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ORIGINAL RESEARCH

Emergence of novel methicillin-resistant *Staphylococcus aureus* strains in a tertiary care facility in Riyadh, Saudi Arabia

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Purpose: There is a need for continuous surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) to identify emergence of new strains. We hypothesize that MRSA strains are evolving with ongoing acquisition of SCC*mec* elements. This study was carried out to evaluate the evolution of MRSA at a tertiary care facility in Saudi Arabia.

Methods: MRSA isolates associated with invasive clinical infection, which were identified in 2017 at the microbiology laboratory, King Khalid University Hospital (KKUH) in Riyadh, Saudi Arabia, were studied. The molecular characterization of isolates was carried out using StaphyType DNA microarray (Alere Technologies GmbH/Abbott, Jena, Germany).

Results: The 125 MRSA isolates studied belonged to 18 clonal complexes (CC) which were distributed into 32 strain assignments. The predominant CC were CC5 (n=30), CC6 (n=17), CC80 (n=13), CC22 (n=12), CC361 (n=12). The findings demonstrated the first identification of CC152, CC361 and CC1153 MRSA as well as ST5-MRSA-[I+fus], "Geraldine Clone", CC6-MRSA-IV (PVL+) and CC88-MRSA-V (PVL+), WA MRSA-117 in Saudi Arabia. Four novel variants were identified: CC5-MRSA-[VI+fus+tirS], CC22-MRSA-[V/VT+fus] (PVL+), CC152-MRSA-[V+fus](PVL+) and CC361-MRSA-[VT+fus]. Fifty-four isolates (n/N=54/125; 43.2%) including the novel strains carried the Q6GD50 SCC*fusC* gene while the Panton-Valentine leukocidin genes were present in 30.4% (n/N=38/125).

Conclusion: The findings demonstrate an expanding MRSA repertoire in our setting including emergence of previously unreported clonal complexes and novel strains. The high carriage of *fusC* gene suggests a role for fusidic acid misuse in driving the evolution of the MRSA genome and underscores the need for increased monitoring of antibiotic use.

Keywords: DNA microarray, fusidic acid, clonal complex, panton-valentine leukocidin

Introduction

Methicillin-resistant *S. aureus* (MRSA) which was first described in the 1960s remains a major etiological agent of community-acquired and nosocomial infections. MRSA infections contribute to the burden of health care accounting for significant morbidity, mortality with significant economic consequences.[1] The epidemiology of MRSA is dynamic and ever evolving with community-acquired MRSA lineages (CA-MRSA) being identified as increasingly relevant also in the health care sector.[2,3]

Antibiotic resistance in MRSA is mediated by the presence of modified penicillin-binding protein (PBP2a), which is encoded for by alleles of the *mecA* gene located on SCC*mec* (staphylococcal cassette chromosome *mec*) element. In addition to the *mecA*

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gene, this large, genetic element also harbors regulatory genes, recombinase genes and a variety of accessory genes. The nomenclature of the SCC*mec* elements is based on the composition of the regulatory and recombinase genes as well as those of additional genes within the *mec* complex which encode for other resistance mechanisms or virulence determinants.[2,4] Pseudo-SCC*mec* elements which are truncated with absence of the *ccr* recombinase genes as well as SCC elements lacking *mec* genes but harboring other markers such as the fusidic acid resistance gene *fusC* have been described in the literature.[5,6] High-resolution typing utilizing newly developed probes been used to differentiate SCC*mec* types into various subtypes.[3,6]

In Saudi Arabia, MRSA infections contribute significantly to the burden of health care delivery with increasing occurrence of CA-MRSA lineages causing nosocomial infections.[7,8] Recently reported work showed the presence of a high degree of diversity and an emergence of both pandemic and rare MRSA strains among isolates obtained from 2009 to 2015 at King Khaled University Hospital (KKUH), Riyadh, Saudi Arabia.[8] Further work also showed that MRSA colonizing health care workers at the facility were of similar population structure as those identified in patients.[9]

