


Emergence of Methicillin-Resistant *Staphylococcus aureus* Belonging to Clonal Complex 15 (CC15-MRSA) in Kuwait Hospitals

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Edet E Udo 
Samar S Boswihi
Bindu Mathew
Bobby Noronha
Tina Verghese
Aisha Al-Jemaz
Fatma Al Saqer

Department of Microbiology, Faculty of
Medicine, Kuwait University, Safat,
Kuwait

Purpose: Methicillin-resistant *S. aureus* (MRSA) belonging to clonal complex 15 (CC15-MRSA) is rare among clinical isolates with few reports from retail camel meat and human patients. This study investigated the genetic relatedness of CC15-MRSA isolated for the first time from patients in Kuwait hospitals.

Methods: Antibiotic susceptibility was tested by the disk diffusion method. Minimum inhibitory concentration was determined using Etest strips. Molecular typing was performed using *spa* typing, multilocus sequence typing and DNA microarray.

Results: Of 1327 MRSA isolates, 42 (3.1%) were identified as CC15-MRSA. The 42 isolates belonged to sequence type ST1535-harbored SCCmec type V and *spa* types t084 (36 isolates), t346 (3 isolates) and one of t114, t228 and t7583. All 42 isolates were resistant to gentamicin, kanamycin, fusidic acid and cadmium acetate; 38 isolates were resistant to tetracycline. The isolates harbored *aacA-aphD* and *fusC* that codes for gentamicin and fusidic acid resistance, respectively. *Tet(K)* was present in the tetracycline-resistant isolates. In addition, the 42 isolates carried *inu(A)* (lincosamide nucleotidyltransferase) that confers resistance to lincomycin and clindamycin although phenotypically susceptible to these antibiotics. The isolates belonged to accessory gene regulator type II and capsular polysaccharide group 8 but lacked genes for *Staphylococcus enterotoxins*, toxic shock syndrome toxin, collagen-binding adhesins and Panton–Valentine leukocidin.

Conclusion: This study revealed the emergence and transmission of a previously rare MRSA clone among human patients in Kuwait hospitals and highlights the increasing infiltration of rare MRSA into the human population.

Keywords: DNA microarray, MRSA, antibiotic resistance, MLST

Introduction

Antibiotic-resistant pathogens continue to threaten the effective delivery of health-care globally due to the associated limited choices of antibiotics for therapy of serious infections caused by these organisms, prolonged illness, prolonged hospitalization leading to increased cost of treatment and mortality.¹

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important antibiotic-resistant pathogen known to cause a wide range of infections including superficial skin and soft tissue infections and serious invasive infections such as pneumonia, osteomyelitis, and septicemia.² Since the initial report in the United Kingdom in the early 1960,³ MRSA strains have been reported from healthcare facilities and in communities worldwide.² Furthermore, the epidemiology of MRSA has changed substantially

Correspondence: Edet E Udo
Department of Microbiology, Faculty of
Medicine, Kuwait University, P. O. Box
24923, Safat 13110, Kuwait
Tel +965 498 36773
Fax +965 2533 2719
Email edet@hsc.edu.kw

since the initial report in the UK. MRSA changed from being primarily associated with infections among elderly patients in healthcare facilities (healthcare-acquired MRSA) to colonizing or infecting young and apparently healthy individuals with no previous history of hospitalization in the community (Community-associated MRSA).^{1,4,5} The emergence of community-associated MRSA (CA-MRSA) was soon followed by reports of MRSA that were associated with livestock or humans associated with livestock which have been described as livestock-associated MRSA (LA-MRSA).^{6–11}

Since their description in the early 1990s, different CA-MRSA clones have emerged in different geographical regions with different capacities for global transmission. Whereas strains belonging to ST30-MRSA-IV and ST80-MRSA-IV have been widely reported in different countries, others such as ST59-MRSA and ST93-MRSA-IV have restricted geographical distribution.^{1,2} In addition, novel MRSA clones as well as variants of the established CA-MRSA clones continue to emerge.^{12,13,15,18} The constant changes in the distribution of MRSA clones warrants regular surveillance of MRSA in local healthcare facilities to detect the newly emerging clones so as to institute effective control measures and prevent their transmission.^{14,15}

MRSA strains belonging to clonal complex 15 (CC15-MRSA) are rare in the literature with only small numbers reported in retail camel meat in Saudi Arabia¹² and in a small number of human patients in Saudi Arabia,^{13,16} Iran^{17,18} and Italy.¹⁹ Whole genome sequencing of four CC15-MRSA isolates cultured from human patients (N=2), and retail meat (N=2) in Saudi Arabia revealed that all four isolates belonged to the same sequence type, ST1535 (13-13-1-1-81-11-13), which is a single locus variant of ST15 and harbored a novel SCC_{mec} V/SCC_{fus} composite genetic element.²⁰

Significant changes in the distribution of MRSA clones have been reported in Kuwait public hospitals in recent years. The number of MRSA clones in Kuwait hospitals increased from one dominant clone in 1992 to 30 diverse clones in 2010.²¹ In addition, novel MRSA clones and variants of well-known clones emerged in the country.¹⁴ This paper reports on the isolation and molecular characteristics of a newly emerging CC15-MRSA clone cultured from clinical samples of patients in Kuwait hospitals.

