play a significant role in being the reservoir within healthcare environment [1,2]. It is often transmissible and the carriage appears to lead to clinical infection with greater frequency [3]. Healthcare workers who are at the interface between hospitals, nursing homes, ambulatory care and long term healthcare facilities on one hand and community on the other, may serve as vectors, source or victims of MRSA cross transmission [4,3].

Healthy carriers of MRSA among the HCWs are at the interface between the healthcare environment and community and are found responsible for shuffling of the HA MRSA and CA MRSA strains. The likely phenomenon of MRSA transmission from HCWs to the patients has been studied extensively. Many studies reported clear molecular (identical strain type) evidence of HCWs being the source of MRSA [5,6,7]. One of the major risk factors associated with increased MRSA nasal carriage rate and its transmission is the vicious cycle comprising of transiently colonized hands of the HCW with MRSA from the patient or the hospital environment, becoming the nasal carrier of the same strain, then contaminating the hands with the endogenous strain and transmitting it again to the patients [8,9]. Poor infection control practices are usually implicated in both acquisition and transmission of MRSA by healthcare personnel [10,11].

Staphylococcal Cassette Chromosome mec is a 21-67 kb mobile genetic element that carries mecA gene. HA-MRSA strains harbor SCCmec (Staphylococcal Cassette Chromosome) type I, II and III that are comparatively large and carry multiple determinants of drug resistance. In addition to methicillin resistance, these strains are usually found resistant to other drugs like aminoglycosides, fluoroquinolones, macrolides or combination of these antibiotics [12,13,14].

Contrary to this, CA-MRSA strains mainly carry a smaller SCCmec type IV or SCCmec type V element [15]. The small size of SCCmec type IV and V attribute to their easy horizontal transfer and adaptability between different genomic backgrounds. These strains usually demonstrate resistance against beta-lactams only [16]. However, CA-MRSA isolates are strongly associated with virulence factors such as Panton-Valentine leukocidin and the arginine catabolic mobile element which are thought to contribute to their pathogenic potential [17].

The expanding accumulation of CA-MRSA within the community has led to its unavoidable infiltration into the hospitals. As a result, CA-MRSA strains have started to arise as a cause of nosocomial infections and hospital outbreaks have also been reported worldwide [18]. In regions with endemically established CA-MRSA clones, such as USA300 clone in the United States, these have started to replace the healthcare-associated MRSA strains which were for long known as the traditional cause of HAI [19].

The emergence of CA-MRSA as a cause of HAI has increased in the number of hospitalised patients. The increase in antibiotic resistance might be the result of exposure of CA MRSA strains to the selective antibiotic pressure in the hospitals. Now there comes a need to make clear the definitions, frequency and epidemiology of CA-MRSA for the development of systems for identifying and controlling such organisms in the community as well as in healthcare facilities, and at the hospital-community interface. [18,20].

A mathematical model was developed to demonstrate the contributing factors for the replacement of HA-MRSA by CA-MRSA. According to it, CA-MRSA strains will become more
dominant within health care facilities as compared to HA MRSA. The reversal will take place as a consequence of the documented increasing community reservoir and inflow into the hospital through patients as well as the healthcare workers who harbour CA-MRSA. Another factor being responsible is that CA-MRSA strains harbour a smaller Staphylococcal Cassette Chromosome mec (SCCmec IV and SCCmec V), responsible for methicillin resistance, in comparison to larger cassettes harbour by HA-MRSA (SCCmec I, II and III). Competitive replacement of HA-MRSA by CA-MRSA with potentially greater biological fitness will ultimately occur, with catastrophic severity because this time CA-MRSA strains will cause infections among immunocompromised, hospitalized patients [21].

To understand the molecular epidemiology of methicillin-resistant *Staphylococcus aureus*, SCCmec typing is essential [22]. Full characterization of MRSA requires definition of the putative bacterial genetic background in addition to the heterologous and complex SCCmec elements. SCCmec typing is a useful molecular tool and its importance in community clonal outbreaks is being recognized with a great increase [23]. International Union of Microbiology Societies recently set the new MRSA nomenclature scheme in which it incorporates SCCmec typing information in addition to that provided by multilocus sequence typing [24].

Keeping in view the threat of multidrug resistant MRSA cross transmission in the hospitals and other healthcare facilities through HCWs, there is a need to get information regarding the prevalence of *Staphylococcus aureus* and MRSA nasal carriage among the healthcare workers. The present situation of the frequency of CA-MRSA and HA-MRSA in the HCWs in Pakistan is important not only epidemiologically but also for local public health. The knowledge of the MRSA prevalence and the current antibacterial profile is important for the selection of the appropriate antimicrobial treatment for these infections. In the same way, the screening and eradication of MRSA from the colonized HCWs should be emphasized and recommended as an important part of a comprehensive infection control policy.

