STAPHYLOCOCCAL CASSETTE CHROMOSOME MEC TYPES IN NASALLY CARRIED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES OF HEALTHCARE WORKERS IN A TERTIARY CARE HOSPITAL: A CROSS-SECTIONAL STUDY

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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. The phenotypic resistance to methicillin was determined using Cefoxitin disk 30 µg. All the isolates showing Cefoxitin resistance were confirmed for mecA gene and typed for SCCmec I, II, III, IV (a, b, c, d) and V by PCR. 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 are harboring Hospital-acquired MRSA more frequently and can act as 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.

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INTRODUCTION

Colonization is a critical 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 the healthcare environment[1,2]. It is often transmissible, and the carriage appears to lead to clinical infection with more significant frequency[3]. Healthcare workers who are at the interface between hospitals, nursing homes, ambulatory care, and long-term healthcare facilities on the one hand and the community on the other may serve as vectors, sources, or victims of MRSA cross-transmission [3,4].

Healthy carriers of MRSA among the HCWs are at the interface between the healthcare environment and community and are responsible for shuffling the HA MRSA and CA MRSA strains. The likely phenomenon of MRSA transmission from HCWs to 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 significant 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 acquiring and transmitting MRSA by healthcare personnel[10,11].

Staphylococcal Cassette Chromosome (SCC) mec is a 21-67 kb mobile genetic element that carries the mecA gene. Hospital-acquired (HA)-MRSA strains harbor SCCmec (Staphylococcal Cassette Chromosome) type I, II, and III that are comparatively large and have multiple drug resistance determinants. In addition to methicillin resistance, these strains are usually found resistant to other drugs like aminoglycosides, fluoroquinolones, macrolides, or a combination of these antibiotics [12,13,14].
Contrary to this, Community-acquired (CA)-MRSA strains mainly carry a minor SCCmec type IV or SCCmec type V element[15]. The small size of SCCmec type IV and V is attributed 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 the USA300 clone in the United States, these have started to replace the healthcare-associated MRSA strains, which were long known as the definitive cause of healthcare-associated infections (HAI)[19].
The emergence of CA-MRSA as a cause of HAI has increased the number of hospitalized patients. Increased antibiotic resistance might result from exposure of CA MRSA strains to the selective antibiotic pressure in hospitals. Now there comes a need to make clear the definitions, frequency, and epidemiology of CA-MRSA for developing systems for identifying and controlling such organisms in the community, healthcare facilities, and at the hospital-community interface[18,20].
A mathematical model was developed to demonstrate the contributing factors for replacing HA- MRSA with CA-MRSA. According to it, CA-MRSA strains will become more dominant within healthcare facilities than HA MRSA. The reversal will occur due to the documented increasing community reservoir and inflow into the hospital through patients and healthcare workers who harbor CA-MRSA. Another responsible factor is that CA-MRSA strains carry a smaller Staphylococcal Cassette Chromosome mec (SCCmec IV and SCCmecV), accountable for methicillin resistance, compared to larger cassettes harbored by HA-MRSA (SCCmec I, II and III). Competitive replacement of HA-MRSA by CA-MRSA with potentially more significant biological fitness will ultimately occur, with catastrophic severity. This time, CA-MRSA strains will cause infections among immunocompromised, hospitalized patients [21].
To understand the molecular epidemiology of methicillin-resistant Staphylococcus aureus, SCC mec typing is essential[22]. Complete characterization of MRSA requires defining the putative bacterial genetic background and the heterologous and complex SCCmecelements. SCCmectyping is a useful molecular tool, and its importance in community clonal outbreaks is being recognized with a significant increase[23]. The International Union of Microbiology Societies recently set the new MRSA nomenclature scheme, which 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 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 healthcare workers. The present frequency of CA-MRSA and HA-MRSA in the HCWs in Pakistan is essential not only epidemiologically but also for local public health. The knowledge of the MRSA prevalence and the current antibacterial profile is vital for selecting the appropriate antimicrobial treatment for these infections. In the same way, screening and eradicating MRSA from the colonized HCWs should be emphasized and recommended as an essential part of a comprehensive infection control policy. The present study is the first study that has been conducted to determine asymptomatic nasal carriage of MRSA in healthcare workers and determine the genotypes of the isolates to reveal whether the isolates were hospital-acquired or community-acquired.