The changing epidemiology of MRSA necessitates the continuous surveillance particularly in Saudi Arabia and the Arabian Gulf region where a dynamic population movement exists. The large expatriate population, increasing numbers of tourists and, specifically in Saudi Arabia, the annual influx of millions of Hajj pilgrims makes it plausible that the Arabian Gulf region might be pivotal in the global transmission of MRSA clones. This need for surveillance is underscored by the recent report of the diversity of CC22-MRSA-IV strains across the Arabian Gulf region.[3] Using high-resolution typing, at least six CC22-MRSA-IV strains differing in SCC*mec* subtypes and toxin carriage were identified from three countries in the region.[3] We speculate that new MRSA strains might continuously evolve in our health care facilities and the acquisition of SCC*mec* elements might occur more commonly than appreciated. The present study was carried out to evaluate the evolution of MRSA at a tertiary care center in the capital of Saudi Arabia, Riyadh, and findings demonstrate the emergence of novel variants and previously unidentified MRSA strains.

Materials and methods

Specimen collection and bacterial strains

The study was carried out at the King Khalid University Hospital (KKUH) in Riyadh, Saudi Arabia. Ethical approval was obtained from the hospital ethics committee. MRSA isolates associated with invasive clinical infection identified in 2017 were studied. Bacterial identification, confirmation of methicillin resistance and antibiotic susceptibility testing were performed in accordance with Clinical and Laboratory Standards Institute guidelines as previously described.[8]

DNA microarray for molecular characterization

All isolates were characterized using microarray assays for *S. aureus* typing and characterization of SCC elements (Alere Technologies GmbH/Abbott, Jena, Germany). The probes, primers and procedures used in the assay for detection of species markers, virulence genes, resistance genes and SCC*mec* subtyping have been described previously.[6,10] Assays were carried out in accordance with manufacturer provided protocols. The analysis of presence or absence of target gene, strain and clonal complex assignment and SCC*mec* subtype was carried out as previously described.[6,10]

Results

A total of 125 MRSA isolates associated with clinical infections obtained in 2017 at KKUH microbiology laboratory were studied. The isolates were obtained from blood (n=25), respiratory aspirates (n=20) and wound swabs (n=80). Based on DNA microarray analysis, these 125 MRSA isolates were grouped into 18 clonal complexes (CC) which were distributed into 32 strain assignments based on CC affiliation, toxin gene carriage and SCC*mec* subtype (Table 1). The predominant CC were CC5 (n=30), CC6 (n=17), CC80 (n=13), CC22 (n=12), CC361 (n=12) (Table 1). The findings demonstrated the first identification of CC152, CC361 and CC1153 MRSA in Saudi Arabia. Furthermore, we report the first identification of ST5-MRSA-[I +*fus*], "Geraldine Clone", CC6-MRSA-IV (PVL+) and CC88-MRSA-V (PVL+), WA MRSA-117 in Saudi Arabia (Table 1). Three isolates identified as MRSA phenotypically were genotypically characterized as CC2250 *S. argenteus*.

Four novel variant of MRSA strains were identified. These strains belong to CC5, CC22, CC152 and CC361. Table 2 shows the molecular characterization of these four MRSA strains. Except for the CC5 strain with SCC*mec*VI, all others harbored SCC*mec*V. Almost half of the total isolates (n/N=54/125; 43.2%) including all the novel strains carried the

