

Emergence of Methicillin-Resistant *Staphylococcus aureus* in the Maternity Hospital, Kuwait

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Key Words

Methicillin-resistant *Staphylococcus aureus* · Neonates · Molecular typing

Abstract

Objective: To establish the relatedness of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in the Maternity Hospital, Kuwait. **Materials and Methods:** A total of 22 MRSA were isolated from 20 neonates and 1 mother in the Special Care Unit, Maternity Hospital, Kuwait. They were characterized using antibiogram, pulsed-field gel electrophoresis (PFGE), SCCmec typing, spa typing and multi locus sequence typing (MLST), and were screened for genes encoding Panton Valentine leukocidin (PVL) and capsular polysaccharide types 5 and 8. **Results:** The isolates were resistant to cadmium acetate (n = 22 or 100%), trimethoprim (n = 13 or 59.1%), gentamicin (n = 7 or 31.8%), ciprofloxacin (n = 5 or 22.7%), erythromycin and clindamycin (n = 2 or 9.1%), tetracycline (n = 2 or 9.1%) and fusidic acid (n = 2 or 9.1%). Eight isolates contained genes for PVL while 15 and 6 carried genes for types 5 and 8 capsular polysaccharide, respectively. Molecular typing distinguished 12 clones. Ten of these clones consisted of 20 isolates belonging to ST60-SCCmec-IV-t3935 (5 isolates), ST6-SCCmec-IV-t6269 (4 isolates), ST194-SCCmec-IV-t6892 (3 isolates), ST1-SCCmec-V-t2962 (2 isolates) and 1 isolate each of ST77-SCCmec-IV-t339, ST935-SCCmec-V-t1084, ST1317-SCC-

mec-V-t1548, ST9-SCCmec-V-t5801, ST627-SCCmec-IV-t1340 and ST2148-SCCmec-IV-t2810. **Conclusion:** The study demonstrated the emergence of MRSA including novel ST60 and ST194 clones at the Maternity Hospital in Kuwait.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing global public health problem with an expanding patient population base [1]. In addition, there are increasing reports of community-associated MRSA (CA-MRSA) colonization, infections and outbreaks of infections among neonates in different countries [2–7].

Despite the endemicity of MRSA in Kuwait hospitals in the 1990s, the Maternity Hospital was free of MRSA prior to 2005 [8, 9]. The absence of MRSA at the Maternity Hospital was attributed to the fact that pregnant women admitted to the hospital for child delivery were usually not sick and only spent a few days in the hospital after delivery, thereby eliminating the risk factor for the acquisition and transmission of healthcare-associated MRSA. However, during the period between October and December 2011, MRSA were isolated from babies admitted to the Special Babies Care Unit of the Maternity Hospital. The objective for this study was to characterize these MRSA isolates to

Table 1. Characteristics of MRSA isolates from Maternity Hospital, Kuwait

Patient No.	Date	Sample	Resistance Profile	PFGE type	SCCmec	Spa type	STs	PVL
10435	5/10/11	UMB	Cd ^a , Tp	A	IV	t3935	60	-
10482	30/10/11	groin	Cd, Tp	A	IV	t3935	60	-
10577	1/12/11	groin	Cd, Tp	A	IV	t3935	60	-
10514	2/11/11	nasal	Cd,	Ab	IV	t3935	60	-
10555	30/11/11	n.s.	Cd, Tp, Tet, Mn	Ac	IV	t3935	60	-
10411	3/10/11	nasal	Cd	Aa	IV	t6892	194	+
10570	1/12/11	nasal	Cd, Gm, Km, Tp, Cip	Aa	IV	t6892	194	+
10525	2/11/11	UMB	Cd, Tp, Cip	Aa	IV	t6892	194	+
10445	1/11/11	groin	Cd	B	IV	t339	77	+
10561	28/11/11	HVS	Cd	C	IV	t6269	6	-
10553	28/11/11	n.s.	Cd	C	IV	t6269	6	-
10572	1/12/11	UMB	Cd	C	IV	t6269	6	-
10554	28/11/11	nasal	Cd	C	IV	t6269	6	-
10407	3/10/11	c/tip	Cd, Gm, Km, Tp	D	V	t2962	1	+
10434	5/10/11	blood	Cd, Gm, Km, Tp	D	V	t2962	1	+
10409	3/10/11	eye	Cd, Gm, Km, Tp, Cip	E	IV	t1084	22	+
10518	6/11/11	UMB	Cd, Gm, Km	Ea	V	t1084	935	-
10571	1/12/11	axilla	Cd, Tp, Em, Cc, Fa, Cip	Eb	V	t1548	1,317	-
10556	28/11/11	nasal	Cd, Tp	Ec	IV	t1340	627	+
10436	6/10/11	blood	Cd, Gm, Km, Fa	F	V	t5801	9	-
10438	11/10/11	nasal	Cd, Tp, Tet, Mn	G	IV	t2810	2,148	-
10575	1/12/11	axilla	Cd, Gm, Km, Tp, Em, Cc, Cip	H	III	t4410	239	-