Materials and Methods

Bacterial Strains

The CC15-MRSA isolates used in this study were collected as part of routine microbiology diagnostic services in Kuwait

public hospitals. It is mandatory to submit all MRSA isolates obtained from clinical samples (infection and colonization sites) in public hospitals in Kuwait to the MRSA Reference Laboratory for molecular typing following primary isolation and identification in the diagnostic microbiology laboratories using standard bacteriological methods including growth on mannitol salt agar, gram stain, tube coagulase test and DNase testing. The isolates used in this study were part of the MRSA isolates obtained from 1 January to 31 December, 2016 in all 14 public hospitals. The CC15-MRSA were isolated only in eight diagnostic microbiology laboratories. The isolates were preserved in 40% glycerol (v/v) in brain heart infusion broth at -80°C . Isolates were subcultured twice on brain-heart infusion agar before processing.

Antibiotic Susceptibility Testing

Antibiotic susceptibility profiles of the isolates were tested by disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI)²² against the following antimicrobial disks (Oxoid, Basingstoke, UK): benzyl penicillin (2U), cefoxitin (30 μg), kanamycin (30 μg), mupirocin (200 μg and 5 μg), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), tetracycline (10 μg), trimethoprim (2.5 μg), fusidic acid (10 μg), rifampicin (5 μg), ciprofloxacin (5 μg), teicoplanin (30 μg), and linezolid (30 μg). Susceptibility to the antibiotics was interpreted according to the CLSI. Minimum inhibitory concentration (MIC) of cefoxitin, vancomycin, teicoplanin, mupirocin, erythromycin and clindamycin were determined with Etest strips (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions. *S. aureus* strains ATCC25923 and ATCC29213 were used as quality control strains for disk diffusion and MIC determination, respectively. Susceptibility to fusidic acid was interpreted according to guidelines of the British Society to Antimicrobial Chemotherapy.²³ Susceptibility to cadmium acetate (50 μg) mercuric chloride (109 μg) and ethidium bromide (50 μg) was performed as described previously.²⁴

Molecular Typing of Isolates

DNA microarray analysis was performed to determine antibiotic resistance genotypes and virulence-related genes, and to assign the isolates to clonal complex (CC) using the Identibac *S. aureus* genotyping Kit 2.0 (Alere Technology, Jena, Germany) and protocols provided by the manufacturer and described previously.²

DNA for PCR amplification was isolated and purified as described previously.¹⁴ *Spa* typing was performed using

protocol and primers published previously.²⁵ Multilocus sequence typing (MLST) was performed using the protocol described by Enright et al.²⁶

Pulsed-field gel electrophoresis (PFGE) was performed as described previously.²⁷ Contour-clamped homogenous electric field (CHEF) electrophoresis of *Sma*I-digested genomic DNA was performed in CHEF-DRIII system (BioRad, Hercules, CA, USA). The run was for 22 h with 5-s initial pulse and 40-s final pulse. *Sma*-I digested *S. aureus* strain NCTC 8325 was used as molecular size marker. Following electrophoresis, the gel was stained in ethidium bromide (0.5 mg/L) and photographed under ultra violet illumination. Strain relatedness was determined using BioNumerics software (version 7.0; Applied Maths, Kortrijk, Belgium). The software established similarity between isolates using band-based dice coefficient method with 1.5% optimization and 1.5% band position tolerance. They were separated into similarity clusters by the unweighted-pair group method using average linkages (UPGMA). Relatedness of PFGE profiles was established at a cutoff level of 100% and 90%. The PFGE profiles of MRSA isolates that showed 100% similarity were considered identical and clustered in a single group, while the PFGE profiles of MRSA isolates that showed less than 100% similarity were considered a subtype of that group. Isolates of PFGE profiles that showed similarity at a cutoff level less than 90% were clustered into different groups.

Plasmid analysis was performed by the CTAB method as described previously.²⁷ Curing, mixed-culture transfer and conjugation experiments were performed as described previously.²⁸

Results

Molecular Typing of Isolates

The results of DNA microarray analysis performed on 1327 MRSA isolates obtained from different clinical samples in 2016 identified 42 (3.1%) isolates as CC15-MRSA-V +SCCfus. The distribution of other MRSA clones obtained in 2016 was reported elsewhere.¹⁴ The 42 isolates were cultured from wound swabs (N=14), nasal swabs (N=10), high vaginal swabs (N=5), groin swabs (N=3) blood (N=2), urine (N=2), tracheal aspirate (N=1), eye swab (N=1) with no clinical sources specified for two isolates in eight hospitals including Mubarak hospital, MUB (N=12), Adan hospital, ADH (N=5), Jahra hospital, JH (N=3), Razi hospital, RAZ (N=3), Maternity hospital, MAT (N=11), Sabah hospital, SAB (N=4), Chest-Disease hospital, CDH (N=1), and

Farwaniya hospital, FAW (N=3). The isolates were obtained from 25 males and 15 females. The gender of two of the patients was not provided.

