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Antimicrobial resistance and virulence markers in methicillin sensitive *Staphylococcus aureus* isolates associated with nasal colonization☆

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Most *Staphylococcus aureus* infections occur in previously colonized persons who also act as reservoirs for continued dissemination. This study aimed to investigate the carriage of antimicrobial resistance and virulence markers in *S. aureus* isolates associated with nasal colonization. The study was conducted from December 2013–April 2014. Nasal swabs were collected and questionnaires administered to 97 medical students in Riyadh Saudi Arabia. Bacterial culture, identification and antimicrobial susceptibility testing were performed by conventional methods and chromogenic agar was used for methicillin resistant *S. aureus* (MRSA) screening. Molecular characterization of isolates was carried out using the StaphyType DNA microarray. Thirty two students (43%) had *S. aureus* nasal carriage (MSSA = 31; MRSA = 1). Seventeen clonal complexes (CC) were identified namely: CC15-MSSA (n = 5), CC1-MSSA-SCC*fus* (n = 4), CC8-MSSA (n = 3), CC22-MSSA (n = 3), CC25-MSSA (n = 3), CC101-MSSA (n = 2). Other CC found as single isolates were CC5-MSSA, CC6-MSSA, CC30-MSSA, CC45-MSSA, CC96-MSSA, CC188-MSSA, CC398-MSSA, CC942-MSSA/*PVL*+, CC1290-MSSA, ST2482-MSSA, CC80-MRSA-IV/*PVL*+. The CC1-SCC*fus* isolates harbored the Staphylococcal cassette chromosome (*SCC*) with *ccr*A-1; *ccr*B-1 and *ccr*B-3 genes plus the putative fusidic acid resistance marker Q6GD50. One MSSA isolate was genotyped as coagulase negative *Staphylococcus* spp with an irregular composite SCC*mec* element. Majority of the isolates harbored various virulence genes including the hemolysin, enterotoxin, and exfoliative genes as well as various adhesive protein producing genes. Although there was low carriage of MRSA, the MSSA isolates harbored various resistance and virulence genes including those usually seen in MRSA isolates. The presence of isolates with incomplete SCC*mec* elements plus putative resistance and virulence genes is of concern.

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1. Introduction

Staphylococcus aureus is an opportunistic pathogen responsible for a diverse spectrum of human and livestock diseases; and it is also associated with asymptomatic colonization of the skin and mucosal surfaces of normal humans. Methicillin resistant *S. aureus* (MRSA) was first described in England in 1961 and is now globally established as an important agent of nosocomial infections with associated significant patient morbidity and mortality [8,13]. MRSA infections were initially described as hospital acquired but in recent years a shift in the epidemiology occurred with increased frequency of infections originating in the community and in absence of predisposing risk factors for MRSA infection [10,31,34]. These community acquired MRSA (CA-MRSA) strains are now recognized as distinct clonal entities which are genotypically and phenotypically distinct from the hospital-acquired MRSA (HA-MRSA) strains. Nasal carriage of *S. aureus* plays a key role in the epidemiology and

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pathogenesis of staphylococcal infections [15]. Most *S. aureus* infections occur in previously colonized persons who also act as reservoirs for continued dissemination in the community and hospital environments [16]. Nasal colonization with high transmissibility clones have been shown to contribute to the accelerated spread of CA-MRSA in the community. Carriage of CA-MRSA is increasingly reported especially in places where people are in close contact including academic institutions such as kindergartens and universities [2,11,16,19,27]. In the USA, studies have shown MRSA nasal carriage rates of 5%–50% among health care personnel depending on the type and degree of patient contact [4,12,14,29]. Health care workers including medical students are a bridge between the hospital and the community. Nasal carriage of *S. aureus* and specifically CA-MRSA, by medical students has recently been described as a possible mechanism of trafficking of these strains between the hospital and the community [19]. With the increasing occurrence of MRSA infections, identification of the reservoir of infection, determining the prevalent clones and understanding the mediators for the effective trafficking of these successful clones in the community has become imperative. Currently there is limited data on the determinants of nasal carriage of *S. aureus* or genotypic characterization of these isolates in the community in Saudi Arabia. This study aimed to investigate molecular characterization of *S. aureus* isolates associated with nasal colonization in young adults in Saudi Arabia.