Positive (+) and negative (-). Cc = Clindamycin; Cd = cadmium acetate; Cip = ciprofloxacin; c/tip = catheter tip; Em = erythromycin; Fa = fusidic acid; Gm = gentamicin; HVS = high vaginal swab; Km = kanamycin; Mn = minocycline; n.s. = not specified; STs = MLST sequence types; Tet = tetracycline; Tp = trimethoprim; UMB = umbilical cord. ^a Cadmium resistance is widespread in *S. aureus* where it may be linked with penicillin resistance [28, 29]. Although not a therapeutic agent, cadmium is toxic to bacteria including *S. aureus* and in response to this toxicity, the bacteria have developed resistance to cadmium [30].

identify the number and type of MRSA clones involved and to establish whether they were related to the MRSA circulating in other hospitals in Kuwait or whether they represented novel clones emerging in this hospital.

Materials and Methods

MRSA Isolates

The Maternity Hospital, Kuwait, is a 500-bed, tertiary hospital which handles 12,000–14,000 deliveries, accounting for approximately 30% of all deliveries in Kuwait [10]. During the period from 1 October to 31 December 2011, 21 MRSA isolates were obtained from 20 babies admitted to the Special Care Unit, a 150-bed facility. One MRSA isolate was obtained from a mother of one of the babies. The MRSA were isolated as part of routine microbiology diagnostic investigations and identified at the Microbiology laboratory of the Maternity Hospital using standard bacteriological techniques. The MRSA isolates were obtained from groins, umbilical stump swabs, nasal swabs, blood, axilla, eye swabs and catheter tips, all representing colonization and infection sources (table 1). The isolates were preserved in 40% glycerol in brain-

heart infusion broth (v/v) at -80°C. Further studies were performed in the Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait.

Antibiotic Susceptibility Testing

Susceptibility to benzyl penicillin, ceftiofur, kanamycin, gentamicin, erythromycin, clindamycin, chloramphenicol, tetracycline, minocycline, trimethoprim, fusidic acid, rifampicin, ciprofloxacin and linezolid was determined by the disk diffusion method [11]. Fusidic acid susceptibility was interpreted according to the British Society for antimicrobial chemotherapy guidelines [12]. Minimum inhibitory concentration was determined for oxacillin, fusidic acid and teicoplanin with Etest strips (BioMerieux, France) according to the manufacturer's instructions. The MIC for vancomycin was performed by the Etest macro method according to the manufacturer's protocol. Susceptibility to cadmium acetate (50 µg), propamidine isethionate (100 µg) and mercuric chloride (109 µg) was performed by disk diffusion as described previously [8]. *S. aureus* strain ATCC25923 was used as the quality control strain for susceptibility testing. Methicillin resistance was confirmed by detecting PBP 2a in culture supernatants using a rapid latex agglutination kit (Denka-Seiken, Japan) according to the manufacturer's instruction. Multiple resistance was defined as resistance to more than three classes of antibacterial agents.

