Genotyping of *Staphylococcus aureus* associated with nasal colonization among healthcare workers using DNA microarray

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**Abstract**

Introduction: Healthcare workers (HCWs) colonized with *Staphylococcus aureus* may serve as a reservoir of infection. This study was carried to determine the genetic make-up of *S. aureus* nasal colonizers in HCWs.

Methodology: Nasal swabs were obtained from 93 HCWs and molecular characterization of identified *S. aureus* isolates was carried out using the StaphyType DNA microarray (Alere Technologies GmbH, Jena, Germany).

Results: Twenty-nine HCWs (31%) were colonized with *S. aureus* (MSSA = 23; MRSA = 6). Thus the overall MRSA carriage rate was 6.5% (n/N = 6/93) and 20.7% (n/N = 6/29) of those colonized with *S. aureus* harboured MRSA. The *S. aureus* isolates belonged to 16 clonal complexes (CC). MSSA isolates included three each for CC15, CC188, ST2867; two each for CC5, CC97, CC367 as well as one each for CC1, CC8, CC30, CC45, CC101, CC121, ST291/813 and CC1153. The staphylococcal cassette chromosome recombinase genes *ccrA-I; ccrB-I* and the fusidic acid resistance gene (*fusC*) were present in two MSSA isolates (CC1 and CC8). The six MRSA isolates included CC5-MRSA-[VI⁺;fusC] (n = 2); one each of CC5-MRSA-V; CC22-MRSA-IV (luxI⁺); CC80-MRSA-IV [pV+] (“European CA-MRSA Clone”) and CC97-MRSA-[V⁺;fusC].

Conclusion: There is wide clonal diversity of *S. aureus* colonizers with associated high MRSA carriage among the HCWs. The presence of genetically stable MSSA isolates with the capability to transform into MRSA isolates is of concern.

**Key words:** *Staphylococcus aureus*; MSSA; MRSA; clonal complex; DNA microarray, Saudi Arabia.


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**Introduction**

*Staphylococcus aureus* is an opportunistic pathogen and persistent colonization of the anterior nares occurs in up to 20% in the human population [1]. *S. aureus* colonization is associated with higher risk of developing staphyloccocal infection. Furthermore, colonized persons serve as reservoir of infection facilitating transmission to others particularly in the healthcare setting. Methicillin resistant *S. aureus* (MRSA) is a major cause of nosocomial infections with significant morbidity and mortality globally [2]. Increasingly, community acquired MRSA lineages (CA-MRSA) are now being identified as the dominant aetiological agents of nosocomial MRSA infections [3⁻⁵]. High prevalence of MRSA colonization in HCWs in Saudi Arabia has been documented in the literature although detailed molecular characterization is lacking [6]. We recently reported the first observation of nosocomial infections by highly successful pandemic clones (CC8, “USA300” and CC22-MRSA-IV) and one rare MRSA clone (CC15-MRSA) in Saudi Arabia [5]. In addition to the emergence of these highly successful epidemic clones, increasing occurrence of CA-MRSA lineages in nosocomial infection was identified [5]. In the light of these recent reports of changes in MRSA epidemiology, this study was carried out to determine the molecular characterization of *S. aureus* isolates from nasal colonization among HCWs.
Methodology
The study was carried out in March 2016 at King Khalid University Hospital, Riyadh, Saudi Arabia. Full time employees who have been employed for at least one year were eligible for inclusion. These included doctors, nurses, laboratory staff, technicians and housekeeping staff from different service units of the hospital. Nasal swabs samples were collected from the anterior nares of consenting HCWs. At the time of sample collection, a standardized questionnaire was completed by each participant to obtain demographic information, data on related risk factors for MRSA colonization and transmission as well as knowledge of infection prevention measures. Ethical approval was obtained from the Institutional Review Board, King Saud University, Riyadh Saudi Arabia. All subjects gave written informed consent.

Bacterial identification: S. aureus identification was performed using standard laboratory techniques and MicroScan Walkaway 96 plus System (Beckman Coulter, Brea California, USA). Methicillin resistance was confirmed on Mueller-Hinton agar using the disk diffusion method and by the Cepheid MRSA GeneXpert test (Cepheid, Sunnyvale California, USA). Antibiotic susceptibility testing was carried out using the Vitek2 automated system (BioMerieux, Marcy-l’Étoile, France). A panel of antibiotics including cefoxitin, oxacillin, gentamicin, ciprofloxacin, erythromycin, clindamycin, linezolid, vancomycin, tetracycline, fusidic acid, mupirocin and trimethoprim-sulfamethoxazole were tested.

