Detection and characterization of an ST97-SCCmec-V community-associated methicillin-resistant *Staphylococcus aureus* clone in a neonatal intensive care unit and special care baby unit

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A total of 29 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were obtained from 15 neonates and three healthcare workers (HCWs) in a neonatal intensive care unit (NICU) and special care baby unit (SCBU) and four patients in a medical ward of a Kuwait hospital between 10 and 30 April 2007. The isolates were characterized using antibiogram results, coagulase gene RFLP (*coa*-RFLP), PFGE, staphylococcal cassette chromosome *mec* (SCCmec) typing and multilocus sequence typing (MLST). All isolates were assessed for the carriage of Panton–Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME) genes. The isolates belonged to three SCCmec types, six *coa*-RFLP types, six pulsotypes and six sequence types. One isolate was positive for PVL. None were positive for ACME. All MRSA isolates from the 15 neonates were phenotypically and genetically different from the MRSA isolates obtained from HCWs and those from patients in other wards. They were resistant to gentamicin, kanamycin and fusidic acid, had identical *coa*-RFLP and PFGE patterns, carried the type V SCCmec element and belonged to MLST sequence type ST97. The results showed the transmission of a rare clone of community-associated MRSA belonging to ST97 with the SCCmec-V genotype among neonates in a NICU and SCBU.

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INTRODUCTION

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) emerged in the 1990s among patients in remote communities with no access to healthcare facilities (Udo *et al.*, 1993; Groom *et al.*, 2001) and without the established risk factors for MRSA infection or colonization such as prior antibiotic use, prior hospitalization and old age (Naimi *et al.*, 2003). Since then, CA-MRSA has become a global public health problem. CA-MRSA has been isolated from different patient populations in recent years including

pregnant women and neonates (Chen *et al.*, 2006; Carey *et al.*, 2010; Gould *et al.*, 2009). CA-MRSA is increasing among children in the USA and elsewhere (Mishaan *et al.*, 2005; Song *et al.*, 2010) with increasing reports of outbreaks of infections in neonatal intensive care units (NICUs) (Mishaan *et al.*, 2005; Gould *et al.*, 2009). Outbreaks of CA-MRSA infections among neonates can be devastating because of prematurity of organ systems, low birth weight and use of invasive devices with significant morbidity and financial burden although not independently associated with increased mortality (Song *et al.*, 2010).

MRSA was not a problem in the NICU and special care baby unit (SCBU) of Farwaniya Hospital in Kuwait in the 5 years prior to 2007 as revealed by a computerized laboratory data search and infection control registry records. However, between 10 and 30 April 2007, MRSA was isolated from 15 neonates in the NICU and SCBU of

Abbreviations: ACME, arginine catabolic mobile element; CA-MRSA, community-associated MRSA; *coa*-RFLP, coagulase gene RFLP; HCW, healthcare worker; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; NICU, neonatal intensive care unit; PVL, Panton–Valentine leukocidin; SCBU, special care baby unit; SCCmec, staphylococcal cassette chromosome *mec*.

this hospital. This paper describes the molecular characterization of these MRSA isolates.

METHODS

Setting. Farwaniya Hospital is a 920-bed general hospital that serves a population of approximately 1 000 000. It has Obstetrics and Gynaecology departments that handle ~7000 deliveries annually. It has a NICU with 19 beds: 8 level III NICU beds in a single large space, 7 intermediate intensive beds in a separate room, 3 single-bed isolation rooms and a single-bed special procedures room. It is separated from the SCBU by a corridor. The standard nurse/bed ratio in the NICU is 2.5:1 and in the SCBU is 1:1.

***S. aureus* isolates.** Between 10 and 30 April 2007, 15 MRSA-positive neonates were identified in the NICU and SCBU. The colonization of two babies was first noticed on 10/4/2007 and 13/4/2007 in the NICU followed by infection in two babies (conjunctivitis, bacteraemia) on 16/4/2007 and 17/4/2007. The detection of MRSA from babies on 13/4/2007 warranted microbiological screening of all neonates, nurses, physicians and support staff in the units. Environmental surfaces including incubators, incubator windows, filters, incubator water, humidifiers, IV stands, infusion pumps, X-ray cassettes, ultrasound probes, nursing counter area, patients' files and computer keyboards were also sampled. This resulted in the detection of MRSA from 11 babies and 3 healthcare workers (HCWs; a neonatologist, staff nurse and cleaner). The medical records of all neonates were reviewed to establish history, treatment and outcomes. All isolates were identified using standard bacteriological techniques, which included Gram stain, catalase test, tube coagulase test and DNase test. Pure cultures of the isolates were preserved in 40 % (v/v) glycerol in brain heart infusion broth at -20°C .