METHODS

The present study was conducted on 380 healthcare workers from various clinical departments of Lahore General Hospital, Lahore, after taking informed consent from December 2016 to August 2017. The nasal swab was taken from the healthcare employees and 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, biochemical tests like Catalase and DNase were performed to confirm 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.SCCmectyping 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 the confirmation of the results, DNA sequencing was done for random isolates for all the SCCmec types recovered in the present study. For DNA sequencing, the PCR Product was purified by gel Elution**.** The required DNA bands were excised from the gel by a sterile razor, 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 an automated sequencer (Applied Biosystems; 3100 DNA Analyzer).

The cycling profiles for sequencing PCR are given below.

The CROMAS program interpreted the sequencing results for SCCmec types, 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/).

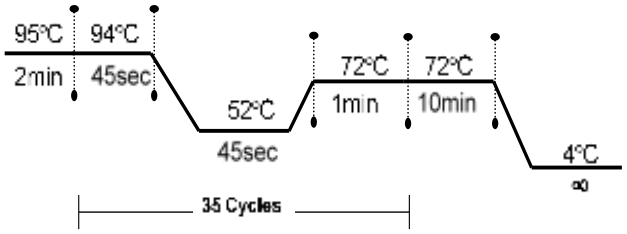
RESULTS

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

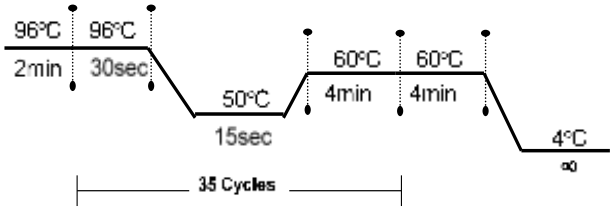
Table 1 Culture results of nasal swabs of the HCWs in the study (n=380)

Culture result	Number	Percentage
Staphylococcus aureus	89	23.42%
CoNS	260	68.42%
No growth	31	8.15%

Of 89 S. aureus 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, more than twenty studies with a sample size of at least 100 HCWs in non-outbreak situations revealed that the mean S.aureusnasal 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, the mec gene could



be amplified in 30 isolates showing phenotypic resistance to Methicillin. However, one isolate did not show the mecA gene on PCR, even after repeating the amplification three times. None of the SCCmec genes could be amplified as well.
Similar findings have been reported elsewhere as well [25]. The likely phenomenon might bemecA, a gene variant reported by García-Álvarez et al. in Denmark[26]. A new homolog, $mecA_{LGA251,}$ was found in 15 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 whole-genome sequencing to verify the observed antibiotic resistance on a genetic basis. Although S. aureus isolates



harboring this novel mecA homolog turned out to be Methicillin-resistant on routine culture and antimicrobial susceptibility testing, PCR with mecA primers failed to amplify this gene, making it mec A negative. LGA251 mecA encoded altered penicillin-binding protein, PBP2a, is recognized as a divergent when compared to other mecA homologs in the public sequence databases.
SCCmec types 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 experiments 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 the majority of the isolates harbored only a 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).

Usually, a single MRSA isolate harbor a single SCCmec element; however, they can be more than one. Zong et al. studied the diversity of SCCmec elements in MRCoNS in which they came across a substantial number of isolates carrying multiple SCCmec elements by PCR. They proposed that the two SCCmec elements likely 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, which recovered seven SCCmec types that 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 SCCmec element in a single isolate of S. aureus. Overall, 47 SCCmec elements were found in 29 MRSA isolates. Out of 47 SCCmec elements, type III was predominant. In the present study, out of 47 SCCmec elements,62% were hospital-acquired. 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 hospitals acquired, and community-acquired origins.

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 healthcare settings[28]. Healthcare facilities are currently functioning as mixing bowls 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 harboring SCCmec elements of both HA MRSA and CA MRSA types and isolates carrying only one type. Similar results were shown by Hanssen et al. However, some authors report MRSA isolates bearing SCCmec of either hospital-acquired or community-acquired origin[31,32].

Our study had a few limitations. We considered SCCmec I to V in our study due to resources constrain; the addition of other SCCmec types might have solved the discrepancy of the isolate we failed to type. Due to the lack of studies conducted in the community regarding MRSA prevalence, we cannot compare the results and frequency of nasal colonization of HCWs with the community members. HCWs were screened only for nasal carriage, considering other colonization sites might have different impacts on the overall percentage. For healthcare-associated infections, especially those caused by MRSA, healthcare workers (HCWs) are essential 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 for the implementation of sound and applicable infection control policies.

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Conflict of interest statement:

None declared

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