Table 1 Distribution of clonal complex and strain affiliations

Clonal complex (CC)	Strain affiliations	# of isolates	Novel variants
CC1 (n=8)	CC1-MRSA-[IV+*fus*+*ccrAB1*], WA MRSA-1/45	8	
CC5 (n=30)	CC5-MRSA-IV (PVL+/*edinA*+), WA MRSA-121 CC5-MRSA-[IV+*fus*+*ccrAB*], Maltese Clone CC5-MRSA-IV (*tst1*+), Paediatric clone ST5-MRSA-[I+*fus*], Geraldine Clone CC5-MRSA-[V/VT+*fus*] CC5-MRSA-[VI+*fus*]	5 4 1 1 12 7	SCC [*mec* VI+*fus*+*tirS*] (Unknown CC5) (n=1)
CC6 (n=17)	CC6-MRSA-IV, WA MRSA-51 CC6-MRSA-IV (PVL+)	15 2	
CC8 (n=1)	ST8-MRSA-[IV+*ACME*] (PVL+), USA300	1	
CC15 (n=1)	CC15-MRSA-[V+*fus*]	1	
CC22 (n=12)	CC22-MRSA-IV (*tst1*+), "Gaza Epidemic Strain" CC22-MRSA-IV (PVL+) CC22-MRSA-IV (PVL+/*tst*+) CC22-MRSA-[V/VT+*fus*] (PVL+)	5 1 5 1	SCC*mec* V/VT+*fus* (Unknown CC22)
CC30 (n=3)	CC30-MRSA-[VI+*fus*] (PVL+/tst1) CC30-MRSA-IV (PVL+), Southwest Pacific Clone	1 2	
CC45 (n=1)	CC45-MRSA-IV, WA MRSA-23	1	
CC72 (n=2)	ST72-MRSA-IV, USA700	2	
CC80 (n=13)	CC80-MRSA-IV (PVL+)	13	
CC88 (n=7)	CC88-MRSA-IV (PVL+) CC88-MRSA-[IV+*fus*] CC88-MRSA-V (PVL+), WA MRSA-117	3 3 1	
CC96 (n=5)	CC96-MRSA-IV	5	
CC97 (n=6)	CC97-MRSA-[V/VT+*fus*]	6	
CC152 (n=1)	CC152-MRSA-[V+*fus*] (PVL+)	1	SCC [*mec* V+*fus*] (Unknown CC152)
CC239 (n=2)	CC239-MRSA-[III+*ccrC*]	2	
CC361 (n=12)	CC361-MRSA-[V/VT] CC361-MRSA-V/VT (PVL+) CC361-MRSA-[V/VT+*fus*]	3 1 8	SCC [*mec* VT+*fus*] (Unknown CC361)
CC1153 (n=1)	CC1153-MRSA-[V/VT+*fus*] (PVL+)	1	
CC2250 (n=3)	CC2250-MRSA-IV, WA MRSA-114	3	

Q6GD50 *(fusC*, SCC-borne fusidic acid resistance) (Table 2). None of the isolates harbored the vancomycin or mupirocin resistance genes. Table 3 shows the distribution of carriage of virulence and antibiotic resistance genes among all 125 isolates. The Panton-Valentine leukocidin (*pvl*) genes were found in 30.4% (n/N=38/125) of isolates. The predominant virulence genes identified were *sak* and *scn* which were present in 96% of isolates.

Discussion

The identification of MRSA strains which have not been previously reported from Saudi Arabia and novel variants of MRSA strains demonstrate the continuing evolution of MRSA in our setting. Over 40% of the MRSA isolates identified harbored the fusidic acid resistance gene *fusC* presumably as additional payload on the SCC*mec* genetic element. A similar high occurrence of co-carriage of *fusC*

Table 2 Characterization of novel variants of methicillin-resistant *Staphylococcus aureus* strains