Molecular genotyping: This was carried out using the StaphyType DNA microarray (Alere Technologies GmbH, Jena, Germany) technology using target genes, primer and probe sequences as published previously [7,8]. The genotyping protocol and assignment to clonal complexes were conducted as previously described [7,8].

Results
A total of 93 randomly selected healthcare workers were screened. These comprised of nurses (n = 44; 47.3%), doctors (n = 16; 17.2%), housekeeping staff (n = 7; 7.5%) and other hospital staff including laboratory technicians, respiratory therapists and pharmacists (n = 26; 28%). Twenty-nine HCWs (31%) were positive for S. aureus nasal colonization (MSSA = 23; MRSA = 6). Thus the overall MRSA carriage rate was 6.5% (n/N = 6/93) and 20.7% (n/N = 6/29) of those colonized harboured MRSA. None of the 29 HCWs with S. aureus colonization had been hospitalized in the preceding six months, owned pets or had contact with farm animals. There was no history of recent antibiotic use, participation in contact sports or skin infections among the MRSA carriers. Of the 93 HCWs screened, 27 (29%) had been involved in direct care of MRSA patients within the preceding six months. All of these 27 HCWs had S. aureus colonization, however only two of them had MRSA carriage.

Genotyping showed a wide clonal diversity with 16 clonal complexes (CC) identified. The MSSA isolates included three each for CC15, CC188, ST2867; two each for CC5, CC97, CC367; and one each for CC1, CC8, CC30, CC45, CC101, CC121, ST291/813, CC1153. The six MRSA isolates were of CA-MRSA lineages and belonged to CC5, CC80, CC22 and CC97. Table 1 shows the strain affiliations and SCCmec types for the MRSA isolates.

The staphylococcal cassette chromosome recombinase genes were present in two MSSA isolates namely CC1-MSSA (ccrA-I/ccrB-I) and CC8-MSSA (ccrA-I). These two isolates also harboured the fusidic acid resistance gene (fusC) which was also present in CC5 and CC97 isolates (Table 2). The vancomycin and mupirocin resistance genes were absent in all isolates. The β-lactamase operon was present in the majority of MSSA (89%) and in all MRSA isolates. There was agreement between carriage of antibiotic resistance genes and phenotypic antibiotic susceptibility profile. The Panton-Valentine leukocidin genes (lukS-PV and lukF-PV) were identified only in two isolates, i.e., in one CC80- MRSA-IV and one CC1153-MSSA. The genes encoding exfoliative toxin D (etD) and epidermal cell differentiation inhibitor (edinB) were present in

Table 1. Distribution of MRSA clonal complexes.

<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>Strain affiliation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC5</td>
<td>CC5-MRSA-V (entD/J/R+), “WA MRSA-11/34/35/90/108” (n=1)</td>
<td>3</td>
</tr>
<tr>
<td>CC22</td>
<td>CC22-MRSA-IV (tstI+)</td>
<td>1</td>
</tr>
<tr>
<td>CC80</td>
<td>CC80-MRSA-IV [pvl+], “European CA-MRSA Clone”</td>
<td>1</td>
</tr>
<tr>
<td>CC97</td>
<td>CC97-MRSA-[V+fusC]</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>
CC80-MRSA and CC398-MSSA while ST2867 had only \(\text{edinB}\) gene (Table 2). The \(\text{egc}\) (enterotoxin gene) cluster was present in CC5, CC22, C30, CC45, CC121 and CC361 isolates (Table 2). The haemolysin genes \(\text{hla}\) and \(\text{hlb}\) were present in all isolates except for three CC15-MSSA isolates which apparently lacked the \(\text{hlb}\) gene. None of the isolates harboured the arginine catabolic mobile element locus (ACME). The distribution of additional virulence and antibiotic resistance genes in MSSA and MRSA isolates is shown in Table 2.