Present study is the first study in our knowledge which has been conducted to determine not only asymptomatic nasal carriage of MRSA in healthcare workers but also to find out the genotypes of the isolates to reveal whether the isolates were hospital acquired or community acquired.

**Materials and Methods**

The present study was conducted on 380 health care workers from various clinical departments of Lahore General Hospital, Lahore after taking informed consent. Nasal swab was taken from each healthcare employee and was brought to the microbiology laboratory of Post Graduate Medical Institute, Lahore.

The specimens were inoculated on Mannitol salt agar plates along with the positive and negative controls. All cultured plates were incubated at 35° C for 24 hours. Mannitol fermenting, yellow colored colonies were subjected to Gram staining. After finding Gram positive cocci in clusters, further biochemical tests like Catalase and DNase were performed for the confirmation of *Staphylococcus aureus*. Phenotypic resistance to Methicillin was determined by disk diffusion method using 30 µg Cefoxitin disk (Oxoid Ltd) according to CLSI guidelines.

DNA extraction from sub-cultured pure isolates was done in CEMB as previously described by Zhang et al. For Polymerase Chain reaction, reconstitution of nine pairs of specific primers as given by Zhang et al (Forward and Reverse) for SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, V and mecA gene, synthesized by OLIGO-USA was done as advised by the manufacturer. SCCmec typing was performed by uniplex PCR. The cycling conditions for PCR were
optimized as follows

The PCR amplicons were visualized using a UV transilluminator after electrophoresis on a 2% agarose gel containing 0.5 µgm/ml ethidium bromide.

For quality control and for the confirmation of the results, DNA sequencing was done for random isolates for all the SCC\textit{mec} types recovered in the present study. For DNA sequencing, purification of PCR Product was done by Gel Elution. The required DNA bands were excised from the gel by a sterile razor and put into corresponding Eppendorf tubes and stored at -20°C. DNA Extraction Kit (Fermentas) was used and the manufacturer’s protocol was followed. Sequence analysis of the PCR amplified fragments was performed using both gene specific reverse and forward primers. Sequencing analysis was performed according to the manufacturer’s instructions (Big Dye Deoxy Terminators; Applied Biosystems, Weiterstadt, Germany). It was performed on automated sequencer (Applied Biosystems; 3100 DNA Analyzer).

The cycling profiles for sequencing PCR are given below.

The sequencing results for SCC\textit{mec} types were interpreted by CROMAS program and their sequence was analyzed for similarity against the GenBank non-redundant nucleotide library maintained by the National Center for Biotechnology Information (NCBI) with the BLAST program. (http://www.ncbi.nlm.nih.gov/BLAST/).
Results and Discussion

The results of 380 nasal swab cultures are shown in Table 1.

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>89</td>
<td>23.42%</td>
</tr>
<tr>
<td>CoNS</td>
<td>260</td>
<td>68.42%</td>
</tr>
<tr>
<td>No growth</td>
<td>31</td>
<td>8.15%</td>
</tr>
</tbody>
</table>

Out of 89 S. aureus being isolated in the present study, 38 % (n=30) were MRSA. The overall frequency of MRSA among hospital employees(n=380) was 8.2% (n=30). According to a meta-analysis done by Gomes et al from 2009 to 2014, including more than twenty studies with a sample size of at least 100 HCWs in non-outbreak situation revealed that the mean S. aureus nasal colonization was 24% ± 8.9%. Mean nasal MRSA colonization was 6.8% ±4.7% for developing countries and 3.5% ± 2.5% for developed nations. Our results fall into the respective percentage.

Methicillin resistance among Staphylococcus aureus isolates was determined by Cefoxitin (30 µg) Disc Diffusion test and PCR. 31 isolates showed Cefoxitin resistance phenotypically. On PCR, mec gene could be amplified in 30 isolates showing phenotypic resistance to methicillin but 1 isolate did not show mecA gene on PCR, even after repeating the amplification for three times. None of the SCCmec gene could be amplified as well.