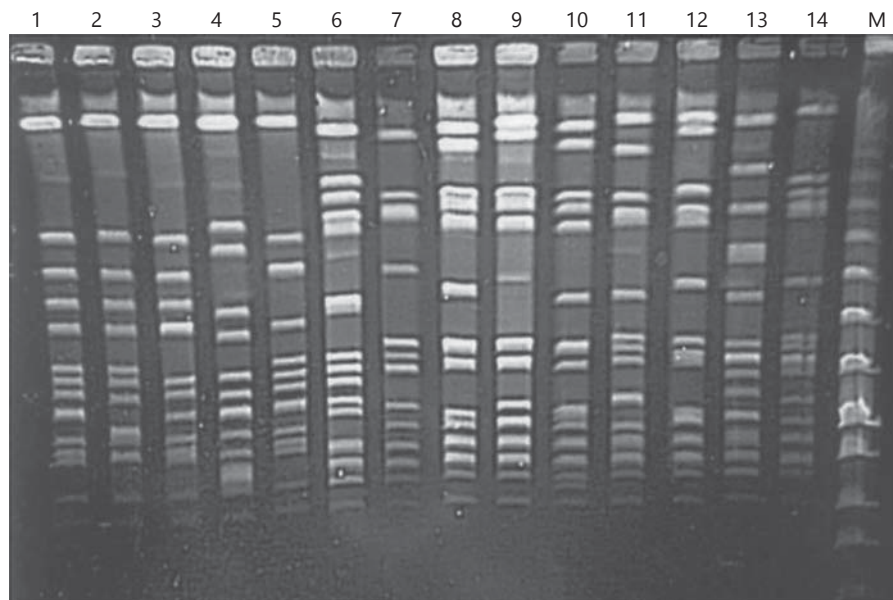


Fig. 1. PFGE patterns of representative MRSA isolates from the Maternity Hospital, Kuwait. Lanes 1–3 and 5: PFGE type A and subtypes. Lane 4: PFGE type B. Lane 6: PFGE type C. Lane 7: PFGE type D. Lanes 8–10: PFGE type E. Lane 11: PFGE type F. Lanes 12–13: PFGE type G and subtype. Lane 14: PFGE type H. Lane M: molecular weight marker.

Detection of Resistance Genes

Resistant isolates were screened for the presence of genes for aminoglycosides, macrolide and tetracycline resistance by PCR. Amplification of *aac6'-aph2'*, *ant4'* and *aph3'* was performed as described previously [13]. Amplification of macrolide resistance genes, *ermA*, *ermB* and *ermC* genes and tetracycline resistance genes was performed as described previously [8, 14].

Pulsed-field gel electrophoresis (PFGE) was performed on all 22 MRSA isolates as described previously [8].

SCCmec typing was performed as described previously [15] with the strains COL (SCCmec type I), XU642 (EMRSA-16, SCCmec type II), WBG525 (EMRSA-1, SCCmec type III), XU1839 (SCCmec type IV) and WBG8318 (SCCmec type V) as controls.

For spa typing, the polymorphic X region of the protein A gene (spa) was amplified as described previously [16]. The spa types were determined using the Ridom Staph Type software (Ridom GmbH, Wurzburg, Germany).

Multilocus sequence typing (MLST) was performed by following previously published protocols [17].

Detection of Genes for Pantan-Valentine Leukocidin and Capsular Polysaccharide

The Pantan-Valentine leukocidin (PVL) gene, *LukS-PV* – *LukF-PV*, [18] and genes for capsular polysaccharide types 5 and 8 [19] were amplified as described previously.

Results

All MRSA isolates were susceptible to linezolid, vancomycin, teicoplanin, tigecycline, rifampicin, mupirocin and chloramphenicol, but were resistant to penicillin G, ceftioxin and the antibacterial agents presented in table 1.

Of the 22 isolates, 4 (18.2%) were resistant to 3 or more classes of antibiotics and were considered multiresistant. All isolates were resistant to the heavy metal ion, cadmium acetate, and 7 (31.8%) were resistant only to cadmium acetate and β -lactam antibiotics.

The 7 gentamicin-resistant isolates were positive for *aac6'-aph2'* and *aph3'*. The 2 tetracycline-resistant isolates were positive for *tetK* and *tetM*. One of the 2 erythromycin-resistant isolates, (#10571), was positive for *ermC* while 1 (#10575) was positive for *ermA*.

Fifteen and 5 of the 22 isolates were positive for genes encoding capsular polysaccharide types 5 and 8, respectively. Eight isolates, obtained from blood, eye swab, umbilical swab, nasal swabs and catheter tip, representing infections and colonization, were positive for PVL.

PFGE identified 8 pulsed-field patterns (pulsotypes) and subtypes designated types A–H (fig. 1). Of the 22 isolates, 8 (36.4%) belonged to PFGE type A while 4 had a type C pattern. The isolates, 10,561 obtained from a mother and 105,531 from her baby had an identical PFGE pattern (type C). The 2 isolates with PFGE type D pattern were obtained from 2 different sites of the same baby.