2. Materials and methods

2.1. Study population

The study was conducted from December 2013–April 2014. During this period, nasal swab samples were collected from 97 medical students at Alfaisal University in Riyadh, Saudi Arabia. The student volunteers were from all academic years i.e. pre-clinical to clinical years. At the time of nasal swab sample collection, a questionnaire was administered to each participant to obtain demographic information and data on related risk factors for staphylococcal colonization and transmission (e.g. recent hospitalization, antibiotic use, etc.). The data was recorded with an assigned identifier (ID) number to every sample to preserve confidentiality. Ethical approval was granted by the Institutional Review Board at Alfaisal University and signed informed consent was obtained from all participants.

2.2. Specimen collection

Nasal swab samples were collected from the anterior nares by cotton wool tipped swabs pre-moistened with sterile saline (Saudi Prepared Media Limited, SPML, Riyadh, Saudi Arabia). The swabs were introduced into the nostrils and gently rotated about four times. Samples were sent to the laboratory within 15 min–1 h of collection in Amies transport media [30].

2.3. Bacterial isolation

The swabs were processed in the laboratory by vortexing them in brain heart infusion broth containing 6.5% salt (SPML, Riyadh, Saudi Arabia) for 2 min. The brain heart infusion broths were subsequently incubated at 37 °C for 24–48 h and subculture made on mannitol-salt agar and MRSA Chromogenic agar (SPML, Riyadh, Saudi Arabia). Characteristic yellowish colonies on mannitol-salt agar and pinkish colonies on MRSA Chromogenic agar were further characterized using conventional methods.

2.4. Antimicrobial susceptibility testing

The susceptibility profiles of the isolates to 10 antibiotics (gentamicin 10 μg, penicillin 10 units, cefoxitin 30 μg, rifampicin 5 μg, erythromycin 15 μg, clindamycin 2 μg, linezolid 30 μg, mupirocin 5 μg, trimethoprim-sulfamethoxazole 1.25/23.75 μg and ciprofloxacin 5 μg) (Oxoid Basingstoke, UK) were determined using the disc diffusion technique as described in the Clinical and Laboratory Standards Institute's performance standards for antimicrobial susceptibility testing [5]. Interpretation of inhibition zone was done according to the CLSI guidelines [5]. *S. aureus* ATCC 25923 control strain was used in parallel in each run. Isolates were stored at −80 °C and later characterized genotypically.

2.5. Genetic characterization

Molecular characterization of isolates was carried out using the StaphyType DNA microarray (Alere Technologies GmbH, Jena, Germany). The included target genes, primer and probe sequences have been published previously [20,22]. This DNA microarray covers ca. 170 genes and their allelic variants inclusive of species markers, typing markers, resistance genes, as well as virulence genes. Procedures were performed according to previously published protocols [20,22,24]. The analysis of presence or absence of target gene as well as the assignment of isolates to CC was carried out as previously described [20,22,24].

3. Results

3.1. Demographic information and risk factors

Sixty two of the participants were females (63.9%) and thirty five were males (36.1%). Sixty-eight students (70.1%) were from pre-clinical years, with limited hospital exposure while 29 (29.9%) were in the clinical years, based mostly in the hospital. Up to 55.7% (n = 54) of participants reported usage of an antibiotic in the six months and less than 8% (n = 7) had a surgical history. About 42.3% (n = 41) reported a family member was recently hospitalized and over half of the respondents (53.6%) had at least one family member who was a hospital staff. Twenty-four percent (n = 23) participated in team sports, and 41.2% (n = 40) had companion animals at home.

3.2. Nasal carriage of S. aureus and antimicrobial susceptibility profiles of isolates

Thirty two students (43%) were identified to have *S. aureus* nasal carriage. Of these, 31 (31/32; 96.9%) were MSSA isolates while only one was MRSA (1/32; 3.1%). The single MRSA isolate was obtained from a male clinical year student. All isolates were susceptible to 8 out of the 10 tested antibiotics: gentamicin, erythromycin, ciprofloxacin, clindamycin, trimethoprim-sulfamethoxazole, rifampicin, linezolid, mupirocin, while susceptibility to penicillin and cefoxitin was 18.8% (6/32) and 96.9% (31/32) respectively.