Spa typing of the 42 isolates revealed five *spa* types. These were t084 (N=36), t346 (N=3), t228 (N=1), t144 (N=1) and t7583 (N=1). MLST was performed on 20 representative isolates selected on the basis of their *spa* types as follows: 14 of t084 isolates, all three of t346 and one each of t228, t144 and t7583 isolate. All 20 isolates belonged to the same sequence type, ST1535 (13-13-1-1-81-11-13). ST1535 is a single locus variant of ST15 at the *pta* locus. These isolates carried *pta* 81 instead of *pta*12 in traditional ST15 isolates.²⁰

PFGE of the 42 isolates yielded four closely related patterns designated types A-D with the majority of the isolates belonging to PFGE type A and its subtypes (Figure 1). The t084 isolates were clustered among PFGE type A (N=28) and its subtypes A1 (N=9), type A2 (N=1) and type D (N=1). Isolates of PFGE types B (N=1) and C (N=3) corresponded to *spa* types t228 and t346, respectively. The t114 isolate belonged to PFGE type A1.

Plasmid analysis of the 42 isolates revealed the presence of a single c.28kb plasmid in 39 isolates. Three isolates harbored a single plasmid of c18kb and one isolate contained a 4.4kb plasmid (Figure 2).

Four isolates representing three plasmid patterns and *spa* types, t084, t114, t346, t228 and t7583 were selected for curing and transfer experiments to determine the resistance phenotypes of the plasmids. Colonies were screened for the loss of resistance to gentamicin, kanamycin, trimethoprim, tetracycline, and cadmium acetate. A minimum of 600 colonies were screened for loss of resistance to the selected antibiotics. None of the resistance screened was lost in any of the four isolates. Similarly, no resistance was transferred from any of the four isolates when used as donors in conjugation and mixed-culture transfer experiments.

Antibiotic Resistance Phenotypes and Genotypes

All 42 isolates were susceptible to vancomycin (MIC: ≤ 2 mg/L), teicoplanin (MIC: ≤ 2 mg/L), linezolid, rifampicin, trimethoprim, chloramphenicol, erythromycin (MIC: ≤ 0.5 mg/L) and clindamycin (MIC: 0.19–0.38 mg/L) but were resistant to penicillin G and cefoxitin (N=42), gentamicin (N=42), Kanamycin (N=42), fusidic acid (N=42), tetracycline (N=36) and ciprofloxacin (N=2). All isolates were resistant to cadmium acetate.