Found in clonal complex	Novel MRSA variants	*SCC mec*-complex-associated genes	Regulatory gene	Capsule gene	Antibiotic resistance genes	Virulence genes
CC5	CC5-MRSA-[VI+*fus*+*tirS*]	*mecA; mecR1; ugpQ; ccrA-4; ccrB-4; Q6GD50 (fusC);tirS*	*agrII*	*cap5*	*blaZ; blaI; blaR; dfrA; tetM; fexA; fosB; tetEfflux*	*entD/G/I/J/M/N/R/U; sak; scn*
CC22	CC22-MRSA-[V/VT+*fus*] (PVL^+)	*mecA, ugpQ; ccrAA; ccrC; Q6GD50 (fusC)*	*agrB-IV*	*cap5*	*blaZ; blaI; blaR; aacA-aphD; dfrA; tetK*	*tst1; entC/G/I/L/M/N/O/U; lukF/S-PVL; sak; chp; scn; fnbB*
CC152	CC152-MRSA-[V+*fus*] (PVL^+)	*mecA, ugpQ; Q6GD50 (fusC); ccrAA; ccrC*	*agrI; agrB-IV*	*cap5*	*aacA-aphD; tetK; tetEfflux*	*lukF/S-PVL; sak; scn; fnbA; fnbB; ica A/D*
CC361	CC361-MRSA-[VT+*fus*]	*mecA; ugpQ; ccrAA; ccrC; Q6GD50 (fusC)*	*agrI; agrB-IV*	*cap8*	*blaZ; blaI; blaR;msrA; aphA3; sat;fosB; tetEfflux*	*entG/I/M/N/O/U; sak; scn; fnbA; fnbB*

and SCC*mec* elements has been shown in MRSA isolates from Kuwait.[5] It is of interest that all the novel variants identified harbored the SCC*mec/fusC* element. This evolution of SCC*mec/fusC* composite element is probably due to a survival benefit for the strains driven in part by misuse of fusidic acid given its availability in our setting as an over-the-counter medication in the community. The presence of composite SCC*mec/fusC* element and chimeras of multiple progenitor cassettes has been demonstrated in fusidic-acid-resistant MRSA strains in the United Kingdom.[11] It was suggested that the occurrence of strains harboring genetic elements which include SCC*mec*, antibiotic resistance and virulence genes may be indicative of novel adaptive mechanisms in MRSA.[11] Indeed, this adaptive mechanism could drive the emergence of new MRSA strains such of the novel variants of MRSA strains shown in this study.

CC5-MRSA is a common clonal complex which encompasses a large variety of MRSA strains.[10] CC5-MRSA is prevalent in the Arabian Gulf region with CC5-MRSA-IV (PVL+/*edinA*+), WA MRSA-121, CC5-MRSA-[IV+*fus*+*ccrAB*], "Maltese Clone" and CC5-MRSA-IV (*tst1*+), "Pediatric clone" previously identified from Saudi Arabia (including from KKUH), Kuwait and Qatar.[5,8,12,13] Although CC5-MRSA-V has been identified in the UAE and Saudi Arabia, the CC5-MRSA-[V/VT+*fus*] reported in this study had only been described from Kuwait.[5,8,13] Our findings demonstrate the emergence of CC5-MRSA-VI in the Arabian Gulf region. CC5-MRSA-VI was first described in Portugal and has been reported in France, Colombia, Argentina and the USA while the PVL-positive CC5-MRSA-VI has been reported from Switzerland.[10,14–16] All the CC5-MRSA-VI identified in this study harbored the SCC*mec/fusC* genetic element. In addition, we describe a novel variant with SCC [*mec* VI+*fus*+*tirS*] harboring the Staphylococcal TIR-protein-binding protein (*tirS*) as additional payload. The presence of the *tirS* gene in *S. aureus* has been associated with enhanced bacterial survival in the host as well as increased virulence through attenuation of the TLR2 immune response.[17] The horizontal transfer of this gene among MRSA isolates circulating in our setting is probable as it is localized on a mobile genetic element. This scenario might drive the evolution of MRSA toward emergence of strains with increased bacterial fitness and virulence.

A single isolate of the ST5-MRSA-[I+*fus*] "Geraldine Clone" was identified and to the best of our knowledge, this is the first description of this strain in the Arabian Gulf region. The ST5-MRSA-[I+*fus*] "Geraldine Clone" was first described in 2003 in France where it is a prevalent clone associated with outbreaks, particularly in the neonatal setting.[18,19] In 2012, a single ST5-MRSA-[I+*fus*] "Geraldine Clone" was reported in Saxony, Germany, in a patient with a history of foreign travel.[6] A travel link could also explain the emergence of this strain in our setting. Although verification of a travel history in this instance was not possible due to lack of access to patient data, the large expatriate population and common occurrences of travel for medical reasons in our setting makes this highly plausible.