3.3. DNA microarray genotyping of MSSA and MRSA isolate(s)

Based on DNA microarray analysis, 31 isolates were grouped into 17 different clonal complexes (CC); CC15-MSSA (n = 5), CC1-MSSA-SCC*fus* (n = 4), CC8-MSSA (n = 3), CC22-MSSA (n = 3), CC25-MSSA (n = 3), CC101-MSSA (n = 2). Other CC occurring as single isolates are CC5-MSSA, CC6-MSSA, CC30-MSSA, CC45-MSSA, CC96-MSSA, CC188-MSSA, CC398-MSSA, CC942-MSSA/*PVL*+, CC1290-MSSA, ST2482-MSSA, CC80-MRSA-IV/*PVL*+. One MSSA strain could not be grouped into any known clonal complex or

sequence type, it was noted to be similar to CC1 but with a different capsule marker (*cap*5 instead of *cap*8). One isolate which was phenotypically identified as MSSA was characterized as coagulase negative *Staphylococcus* spp (CoNS) by identification of species specific genes by DNA microarray. This isolate was also found to have an irregular composite SCC*mec* element and harbored *mec*A (*ccrA-2*; *ccrB-2*; *ccrAA; ccrB-4*) cassette chromosome recombinase genes and arginine catabolic mobile element (ACME). This isolate was confirmed to be *S. epidermidis* by DNA microarray targeting the rpo*B* sequence [23].

3.4. Antimicrobial resistance and virulence genes

The prevalence of the Panton-Valentine leucocidin *(pvl)* gene and MRSA was low in the study population: only two isolates, CC942-MSSA and CC80-MRSA were positive for the *pvl* gene. The predominant clones of MSSA in this study were CC15 (16.1%) and CC1 (12.9%). CC1 isolates harbored a combination of staphylococcal cassette chromosome with the cassette chromosome recombinase genes *ccrA-1*; *ccrB-1* and putative fusidic resistance marker Q6GD50 (*fus*C). One isolate in the CC1-SCC*fus* group also harbored the *ccrB-3* cassette chromosome recombinase gene and the *vga* (streptogramin-A-resistance) gene. The beta-lactamase operon was present in all isolates except the following four: CC1-MSSA-SCC*fus*, CC1290-MSSA, CC80-MRSA-IV/*PVL*+ and the strain not assignable to any known clonal complex. None of the isolates haboured vancomycin resistance genes (*vanA*, *vanB* and *vanC*).

Accessory regulatory genes *agr*I was the predominant allele present in 16 isolates, followed by *agr*III which was present in 8 isolates, and *agr*II present in 7 isolates. The unclassified strain carried *agr*III allele while *agr*IV was not present in any isolate. Majority of the isolates harbored various virulence genes including the hemolysin, enterotoxin, and exfoliative genes as well as various adhesive protein producing genes. The distribution of some of these regulatory and virulence genes are shown in Table 1.

4. Discussion

Human colonization by *S. aureus* is variable and about 30–50% of healthy humans could be colonized by the organism but colonization rates varies from region to region and also among different study populations [17]. Colonization rate in our study was 43.2% (32/74) which is a relatively higher rate than 19.1% reported from Spain by Lozano et al.; 26.1% from Abha, Saudi Arabia by Algahaithy et al. and 29% by Bischoff et al. from the US [1,3,18]. However similar rates of 32% were reported from Port Harcourt, Nigeria and 40.8% from Brazil [26,27].

S. aureus has a clonal population structure and while some clones tend to predominate in some geographical regions, global dissemination is observed with some other clones [21]. In our study, CC15 and CC1 MSSA were the top two clones identified. CC1 lineages of *S. aureus* have a global distribution and are also associated with nasal carriage and methicillin resistance, while CC15-MSSA, although associated with nasal carriage, is rarely associated with methicillin resistance [21]. The prevalence of MRSA was low in our study population. This finding is similar to the observation reported from Abha (Qassim region) of Saudi Arabia by Algahaithy et al. [1]. The single MRSA strain (CC80) isolated is a well-known and characterized CA-MRSA strain prevalent in the Middle East and Europe [21]. It was susceptible to all non-beta lactam antibiotics tested and carried the *pvl* gene.