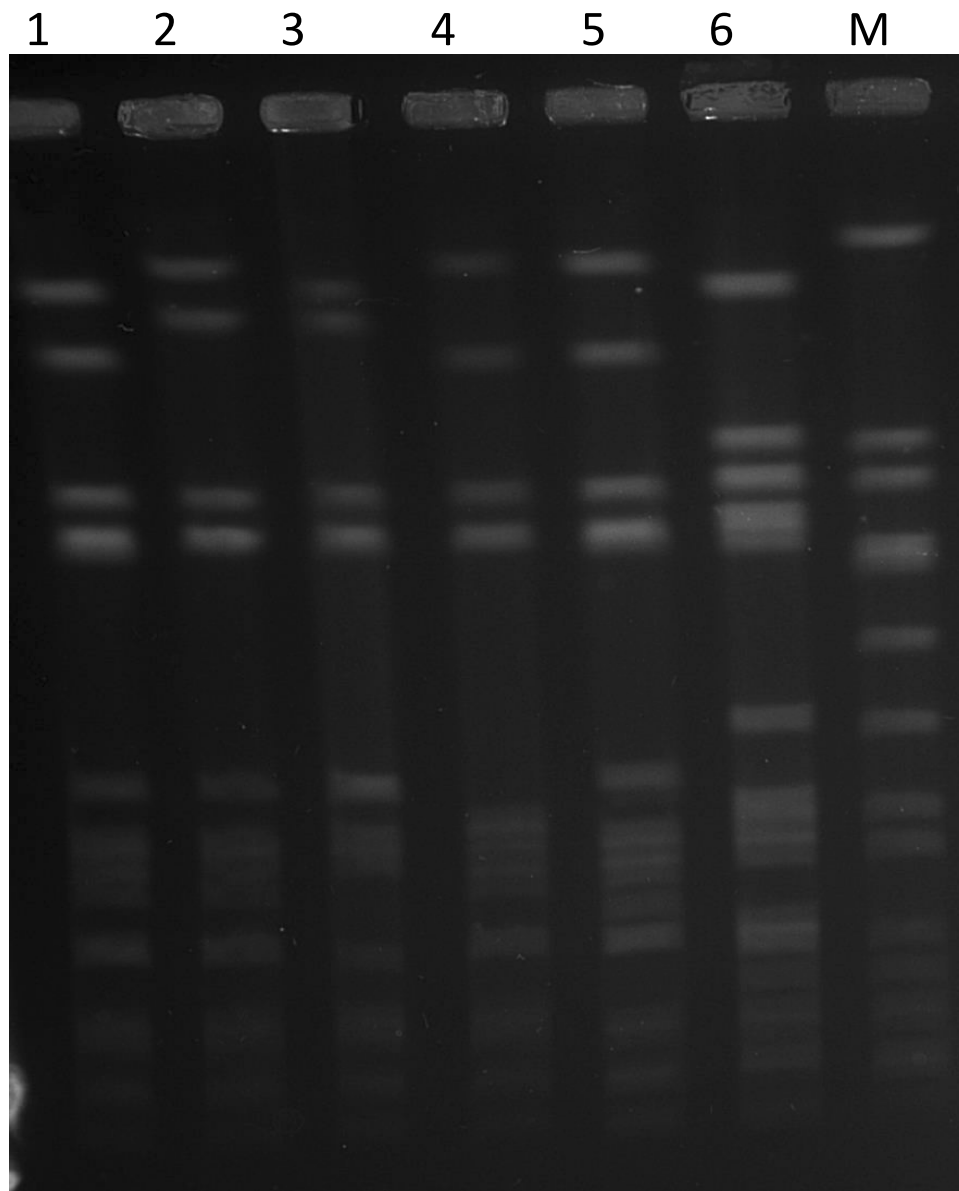


Figure 1 PFGE patterns of representative CC15-MRSA-V+SCCfus isolates. Lane 1, PFGE type D. Lane 2, *S. aureus* strain NCTC8325. Lane 3, PFGE type A1. Lane 4, PFGE type A2. Lane 5, PFGE type A. Lane 6, PFGE type B. Lane M, PFGE type C.

The resistance genotypes of the isolates are summarized in [Table 1](#). The resistance genotypes matched the corresponding resistance phenotypes with the exception of the presence of *inu(A)*. *inu(A)* (lincosamide nucleotidyl-transferase), which confers resistance to lincomycin and clindamycin, was detected in all 42 isolates although they were phenotypically susceptible to clindamycin (MIC: 0.19–0.38 mg/L).

All isolates carried *fusC* which mediates fusidic acid resistance. In addition to *aacA-aphD* which mediates resistance to gentamicin, kanamycin and tobramycin; 39 of the isolates were positive for *aadD* which encodes resistance

to kanamycin, neomycin and tobramycin. None of the isolates was positive for the *aphA3*, which mediates resistance to neomycin and kanamycin. One isolate was positive for *erm(C)*.

Prevalence of Virulence-Related Genes

Results of DNA microarray analysis revealed that, with few exceptions, the isolates were remarkably homogeneous in the carriage of virulence-associated genes as presented in [Table 1](#).

All 42 isolates were positive for genes for accessory gene regulator (*agr*) type II, and capsular polysaccharide

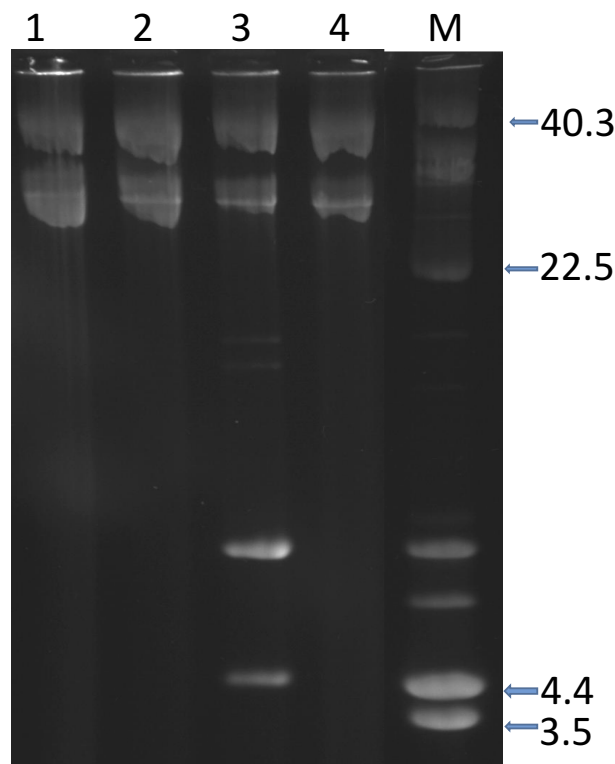


Figure 2 Plasmid content of representative CC15-MRSA-V+SCCfus isolates. Lanes 1, 2, and 4 contain single plasmid of c.28-kb. Lane 3, contains 2 plasmids of c.28-kb and 4.4-kb. Lane M, contains plasmid size markers. Sizes are in Kb.

type 8 (*cap8*), clumping factor A (*clfA*), clumping factor B (*clfB*), cell-wall-associated fibronectin-binding proteins (*ebh*), fibronectin-binding protein A and B (*fnbA/B*), bio-film-associated genes, *icaA*, *icaC* and *icaD*, and genes encoding microbial surface compounds recognizing adhesive matrix molecules, enolase, and hyaluronate lyase (*hysAI*). The isolates were also positive for transferrin binding protein, *isdA* and the type 1 site-specific deoxyribonuclease subunit, *hsdSx* (CC15) and *hsdSx* (CC25) but not *hsdS1*, *hsdS2* and *hsdS3*.

All isolates lacked genes for Pantone–Valentine leucocidin (PVL), staphylococcal enterotoxins, toxic-shock syndrome toxin (TSST), exfoliative toxins, and epidermal cell differentiation inhibitors, *edinA*, *edinB* and *edinC*, arginine catabolic mobile element (ACME) and collagen-binding adhesins (*cna*).