SCCmec typing showed that 21 of the 22 isolates carried SCCmec type IV (16 isolates) or type V (5 isolates) genetic elements. One isolate carried the type III genetic element. Spa typing revealed 11 spa types with spa types t3935 (5 isolates), t6892 (3 isolates) and t6269 (4 isolates) as the more common spa types. MLST

yielded 12 sequence types with ST60, ST6, ST194 and ST1 detected in 5, 4, 3 and 2 isolates, respectively. The other sequence types were represented by single isolates.

The results of molecular typing summarized in table 1 showed that the 8 PFGE type A isolates belonged to 2 clones: ST60-SCC*mec*-IV-t3935 (5 isolates) and ST194-SCC*mec*-IV-t6892 (3 isolates) whereas the PFGE type C (ST6-SCC*mec*-IV-t6269) and type D (ST1-SCC*mec*-V-t2962) isolates were homogeneous and had the same spa and sequence types. Based on the results of SCC*mec* typing, spa typing and MLST, 20 of the 22 MRSA isolates were classified as community-associated MRSA whereas 2 isolates, 1 ST239-SCC*mec*-III and 1 ST22-SCC*mec*-IV, were classified as healthcare-associated MRSA.

Discussion

The result that 20 of the 21 babies were colonized or infected with CA-MRSA mirrors recent global trends where CA-MRSA has become a major cause of infection and outbreaks in neonatal units [2, 6, 7]. Furthermore, the isolation of the CA-MRSA isolates from blood and other clinical materials in this study supports the argument for their involvement in colonization as well as invasive infections in neonates [3, 20].

Previously CA-MRSA isolates were described as non-multiresistant MRSA because of their susceptibility to non- β -lactam agents [1]. Although most of the CA-MRSA isolates (11/20) in this study were non-multiresistant, 8 of them (40%) were multiresistant to non- β -lactam agents. Similarly, increasing numbers of CA-MRSA clones obtained at different centers are becoming multiresistant to antibacterial agents [21–23]. A remarkably high proportion (59%) of our isolates – spanning 6 genetic backgrounds – was resistant to trimethoprim. Our report, together with a recent report by Chen et al. [24] that 9% of CA-MRSA isolates obtained from children in Taiwan were trimethoprim-resistant, may signal an emerging trimethoprim resistance problem among CA-MRSA isolates.

The CA-MRSA isolates belonged to diverse genetic backgrounds, suggesting that they were acquired independently. However, the recovery of the ST60-SCC*mec*-IV, ST6-SCC*mec*-IV and ST194-SCC*mec*-IV isolates from many babies suggested that local transmission of these clones had also occurred in the unit. To the best of our knowledge, this is the first report of ST60-SCC*mec*-IV and ST194-SCC*mec*-IV CA-MRSA clones in a Kuwait hospital. Previous reports of CA-MRSA in Kuwait hospitals showed that 2 CA-MRSA clones (ST80-SCC*mec*-IV

and ST30-SCC*mec*-IV) were dominant [23, 25]. Significantly, in our study, neither of these 2 dominant clones was detected among the isolates from the Maternity Hospital. The absence of the ST60-SCC*mec*-IV and ST194-SCC*mec*-IV clones in the other hospitals in Kuwait diminishes the possibility that they originated from these hospitals and rather suggests that they were unique to the Maternity Hospital at this time.

Although the sources of the isolates were not investigated, the one mother that was sampled carried the same CA-MRSA strain as her baby, suggesting a mother-to-baby transmission. It is possible that some of the babies acquired their CA-MRSA from their mothers at birth as has been reported elsewhere [26]. Healthcare workers in the neonatal unit present a possible alternative source of the CA-MRSA isolates [27], but in our study, none of the healthcare workers was screened for carrying MRSA.

Conclusion

This study has highlighted the emergence of CA-MRSA, including novel ST60 and ST194 clones, with multiresistance and non-multiresistance phenotypes in the Maternity Hospital, Kuwait, which had previously been free of MRSA. There is, therefore, an urgent need to develop and implement a surveillance program to prevent the establishment of these clones and their spread to other units of the hospital.

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