The recovered isolates were susceptible to 8 out of the 10 tested antibiotics (mainly non-beta lactam antibiotics), but vancomycin susceptibility testing through MIC determination was not done. The numbers of vancomycin resistant *S. aureus* (VRSA) reported in the literature are limited worldwide and VRSA isolates have not been reported from our setting [25]. In addition, none of our isolates were found to harbor vancomycin resistance genes. The microarray analysis revealed the presence of *aphA3* (kanamycin/neomycin resistance) in two isolates and Q6GD50 (*fusC*) (a hypothetical protein associated with fusidic acid resistance) in four isolates. As these antibiotics were not part of the panel of antibiotics tested the phenotypic resistance profile for these antibiotics could not be ascertained. An interesting observation was the absence of erythromycin resistance phenotypically despite the presence of the *msrA* gene in CC30-MSSA. We hypothesize that this could be due to non-expression of the gene, and further tests will be needed to confirm this.

Some of the identified MSSA isolates carried virulence genes usually reported in MRSA; the presence of staphylococcal cassette

Table 1
Distribution of various virulence, regulatory and toxin genes.

Strain (n)	Strain type	*agr*	Resistance genes	Virulence genes			
				cap	Enterotoxins/exfoliative	Toxic shock syndrome	Others
CC1(4)	MSSA	III	*blaZ*, *blaI*, *blaR** *Q6GD50 (fusC)*	8	*ent*A,B,H,K,Q		*sak*, *scn*
CC5(1)	MSSA	II	*blaZ*, *blaI*, *blaR*,,*tet*M,*fos*B	5	*ent*G,I,M,O,U		*sak*, *scn*,*chp*
CC6(1)	MSSA	I	*blaZ*, *blaI*, *blaR*, *fos*B	8	*ent*A		*sak*, *scn*
CC8(3)	MSSA	I	*blaZ*, *blaI*, *blaR*, *fos*B	5	*ent*B,K,L,Q*	*tst*1*	*sak*, *scn*
CC15(5)	MSSA	II	*blaZ*, *blaI*, *blaR*, *fos*B	8			*scn*,*chp*
CC22(3)	MSSA	I	*blaZ*, *blaI*, *blaR*	5	*ent*G,I,M,O,U		*sak*, *scn*,*chp*
CC25(3)	MSSA	I	*blaZ*, *blaI*, *blaR*, *fos*B	5	*ent*G,I,M,O,U		*sak*, *scn*,*chp*
CC30(1)	MSSA	III	*blaZ*, *blaI*, *blaR*, *msr*A	8	*ent*A, G,I,M,O,U	*tst*1	*sak*, *scn*,*chp*
CC45(1)	MSSA	I	*blaZ*, *blaI*, *blaR*	8	*ent*G,I,M,O,U		*sak*, *scn*,*chp*
CC96(1)	MSSA	III	*blaZ*, *blaI*, *blaR*	8	*ent*A		*sak*, *scn*,*chp*
CC101(2)	MSSA	I	*blaZ*, *blaI*, *blaR*, *fos*B	8			*sak*, *scn*
CC188(1)	MSSA	I	*blaZ*, *blaI*, *blaR*, *aph*A3,*sat*	8			*sak*, *scn*
CC398(1)	ST291/813MSSA	I	*blaZ*, *blaI*, *blaR*	5			*sak*, *scn*,*chp*
CC942(1)	MSSA/PVL	III	*blaZ*, *blaI*, *blaR*	5			*sak*, *scn*,*chp*
CC1290(1)	MSSA	I		8			*sak*, *scn*
ST2482(1)	MSSA	II	*blaZ*, *blaI*, *blaR*	5			*sak*, *scn*
Unassigned(1)	MSSA	III		5	*ent*H		*sak*, *scn*
CC80(1)	MRSA/PVL	III	*mec*A, *aph*A3,*sat*	8	*et*D		*sak*, *scn*