CC152-MRSA-V has been reported from Germany, Australia, Sweden, Switzerland and Kuwait.[5,10] In keeping

Table 3 Distribution of virulence and resistance gene markers

Virulence and resistance gene markers		Number of positive isolates (N=125)	Percentage of positive isolates
Accessory gene regulator allele I	***agrI***	53	42.4
Accessory gene regulator allele II	***agrII***	32	25.6
Accessory gene regulator allele III	***agrIII***	36	28.8
Accessory gene regulator allele IV	***agrIV***	7	5.6
Alternate penicillin-binding protein 2, defining MRSA	***mecA***	125	100
Mercury resistance operon	***merA***	0	0
	merB	0	0
Scc*mec* XI	***mecC***	0	0
	blaZ-SCCmec XI	0	0
Beta-lactamase	***blaZ***	115	92
Beta lactamase repressor (inhibitor)	***blaI***	115	92
Beta-lactamase regulatory protein	***blaR***	114	91.2
Rrna adenine N-6-methyl-transferase, erythromycin/clindamycin resistance	***ermA***	4	3.2
Erythromycin/clindamycin resistance	***ermB***	0	0
Erythromycin/clindamycin resistance	***ermC***	36	28.8
Lincosamide nucleotidyltransferase	***linA***	1	0.8
Energy-dependent efflux of erythromycin	***msrA***	13	10.4
Bifunctional enzyme Aac/Aph, gentamicin resistance	***aacA-aphD***	22	17.6
Amino-glycoside adenyl-transferase,tobramycin resistance	***aadD***	7	5.6
3'5'-aminoglycoside phospho-transferase, neo-/ kanamycin resistance	***aphA3***	23	18.4
Streptothricine-acetyl-transferase	***sat***	23	18.4
Dihydrofolate reductase type I	***dfrA***	18	14.4
Fusidic acid resistance	***farI***	13	10.4
Hypothetical protein associated with fusidic acid resistance	***Q6GD50 (fusC)***	54	43.2
Mupirocin resistance protein	***mupR***	0	0
Tetrazyklin resistance	***tetK***	22	17.6
Tetracycline resistance	***tetM***	9	7.2
Chloramphenicol acetyltransferase	***cat***	0	0
23S Rrna methyltransferase	***cfr***	0	0
Chloramphenicol/florfenicol exporter	***fexA***	7	5.6

(*Continued*)

Table 3 (Continued).

Virulence and resistance gene markers		Number of positive isolates (N=125)	Percentage of positive isolates
Metallothiol transferase	***fosB***	71	56.8
	fosB-plasmid	0	0
Quaternary ammonium compound resistance protein A	***qacA***	2	1.6
Quaternary ammonium compound resistance protein C	***qacC***	0	0
Transport/effluxprotein	***tetEfflux***	110	88
Vancomycin resistance gene	***vanA***	0	0
Vancomycin resistance gene from enterococci and Clostridium	***vanB***	0	0
Teicoplanin resistance gene from enterococci	***vanZ***	0	0
Toxic shock syndrome toxin 1	***tst1 (consensus)***	14	11.2
Panton Valentine leukocidin F component	***lukF-PV***	38	30.4
Panton Valentine leukocidin S component	***lukS-PV***	38	30.4
Staphylokinase	***sak***	120	96
Chemotaxis-inhibiting protein	***chp***	38	30.4
Staphylocccal. Complement inhibitor	***scn***	121	96.8
Exfoliative toxin serotype A	***etA***	0	0
Exfoliative toxin serotype B	***etB***	0	0
Exfoliative toxin D	***etD***	13	10.4
Epidermal cell differentiation inhibitor	***edinA***	5	4
Epidermal cell differentiation inhibitor B	***edinB***	14	11.2
Epidermal cell differentiation inhibitor C	***edinC***	0	0
Arginine catabolic mobile element locus	***ACME***	1	0.8