Similar findings have been reported elsewhere as well[25]. The likely phenomenon might bemecA gene variant as reported by García-Álvarez et al in Denmark [26]. A new homologue, mecA_LGA251 was found in15 of 26 isolates from England, 12 of 16 isolates from Scotland and 24 of 32 from Denmark, which were methicillin resistant on phenotypic detection methods. They applied sequencing of whole-genome to verify the observed antibiotic resistance on the genetic basis. Although S. aureus isolates harbouring this novel mecA homologue turned out to be methicillin resistant on routine culture and antimicrobial susceptibility testing, PCR with mecA primers failed to amplify this gene, making it mecAnegative. LGA251 mecA encoded altered penicillin binding protein, PBP2a, is recognized as a divergent when compared to other mecA homologues in the public sequence databases.

SCCmec types, being considered in this study, could be assigned to 29 isolates. One isolate could not be typed, though mecA gene could be amplified in this isolate. It showed no amplification band on repeated experiment and thus was labeled as “untypable”. The isolate most probably carried SCCmec type(s) not being considered in the study. PCR amplification of the remaining 29 isolates showed that majority of the isolates harboured only single SCCmec element, including type I (n=2), II (n=5), III (n=6), IV (n=4; IVa subtype =4) and V (n=3). 2 isolates had two types including II + IV (n=1) and II + IVb (n=1). 5 isolates were found to have 3 types including I+III+V (n=1) (Figure 1), II+III+V (n=2), II+III+IVa (n=1), and III+IVa+V (n=1). The remaining two isolates had four SCCmec elements including I+II+III+V (n=1) and I+III+IVa+V (n=1).
Figure 1 Gel electrophoresis showing $mec\text{A}$ and SCC$mec\text{I}$, III and V

Usually, single MRSA isolate harbours single SCC$mec$ element, however they can be more than one. Zong et al studied diversity of SCC$mec$ elements in MRCoNS in which they came across a substantial number of isolates carrying multiple SCC$mec$ elements by PCR. They proposed it to likely that the two SCC$mec$ elements actually constituted a composite rather than two independent units. Gill et al reported three MRSA isolates with multiple ccr genes.

Hanssen et al [27] reported Staphylococcal strains, in which they recovered seven SCC$mec$ types which had not been reported previously, and multiple ccr genes were found in most of them. They reported six different SCCs in one Staphylococcal isolate. Singh et al recently reported more than one SCC$mec$ elements in a single isolate of $S.\text{ aureus}$. Overall 47 SCC$mec$ elements were found in total 29 MRSA isolates. The distribution of these $mec$ elements is given in Figure 2.

Figure 2 Distribution of various SCC$mec$ types harboured by MRSA isolates among HCWs ($n=47$)
Out of 47 SCCmec elements, type III was predominant. In the present study, out of 47 SCCmec elements, 62% were hospital acquired (Figure 3).

![Figure 3](image)

**Figure 3** Frequency of SCCmec types among HCWs according to their origin (n=47)

Out of 29 MRSA isolates, 44.82% had SCCmec type(s) of hospital acquired origin, 24.13% had SCCmec type(s) of community acquired origin and 31.03% isolates had SCCmec types of both hospital acquired and community acquired origins. (Figure 4).

![Figure 4](image)

**Figure 4** MRSA Isolates among HCWs according to the origin of mecTypes they harbour (n=29)

The hospital epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) has changed in the past few years due to the encroachment of community-associated MRSA (CA-MRSA)
strains into health care settings [28]. Healthcare facilities are currently functioning as mixing bowl for CA MRSA and HA MRSA, community acquired strains being brought in by HCWs and outpatients [29]. Zong et al [30] in China also reported Staphylococcal isolates harbouring SCC\(_{mec}\) elements of both HA MRSA and CA MRSA types in addition to isolates harbouring only one type. Similar results were shown by Hanssen et al. However, some authors report MRSA isolates bearing SCC\(_{mec}\) of either hospitalacquired or community acquired origin [31,32].

Our study had a few limitations. We considered SCC\(_{mec}\) I to V in our study due to resources constrain, addition of other SCC\(_{mec}\) types might have solved the discrepancy of the isolate that we failed to type. Due to lack of studies conducted in community regarding MRSA prevalence, we are unable to compare the results and frequency of nasal colonization of HCWs with the community members. HCWs were screened only for nasal carriage, taking other colonization sites into consideration might have some additional impact on the overall percentage. For healthcare associated infections, especially those caused by MRSA, health care workers (HCWs) are important in the nosocomial transmission dynamics. HCWs who become persistently colonized with MRSA, e.g., in the nose, act as a constant source for MRSA transmission. This calls out for the implementation of the sound and functional infection control policies.

**Acknowledgements**

This research was supported by Postgraduate Medical Institute, Lahore, Lahore General Hospital, Lahore and Centre of Excellence in Molecular Biology, Lahore, Pakistan.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Conflict of interest statement:**

None declared

**References**