Key: * presence of genes variable among the isolates, *sak*-staphylokinase,*scn*-staphylococcal complement inhibitor, *chp*-chemotaxis-inhibiting protein,*et*D-exfoliative toxin D, *ent* A, B, H, K, L, Q, G,I,M,H,U- enterotoxin A, B, H, K, L, Q, G,I,M,H,U, *bla*Z-beta-lactamase, *bla*I- beta lactamase repressor, *bla*R-beta-lactamase regulatory protein, *fos*B- metallothiol transferase, *Q6GD50 (fusC)* - hypothetical protein associated with fusidic acid resistance, *msr*A-energy-dependent efflux of erythromycin, *aph*A3 – 3′5′-aminoglycoside phosphotransferase, neo-/kanamycin resistance, *cap*-capsule type, *sat*-streptothricine acetyltransferase.

chromosome recombinase gene *ccrA-1*; *ccrB-1* and *fus*C resistance gene was observed in CC1-MSSA isolates. While this observation is rare, Corkill et al. reported the presence of methicillin-susceptible (*mec*A gene negative) but fucidin-resistant *S. aureus* harboring recombinase genes in a cluster of invasive infections among intravenous drug users [6]. While Corkill et al. were the first to give a description of cassette chromosome recombinase genes *ccrA-1* and *ccrB-1* in MSSA, our study is the first to give this description in Saudi Arabia along with the clonal strain in which the gene is harbored. The presence of *S. aureus* population with cassette chromosome recombinase and *fus*C genes as observed in our study, may represent a genetically stable pool of MSSA isolates capable of transforming to MRSA by the acquisition of the *mec*A gene, or that these MSSA clones could represent MRSA lineages which have lost their *mec*A gene in storage [33]. In contrast to the low prevalence of the *pvl* genes, the staphylococcal complement inhibitor (*scn*), staphylokinase (*sak*) and chemotaxis inhibiting protein (*chp*) were widely distributed among the isolates although it is interesting to note that the CC15 strains lacked the *sak* gene. The exfoliative toxin D (*et*D) which has been associated with blister formation in the epidermis of newborn mice was found only in the single MRSA isolate and was not detected in any of the MSSA [32]. The colonization of a clinical year student by this toxin producing strain is worrisome. In the absence of effective infection control practices and procedures, this strain may become widely disseminated in the hospital. The CC398-MSSA isolate was recovered from a student with no history of exposure to companion animals or livestock. This isolate harbored the immune evasion gene cluster comprising of *sak*, *scn* and *chp* genes. This finding is quite unusual as CC398-MSSA have been shown to possesses *chp* and *scn* but not *sak* [7]. Further work on the genetic evolution/origin of this strain in our setting is needed.

The presence of the CoNS isolate (speciated as *S. epidermidis*) harboring *mec*A (*ccrA-2*; *ccrB-2*; *ccrAA*; *ccrB-4*) cassette chromosome recombinase genes and arginine catabolic mobile element (ACME) is noteworthy and worrisome. *mec*A is believed to be transferred to MSSA from CoNS strains and the ACME gene is associated with bacterial adaptability and colonization ability [9]. Thus the presence of this CoNS strain in a *S. aureus* population with suitable genetic background favorable for *mec*A acquisition may lead to the eventual emergence of MRSA strains, particularly if infection control procedures are not well established in the hospital. While CC5, CC6, CC30, CC45, CC96, CC188, CC398 have been reported in the literature, the occurrence of CC942-MSSA/*PVL*+, CC1290-MSSA, ST2482-MSSA appears sporadic. CC5, CC30 and CC45 are known lineages associated with CA-MRSA, thus these MSSA clones could eventually evolve to the latter through *mec*A gene acquisition [21,28]. The identification of one MSSA isolate that could not be typed may suggest the presence of novel clones of *S. aureus* circulating in our setting. However, this is an anecdotal observation based on a small sample size, further studies evaluating larger number of participants may be required. The presence of novel clones of MSSA may lead to the evolution of novel MRSA clones with epidemic/pandemic potential, thus the significance of population dynamics-based studies cannot be over emphasized.

In conclusion, this study demonstrates a low prevalence of *pvl* genes and nasal carriage of MRSA in the study population. A limitation of our work is the small sample size but the significance of our findings indicates the urgent need for larger studies in our setting. The presence of CoNS harboring *mec*A gene recombinase elements with resistance mediator to fusidic acid, as well as the presence of un-typeable MSSA with the potential to evolve into novel MRSA clones with expanded drug resistance is of concern.

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