**Discussion**

The \(S.\ \text{aureus}\) colonization rate as well as carriage of MRSA shown in this study is similar to that previously reported among healthcare workers in Saudi Arabia [9]. Of the 27 HCWs who had cared for MRSA patients in the preceding six months only two had

<table>
<thead>
<tr>
<th>Strain (n)</th>
<th>Strain type</th>
<th>(\text{agr})</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1 (1)</td>
<td>MSSA</td>
<td>III</td>
<td>(\text{blaZ, blaA, blaR, msrA, aphA3, fusC})</td>
</tr>
<tr>
<td>CC5 (2)</td>
<td>MSSA</td>
<td>II</td>
<td>(\text{blaZ*, blaA*, blaR*, fosB, fusC, tetM, lmrA, lmrB, lmrC, lmrD, lmrE})</td>
</tr>
<tr>
<td>CC5 (3)</td>
<td>MRSA</td>
<td>II</td>
<td>(\text{fosB, fusA, fusC, dfrA*, tetM*, meca})</td>
</tr>
<tr>
<td>CC8 (1)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, fusC, fosB})</td>
</tr>
<tr>
<td>CC15 (3)</td>
<td>MSSA</td>
<td>II</td>
<td>(\text{blaZ*, blaA*, blaR*, fosB})</td>
</tr>
<tr>
<td>CC22 (1)</td>
<td>MRSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, dfrA, meca})</td>
</tr>
<tr>
<td>CC30 (1)</td>
<td>MSSA</td>
<td>III</td>
<td>(\text{blaZ, blaA, fosB})</td>
</tr>
<tr>
<td>CC45 (1)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, fosB})</td>
</tr>
<tr>
<td>CC80 (1)</td>
<td>MRSA</td>
<td>III</td>
<td>(\text{blaA, blaR, aphA3, sat, far1, meca})</td>
</tr>
<tr>
<td>CC97 (2)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, tetK*})</td>
</tr>
<tr>
<td>CC97 (1)</td>
<td>MRSA</td>
<td>I</td>
<td>(\text{blaA, blaR, aphA3, sat, meca})</td>
</tr>
<tr>
<td>CC101 (1)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, fosB})</td>
</tr>
<tr>
<td>CC121 (1)</td>
<td>MSSA</td>
<td>IV</td>
<td>(\text{blaZ, blaA, fosB})</td>
</tr>
<tr>
<td>CC188 (3)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, aphA3, sat, far1, meca})</td>
</tr>
<tr>
<td>CC361 (2)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{blaZ, blaA, aphA3, aacA-aphD*, sat, tetK*, meca})</td>
</tr>
<tr>
<td>CC398 [ST291/813]; (1)</td>
<td>MSSA</td>
<td>I</td>
<td>(\text{claZ, lmrA, lmrB})</td>
</tr>
<tr>
<td>CC1153 (1)</td>
<td>MSSA</td>
<td>II</td>
<td>(\text{blaZ, blaA, lmrA, aadD, far1, qacC})</td>
</tr>
<tr>
<td>ST2867 (3)</td>
<td>MSSA</td>
<td>II</td>
<td>(\text{blaZ, blaA, lmrA, meca, aadD, far1, qacC})</td>
</tr>
</tbody>
</table>

\(^{*}\): carried by one isolate; \(^{*}\): carried by two isolates; \(\text{agr}\): accessory gene regulator allele; \(\text{cap}\): capsule type; \(\text{blaZ}\): beta-lactamase; \(\text{blaA}\): beta-lactamase repressor, \(\text{blaR}\): beta-lactamase regulatory protein, \(\text{fosB}\): mmetaboliloh transferase, \(\text{aphA3}\): 3'5'-aminoglycoside phosphotransferase, \(\text{aacA-aphD*}\): bifunctional enzyme, \(\text{mrA}\): energy-dependent efflux of erythromycin, \(\text{tetK}\): kanamycin resistance, \(\text{sat}\): streptomethine acetyltransferase, \(\text{fusC}\): hypothetical protein associated with fusidic acid resistance, \(\text{tetK} & \text{tetM}\): tetracycline resistance, \(\text{ent}\): enterotoxin, \(\text{pvl}\): Panton-Valentine leukocidin, \(\text{qacC}\): quaternary ammonium compound resistance protein C, \(\text{aadD}\): amino-glycoside adenyltransferase, tobramycin resistance, \(\text{far1}\): fusidic acid resistance; \(\text{dfrA}\): dihydro-folate reductase type 1.