The isolates were variable in the carriage of genes for leukocidins, haemolysins; Immune evasion clusters and proteases as summarized in Table 1. Whereas all isolates were negative for *lukM* components, they were all positive for *lukF*, *lukS*, *lukD*, but differed in the carriage of *luKE*, *lukX* and *hlgA*. For the Immune evasion cluster genes, all 42 isolates were positive for *Chp* (chemotaxis-inhibiting

proteins) and *scn* (staphylococcal complement inhibitor) and were negative for *sak* (staphylokinase). The haemolysins encoding gene *hlyB* (haemolysin B) was absent in one t084 and in the t346 isolates (Table 1).

Distribution of the CC15-MRSA-V+SCCfus Isolates in the Hospitals

The time line for the isolation of the CC15-MRSA-V isolates is summarized in Table 2. The isolates were obtained from eight hospitals with most of them identified in two hospitals, namely, MUB (N=13) and MAT (N=11). The first isolate was obtained in January from a High Vaginal Swab sample of a patient at the MAT. The numbers that were isolated in subsequent months varied with higher numbers isolated in November (N=8), August (N=7), April (N=5) and December (N=5).

Table 3 shows the distribution of the 36 *spa* type t084 isolates. The t084 isolates were widely distributed with 19 of the 36 isolates originating from two of the eight hospitals. The isolates were obtained from different clinical samples representing colonization and infection sites with most of the isolates cultured from nasal (N=9) and wound (N=8) samples.

Discussion

This study reports the isolation of CC15-MRSA-V+SCCfus isolates from human patients in Kuwait hospitals. Prior to this report, CC15-MRSA-V+SCCfus isolates were isolated sporadically from retail camel meat¹² and human patients in Saudi Arabia.^{13,20} The report of 42 CC15-MRSA-V+SCCfus isolates in this study represents the largest collection of CC15-MRSA-V+SCCfus isolates reported in the literature among human patients and represents a significant change in the epidemiology of this clone and its expansion into the human patient population.

The 42 CC15-MRSA-V+SCCfus in this study belonged to ST1535, harbored SCCmec V, agr II, and the novel SCCfus composite genetic element similar to the isolates obtained from retail camel meat¹² and human patients reported previously in Saudi Arabia²⁰ and may be related to them. However, although the isolates obtained from Kuwait and Saudi Arabia belonged to the same genotype, CC15-MRSA-V+SCCfus, they were not homogeneous. Although the majority of the isolates from Kuwait belonged to *spa* type t084, few isolates of *spa* types, t114, t228, t346 and t7583 were also detected. Similarly, in addition to *spa* type t084, the CC15-MRSA-V+SCCfus identified in Saudi Arabia also

Table 1 Characteristics of CC15-MRSA-V+SCCfus Isolates

Relevant Genes	Spa Types									
	t084, N=31	t084, N=1	t084, N=1	t084, N=1	t084, N=1	t084, N=1	t228, N=1	t114, N=1	t346, N=1	t7583, N=1
Regulatory Genes										
<i>SarA</i>	+	+	+	+	+	+	+	+	+	+
<i>Saes</i>	+	+	+	+	+	+	+	+	+	+
<i>Agr II</i>	+	+	+	+	+	+	+	+	+	+
Resistance Genotype										
<i>mecA</i>	+	+	+	+	+	+	+	+	+	+
<i>BlaZ</i>	+	+	+	+	+	+	+	+	+	+
<i>BlaI</i>	+	+	+	+	+	+	+	+	+	+
<i>aacA-aphD</i>	+	+	+	+	+	+	+	+	+	+
<i>aphA3</i>	-	-	-	-	-	-	-	-	-	-
<i>aadD</i>	+	-	+	+	+	-	+	+	+	+
<i>fusC</i>	+	+	+	+	+	+	+	+	+	+
<i>Inu(A)</i>	+	+	+	+	+	+	+	+	+	+
<i>erm(C)</i>	-	-	-	-	+	-	-	-	-	-
<i>Tet(K)</i>	+	-	+	+	-	-	+	+	+	+
<i>fosB</i>	+	+	+	+	+	+	+	+	+	+
Toxins										
<i>tst I</i>	-	-	-	-	-	-	-	-	-	-
Enterotoxins	-	-	-	-	-	-	-	-	-	-
Leukocidins										
<i>lukF</i>	+	+	+	+	+	+	+	+	+	+
<i>lukS</i>	+	+	+	+	+	+	+	+	+	+
<i>hlgA</i>	+	+	+	+	-	+	+	+	+	+
<i>lukD</i>	+	+	+	+	+	+	+	+	+	+
<i>lukE</i>	+	+	+	+	-	+	+	+	+	+
<i>lukX</i>	+	+	+	+	-	+	+	+	+	+
<i>lukY</i>	+	+	+	+	+	+	+	+	+	+
Haemolysins										
<i>hl</i>	+	+	+	+	+	+	+	+	+	+
<i>hla</i>	+	+	+	+	-	+	+	+	+	+
<i>hIb</i>	+	+	-	+	-	+	+	+	-	+
<i>hIII</i>	+	+	+	+	+	+	+	+	+	+
Immune Evasion Clusters (IEC)										
<i>Sak</i>	-	-	-	-	-	-	-	-	-	-
<i>Chp</i>	+	+	+	+	+	+	+	+	+	+
<i>Scn</i>	+	+	+	+	-	+	+	+	+	+
Proteases										
<i>spIA</i>	+	+	+	+	+	+	+	+	+	+
<i>spIB</i>	+	+	+	+	+	+	+	+	+	+
<i>spIE</i>	+	+	+	+	-	+	+	+	+	+

(Continued)

Table 1 (Continued).