with reported literature on CC152-MRSA-V, our isolate harbored the *pvl* and *edinB* genes, and lacked enterotoxin genes.[10] The CC152-MRSA-[V+*fus*] (PVL+) identified in this study is very similar to that recently reported as a novel variant with the carriage of the SCC*fusC* element. However, our isolate had the fibronectin-binding protein genes (*fnbA, fnbB*) which was not present in the previously described isolates.[5]

The findings in this study demonstrate the first identification of CC361 strains in Saudi Arabia. CC361-MRSA-V has been described in the UAE and recently CC361-MRSA-[V/VT+*fus*] was reported in Kuwait.[5,10] The CC361-MRSA-*[*V/VT+*fus*] harboring the SCC*mec* VT+*fus* genetic element identified in this study is similar to the novel variant recently described from Kuwait, thus indicating its second description in our region.[5]

We had previously reported CC1153-MSSA as nasal colonizer in health care workers at KKUH but this is the first identification of CC1153-MRSA in this facility.[9] In our region, the two CC1153-MRSA isolates reported have been from Kuwait.[5,20] Both isolates were pvl^+ while one carried the SCC*mec-V* and the other had SCC*mec* I+*fus*. Similar to these two, our isolate is pvl^+. Thus, all three CC1153 strains identified so far in the region show variability in their SCC*mec* types/ subtypes. It has been suggested that emergence of novel strains as reported for CC5-MRSA-IV, CC22-MRSA-IV and PVL-positive CC30-MRSA-IV might be due to the independent

acquisition of SCC*mec* types/subtypes by the parental MSSA strains.[6] This observation with CC1153-MRSA supports this premise for the emergence of novel MRSA strains.

CC22 is a widespread clonal group and six distinguishable strains based on SCC*mec* IV subtypes and virulence factors have been reported from this region.[3] In this study, three of the previously reported CC22-MRS-IV strains were identified as well as a novel variant of CC22-MRSA-V characterized by carriage of the SCC*mec V/VT+fus and pvl*$^{+}$. Previously reported CC22-MRSA-[V+*fus*] from Saxony, Germany, were negative for *pvl* gene.[6] In Saudi Arabia, a single isolate of CC22-MRSA-V which did not carry the *pvl* gene was reported as a nasal colonizer.[21] The finding of this novel CC22-MRSA variant suggests the expanding repertoire of CC22 strains in this region.

S. argenteus is a newly identified staphylococcus species previously misidentified as *S. aureus*.[22] The isolates identified in this study were phenotypically classified as *S. aureus* but found to be CC2250 *S. argenteus* on genotyping. This represents the second report of *S. argenteus* CC2250-MRSA-IV, WA MRSA-114 in Saudi Arabia having been previously identified from clinical infection in this health care facility.[8] Indeed, *S. argenteus* has been shown to be of clinical relevance and isolates belonging to CC75, CC1233, CC2198, CC2250 and CC2854 have been described.[22,23]

Conclusion

Our findings demonstrate the emergence of novel variants of MRSA strains belonging to CC5, CC22, CC152 and CC361 as well as the first identification of other MRSA strains in Saudi Arabia. These findings are significant and indicative of an expanding repertoire of MRSA strains in our setting. This continued evolution of the MRSA genome arising from acquisition of resistance and virulence genes poses significant challenges for treatment and infection control. The role of fusidic acid misuse in driving this evolution underscores the need for increased monitoring of antibiotic use.

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Disclosure

EM, AR, DG, RE, SM were employees at Alere Technologies GmbH/Abbott, Jena, Germany, at the time the study was carried out. The authors report no other conflicts of interest in this work.

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