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MRSA carriage while the others had MSSA colonization. We offer two hypotheses to explain this finding. One is the possibility that transmission rate of MRSA from patients to the healthcare workers or vice-versa is low in this particular facility. The other is that MSSA colonization is protective hindering acquisition of MRSA carriage because all suitable ecological niches are already occupied. There was no MRSA outbreak in the healthcare facility in the period preceding the study. However, it is of concern that most of those with MRSA carriage have not been involved in the direct care of MRSA patients and they did not have any concomitant risk factor for MRSA carriage such as recent use of antibiotics. This indicates the need for stringent surveillance and routine screening for MRSA colonization with application of an appropriate decontamination protocol.

We had previously reported on the molecular characterization of MRSA isolates associated with nosocomial infection in this facility [5]. With the exception of CC5-MRSA-V (entD/J/R+) strain, all the MRSA strains identified among HCWs in this study had also been documented among patients with clinical infections in this hospital [5]. This CC5-MRSA-V strain which harbours the enterotoxin genes entD/J/R (identical to WA MRSA-11/34/35/90/108) is also being described for the first time in Saudi Arabia. Previous reports from Saudi Arabia (including that carried out in this hospital) had shown wide clonal diversity and emergence of new MSSA and MRSA strains [5,10,11]. The findings from the present study confirms this clonal diversity but interestingly also shows that the clonal expansion of S. aureus continues to occur as demonstrated by the first identification of CC367, CC1153, ST2867 and ST291/1813 MSSA as well as CC5-MRSA-V (sed/j/r+). Furthermore, CC15, CC188 and ST2867 were identified as being predominant which contrasts with the previously reported predominance of CC15 and CC1 MSSA [10]. It is however evident that CC15 MSSA remains a ubiquitous nasal colonizer in our population [10]. Indeed this might explain why CC15-MRSA which has hitherto only been sporadically identified in the literature has been shown with higher occurrence in Saudi Arabia [5,12,13]. It is probable that genetic changes which facilitate the acquisition of mobile genetic elements by CC15-MSSA colonizers is driving the emergence of this rare CC15-MRSA in our setting [14].

The MSSA strains carried a diversity of virulence and resistance genes. We identified one CC8 MSSA isolate with ccrA-1/fusC and CC1 MSSA harbouring ccr1-A/ccrB-1/fusC. The presence of staphylococcal recombinase genes and fusC resistance gene in CC8 MSSA is an unusual finding. This is the second report of this combination in MSSA colonizers in our setting [10]. The ccrA and ccrB recombinase genes are important for the recombination events of SCCmec with S. aureus. MSSA isolates harbouring a combination of ccrA-1, ccrB-1 and fusC genes in the mobile genetic elements are indicative of a pool of genetically stable isolates with the capability to transform into MRSA isolates. Their carriage by HCWs who are exposed to MRSA is of concern as this could drive the evolution of new MRSA isolates, and indeed a high number of Middle Eastern MRSA strains harbor composite SCCmec/SCCfus elements.

In our previous evaluation of S. aureus colonizers, etD and edinB genes were found in MRSA isolates [10,11]. The etD has been associated with epidermal blister formation in mouse models while presence of edinB gene has been linked with onset of bacteremia during the course of pneumonia [15,16]. Our findings indicate the first report of these virulence genes in MSSA isolates from this population. Previous report from Germany has shown presence of etD and edinB in CC25/28 MSSA [17]. The egc cluster which is found on the genomic island vSAβ has been shown to be associated with specific S. aureus clonal types namely (C5, CC25, CC30, CC45 and CC121) irrespective of geographical origin [18-21]. In this study, we also observed the egc cluster in CC361-MSSA. We had previously reported CC398 [ST291/813]-MSSA harbouring sak, scn and chp genes as a nasal colonizer [10]. This isolate was again identified with these three genes in the present report which is in contrast with other report which showed that CC398-MSSA carried the scn and chp genes but not sak [22].

**Conclusion**

Our findings show wide clonal diversity of S. aureus colonizers among HCW with associated high MRSA carriage. The presence of genetically stable MSSA isolates with the capability to transform into MRSA isolates is of concern. Further work across multiple healthcare facilities and with larger sample size is recommended to get a better understanding of the genetic profile of the S. aureus colonizers among healthcare workers in Saudi Arabia.

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