Relevant Genes	Spa Types									
	t084, N=31	t084, N=1	t084, N=1	t084, N=1	t084, N=1	t084, N=1	t228, N=1	t114, N=1	t346, N=1	t7583, N=1
Proteases										
<i>sspA</i>	+	+	+	+	+	+	+	+	+	+
<i>sspB</i>	+	+	+	+	+	+	+	+	+	+
Restriction Modification System										
<i>hsdS1</i>	-	-	-	-	-	-	-	-	-	-
<i>hsdS2</i>	-	-	-	-	-	-	-	-	-	-
<i>hsdS3</i>	-	-	-	-	-	-	-	-	-	-
<i>hsdSx (CC15)</i>	+	+	+	+	+	+	+	+	+	+
<i>hsdSx (etd)</i>	-	-	-	-	-	-	-	-	-	-

Notes: +, gene present; -, gene not detected.

Abbreviations: lukF, leukocidin component B (F); lukS, leukocidin component C (S); hlgA, haemolysins gamma, component A; lukD, leukocidin D component; lukE, leukocidin E component; lukX, leukocidin/haemolysin toxin family protein (lukB or lukG); lukY, leukocidin/haemolysin toxin family protein (lukA or lukH); hl, putative membrane protein; hla, haemolysins alpha; hlb, haemolysins beta; hlll, putative membrane protein; sak, staphylokinase; chp, chemotaxis-inhibiting protein; scn, staphylococcal component inhibitor; splA, serine protease A; splB, serine protease B; splE, serine protease E; sspA, glutamylendopeptidase; sspB, staphopain B, protease.

included isolates with different *spa* types, t328, t393 and t385²⁰ suggesting that the isolates from both countries may have evolved differently. The detection of CC5-MRSA-V+SCCfus isolates with diverse *spa* types provides evidence of an ongoing diversification of the clone. The report of CC15-MSSA belonging to *spa* type t346 among *S. aureus* isolated in different European countries²⁹ supports the independent acquisition of the SCCmec V and *fusC*, as seen in this study, by CC15-MSSA isolates. Therefore, it is important to continue to monitor the evolution of this clone in the region.

Prior to the report of CC15-MRSA-V+SCCfus isolates in Saudi Arabia, CC15-MRSA-IV isolates belonging to ST15 and accessory gene regulator group II (*agr* II), were reported in a small number of human patients in Saudi Arabia¹⁶ and Iran,^{17,18} and an isolate of ST15-MRSA-I was reported in a patient in Italy.¹⁹ Therefore, the CC15/ST1535-MRSA-V+SCCfus isolates in this study and those obtained in Saudi Arabia^{12,13} represent newly emerging variants of the CC15-MRSA lineage. Further support for the new emergence of the CC15-MRSA-V+SCCfus isolates is the report of the presence of *hsdM*/

Table 2 Isolation and Distribution of CC15-MRSA-V+SCCfus Isolates

Months	Hospitals								
	MUB	ADH	JH	RAZ	MAT	SAB	CDH	FAW	Total
January					1				1
February	1				1				2
March	2	1							3
April			1		1				2
May	3		1		1				5
June	1				2				3
July				1	1				2
August	3	1		1	1			1	7
September			1			1			2
October					1	1			2
November	3	1				1	1	2	8
December		1		1	2	1			5
Total	13	4	3	3	11	4	1	3	42

Abbreviations: MUB, Mubarak hospital; ADH, Adan hospital; JH, Jahra hospital; RAZ, Razi hospital; MAT, Maternity hospital; SAB, Sabah hospital; CDH, Chest-Disease hospital; FAW, Farwaniya hospital.

Table 3 Distribution of t084 in Clinical Samples in Different Hospitals

Hospitals	Blood	Groin	Nasal	Wound	HVS**	Skin	Others*	Total
MUB	2	1	4	–	–	–	3	10
ADH			1	2		1		4
JH		1	1		1			3
RAZ			1			2		3
MAT			1	4	3		1	9
SAB		1				1	2	4
CDH			1					1
FAW				2				2
Total	2	3	9	8	4	4	6	36

Notes: *Include sputum, tracheal aspirate, Urine, unspecified. **high vaginal swab.

hsdS variants of the type 1 site-specific deoxyribonuclease subunit in the isolates from Saudi Arabia.²⁰ The *hsdM/hsdS* variants were suggested to facilitate the uptake of new foreign DNA that promoted the emergence of CC15-MRSA.²⁰ The CC15/ST1535-MRSA-V+SCCfus isolates carried one SCCfus composite genetic element which has recently been reported in fusidic acid-resistant MRSA isolates belonging to different genetic backgrounds including CC1, CC5, CC8, CC22, CC30, CC45, CC152, CC97 and CC913^{14,21,30–33} and appears to have contributed to the recent increases in the prevalence of fusidic acid resistance in MRSA isolates.

Surprisingly, although phenotypically susceptible to erythromycin (MIC: ≤ 0.5 mg/L) and clindamycin (MIC: MIC: 0.19–0.38 mg/L), the isolates were positive for *inu(A)* that confers resistance to clindamycin and lincocmycin, and one of the isolates was positive for *erm(C)* that encodes erythromycin resistance suggestive of the presence of unexpressed resistance genes. Similarly, Raji et al¹² reported the presence of *mecA* in CC15-MRSA-V+SCCfus isolates that were phenotypically susceptible to ceftiofloxacin.

Our results, obtained with DNA microarray, mirrored the results obtained with whole genome sequencing of the isolates from Saudi Arabia with regards to the presence of some virulence-related genes.²⁰ Similar to the results with isolates from Saudi Arabia,²⁰ the isolates in this study harbored a variety of virulence genes except genes for staphylococcal enterotoxins, toxic shock syndrome toxin and exfoliative toxins (Table 1). Similarly, non-human clones of Livestock-associated MRSA isolates such as CC398^{8,34,35} and CC97³⁶ also lack genes for staphylococcal enterotoxins and toxic shock syndrome toxin suggesting that the CC15-MRSA-V+SCCfus may be related to LA-MRSA lineage.

The results also revealed the presence of the Immune evasion cluster genes, *scn* and *chp*, and the absence of *sak*. Since *sak* is thought to be associated with tissue invasion, its absence in the CC15 lineage has been used to explain their association with carriage rather than invasive infections.²⁰ However, a few of the isolates in this study were obtained from blood cultures and other invasive clinical samples suggesting that at least some of the CC15-MRSA isolates can also cause invasive infections. Unfortunately, no information was available to indicate whether these patients had catheter or IV lines which could explain their presence in the bloodstreams. It is possible that because only few CC15-MRSA isolates have been reported till date, the range of infections they are capable of causing has not been fully appreciated. As more CC15-MRSA-V+SCCfus are isolated from human patients and studied, more will be known about their capacity to cause infections.

Although isolated in eight hospitals, most of the isolates were obtained from two hospitals. However, the pattern of isolation shown in Table 2 suggests that the isolates were not associated with outbreaks. This is supported by the fact that the isolates were obtained from single patients except in November where two isolates were obtained from two different sites in the same patient. Nevertheless, the isolation of *spa* type t084 in each of the eight hospitals suggests that, given the right conditions, patient-to-patient transmission can occur. It will be interesting to see how this clone will evolve in the future.

Limitations of this study include lack of information regarding travel history of the patients who harbored these strains which would help establish any epidemiological relationship with the isolates from Saudi Arabia. We also do not have information regarding animals contact or consumption of camel meat. Information is also not available regarding

whether the patients were colonized or infected at the time of admission to the hospital. Furthermore, it is not known whether patients whose blood cultures yielded CC15-MRSA-V+SCCfus had catheters or IV lines which could aid the survival of these strains in the blood stream.

Conclusion

We report the isolation and characterization of an emerging CC15-MRSA-V+SCCfus clone from patients in eight hospitals in Kuwait. The isolates belonged to sequence type, ST1535 and closely related pulsotypes. The isolates belonged to different *spa* types with t084 as the dominant *spa* types. The isolates expressed resistance to multiple antibiotics including fusidic acid, gentamicin, and tetracycline, harbored genes for multiple virulence factors but lacked genes for staphylococcal enterotoxins, toxic shock syndrome toxins and exfoliative toxins. Future surveillance is required to monitor the isolation of this clone in other hospitals in the country.

Ethical Approval

This study does not require ethical approval.

Data Sharing Statement

All data are available in the text.

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Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the final version for publication, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare that there is no conflict of interest.

References

- Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev.* 2018;31:4.

- Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One.* 2011;6(4):e17936. doi:10.1371/journal.pone.0017936
- Jevons MP. "Celbenin"-resistant staphylococci. *Br Med J.* 1961;1(5219):124–125. doi:10.1136/bmj.1.5219.124-a
- Udo EE, Pearman JW, Grubb WB. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. *J Hosp Infect.* 1993;25(2):97–108. doi:10.1016/0195-6701(93)90100-E
- David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev.* 2010;23(3):616–687. doi:10.1128/CMR.00081-09
- Armand-Lefevre L, Ruimy R, Andrement A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis.* 2005;11(5):711–714. doi:10.3201/eid1105.040866
- Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis.* 2005;11(12):1965–1966. doi:10.3201/eid1112.050428
- Fluit AC. Livestock-associated *Staphylococcus aureus*. *Clin Microbiol Infect.* 2012;18(8):735–744. doi:10.1111/j.1469-0691.2012.03846.x
- Fitzgerald JR. Human origin for livestock-associated methicillin-resistant *Staphylococcus aureus*. *MBio.* 2012;3(2):e00082–e00012. doi:10.1128/mBio.00082-12
- Liu CM, Price LB, Hungate BA, et al. *Staphylococcus aureus* and the ecology of the nasal microbiome. *Sci Adv.* 2015;1(5):e1400216. doi:10.1126/sciadv.1400216
- Larsen J, Stegger M, Andersen PS, et al. Evidence for human adaptation and foodborne transmission of livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis.* 2016;63(10):1349–1352. doi:10.1093/cid/ciw532
- Raji MA, Garaween G, Ehrlich R, Monecke S, Shibl AM, Senok A. Genetic characterization of *Staphylococcus aureus* isolated from retail meat in Riyadh, Saudi Arabia. *Front Microbiol.* 2016;7:911. doi:10.3389/fmicb.2016.00911
- Senok A, Ehrlich R, Monecke S, Al-Saedan R, Somily A. Molecular characterization of methicillin-resistant *Staphylococcus aureus* in nosocomial infections in a tertiary-care facility: emergence of new clonal complexes in Saudi Arabia. *New Microbes New Infect.* 2016;14:13–18. doi:10.1016/j.nmni.2016.07.009
- Boswih SS, Udo EE, Monecke S, et al. Emerging variants of methicillin-resistant *Staphylococcus aureus* genotypes in Kuwait hospitals. *PLoS One.* 2018;13(4):e0195933. doi:10.1371/journal.pone.0195933
- Senok A, Somily AM, Nassar R, et al. Emergence of novel methicillin-resistant *Staphylococcus aureus* strains in a tertiary care facility in Riyadh, Saudi Arabia. *Infect Drug Resist.* 2019;12:2739–2746. doi:10.2147/IDR.S218870
- Abou Shady HM, Bakr AE, Hashad ME, Alzohairy MA. *Staphylococcus aureus* nasal carriage among outpatients attending primary health care centers: a comparative study of two cities in Saudi Arabia and Egypt. *Braz J Infect Dis.* 2015;19(1):68–76. doi:10.1016/j.bjid.2014.09.005
- Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran. *Int J Infect Dis.* 2013;17(11):e949–e954.
- Goudarzi M, Seyedjavadi SS, Nasiri MJ, Goudarzi H, Sajadi Nia R, Dabiri H. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis. *Microb Pathog.* 2017;104:328–335. doi:10.1016/j.micpath.2017.01.055
- Campanile F, Bongiorno D, Borbone S, Stefani S. Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Italy. *Ann Clin Microbiol Antimicrob.* 2009;8:22. doi:10.1186/1476-0711-8-22

20. Senok AC, Somily AM, Slickers P, et al. Investigating a rare methicillin-resistant *Staphylococcus aureus* strain: first description of genome sequencing and molecular characterization of CC15-MRSA. *Infect Drug Resist.* 2017;10:307–315. doi:10.2147/IDR.S145394
21. Boswihi SS, Udo EE, Al-Sweih N. Shifts in the clonal distribution of methicillin-resistant *Staphylococcus aureus* in Kuwait Hospitals: 1992–2010. *PLoS One.* 2016;11(9):e0162744. doi:10.1371/journal.pone.0162744
22. Clinical and Laboratory Standard Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement M100-S25.* Wayne, PA, USA: CLSI; 2015.
23. British Society to Antimicrobial Chemotherapy (BSAC) (BSAC, 2013). Available from: <http://bsac.org.uk/susceptibility>. Accessed January 30, 2020.
24. Udo EE, Jacob LE, Mathew B. Genetic analysis of methicillin-resistant *Staphylococcus aureus* expressing high- and low-level mupirocin resistance. *J Med Microbiol.* 2001;50(10):909–915. doi:10.1099/0022-1317-50-10-909
25. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol.* 2003;41(12):5442–5448. doi:10.1128/JCM.41.12.5442-5448.2003
26. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol.* 2000;38(3):1008–1015. doi:10.1128/JCM.38.3.1008-1015.2000
27. Udo EE, Al-Bustan MA, Jacob LE, Chugh TD. Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning. *J Med Microbiol.* 1999;48(9):819–823. doi:10.1099/00222615-48-9-819
28. Udo EE, Jacob LE. Conjugative transfer of high-level mupirocin resistance and the mobilization of non-conjugative plasmids in *Staphylococcus aureus*. *Microb Drug Resist.* 1998;4(3):185–193.
29. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med.* 2010;7(1):e1000215. doi:10.1371/journal.pmed.1000215
30. Oliveira DC, Milheirico C, de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* type VI. *Antimicrob Agents Chemother.* 2006;50(10):3457–3459. doi:10.1128/AAC.00629-06
31. Williamson DA, Monecke S, Heffernan H, et al. High usage of topical fusidic acid and rapid clonal expansion of fusidic acid-resistant *Staphylococcus aureus*: a cautionary tale. *Clin Infect Dis.* 2014;59(10):1451–1454. doi:10.1093/cid/ciu658
32. Ellington MJ, Reuter S, Harris SR, et al. Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid and methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents.* 2015;45(5):477–484. doi:10.1016/j.ijantimicag.2015.01.009
33. Monecke S, Jatzwauk L, Muller E, et al. Diversity of SCC*mec* elements in *Staphylococcus aureus* as observed in South-Eastern Germany. *PLoS One.* 2016;11(9):e0162654. doi:10.1371/journal.pone.0162654
34. Heikinheimo A, Johler S, Karvonen L, et al. New dominant spa type t2741 in livestock-associated MRSA (CC398-MRSA-V) in finnish fattening pigs at slaughter. *Antimicrob Resist Infect Control.* 2016;5:6. doi:10.1186/s13756-016-0105-8
35. Cuny C, Abdelbary MMH, Kock R, et al. Methicillin-resistant *Staphylococcus aureus* from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans. *One Health.* 2016;2:11–17. doi:10.1016/j.onehlt.2015.11.004
36. Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehrlich R. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Vet Microbiol.* 2007;125(1–2):128–140. doi:10.1016/j.vetmic.2007.05.016

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