Antibiotic susceptibility testing. Testing of susceptibility to oxacillin, benzylpenicillin, cefoxitin, kanamycin, gentamicin, erythromycin, clindamycin, chloramphenicol, tetracycline, trimethoprim, fusidic acid, rifampicin, ciprofloxacin and linezolid was determined by the disc diffusion method following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2007) with *S. aureus* strain ATCC 25923 as quality control strain. Fusidic acid susceptibility was interpreted according to the British Society for Antimicrobial Chemotherapy guidelines (BSAC, 2005). The MICs of oxacillin, fusidic acid, vancomycin and teicoplanin were determined with Etest strips. Meticillin resistance was confirmed by detecting PBP2a in culture supernatants using a rapid latex agglutination kit (Denka-Seiken) according to the manufacturer's instructions.

Molecular typing of isolates. A combination of coagulase gene typing (Goh *et al.*, 1992) and PFGE (Udo *et al.*, 1999) was used to establish the relatedness of strains. Multiplex PCR was employed to assign SCCmec types I–V as described by Zhang *et al.* (2005).

Multilocus sequence typing (MLST) was performed by following previously published protocols (Enright *et al.*, 2000).

The *lukS-PV* and *lukF-PV* genes, which encode Pantone–Valentine leukocidin (PVL), were amplified as described previously (Lina *et al.*, 1999). The presence of genes for the arginine catabolic mobile element (ACME) (*arcA*) was investigated by PCR using the primers and protocol described by Ellington *et al.* (2008).

RESULTS AND DISCUSSION

In total, 29 MRSA isolates obtained from 15 neonates (21 isolates), three HCWs (three isolates) and four inpatients

(five isolates) residing in other wards during the study period were subjected to phenotypic and genotypic typing to establish their clonal relatedness.

All 29 isolates were positive for PBP2a, and were resistant to penicillin G, oxacillin (MIC $48\text{--}>256\text{ mg l}^{-1}$) and the antibacterial agents shown in Table 1. All isolates were susceptible to vancomycin and teicoplanin (MIC $\leq 2\text{ mg l}^{-1}$), linezolid and rifampicin. All MRSA isolates from colonized or infected babies were resistant to gentamicin, kanamycin and fusidic acid (MIC 8 mg l^{-1}), which was different to the resistance profiles of isolates obtained from HCWs and the patients in other wards.

SCCmec typing revealed that the MRSA isolates from the 15 babies contained the SCCmec type V genetic element, which is a characteristic feature of CA-MRSA. The three MRSA isolates from HCWs contained a type IVa SCCmec element while isolates from patients in other wards carried type IV, IVa and III genetic elements.

Coagulase gene typing and PFGE classified the 29 isolates into six separate groups. As shown in Table 1, all MRSA isolates from the babies had the same *coa*-RFLP and PFGE patterns. Isolates from HCWs showed two patterns while the five MRSA isolates from other inpatients defined three *coa*-RFLP and PFGE patterns.

MLST was performed on eight isolates consisting of three isolates representing PFGE pattern A (isolates were from blood, nose and catheter tip) and one isolate representing each of the remaining five PFGE patterns. The results showed that the representatives of MRSA from the babies belonged to sequence type 97 (ST97) and were different from MRSA from HCWs and MRSA isolated from other patients.

None of the MRSA isolates from the babies were positive for PVL genes. Only one MRSA isolate, obtained from one of the patients in a different ward, was positive for PVL genes (Table 1). None of the isolates were positive for genes encoding the ACME.

These results indicated a nosocomial transmission of a PVL-negative, ST97-MRSA-SCCmec-V clone among 15 neonates in a NICU and SCBU in a Kuwaiti hospital. Although the number of reports associating CA-MRSA carrying the SCCmec type IV or V genetic element with infections in NICUs has increased worldwide (Mishaan *et al.*, 2005; Song *et al.*, 2010; Gould *et al.*, 2009), to our knowledge, this is the first report associating ST97-MRSA with nosocomial transmission in a neonatal unit. It is also the first report, to our knowledge, of CA-MRSA with the SCCmec type V genetic element in a Kuwait hospital. CA-MRSA isolates reported previously in Kuwait hospitals all carried the SCCmec IV genetic element (Udo *et al.*, 2008).

Prior to this report, ST97-MRSA was reported sporadically in human infections. A few isolates of ST97-CA-MRSA have been reported in the USA (Chung *et al.*, 2004), UK (Ellington *et al.*, 2008), Sweden (Fang *et al.*, 2008), Brazil

Table 1. Characteristics of MRSA isolates

Isolate	Site*	Resistance pattern†	MIC (mg l ⁻¹)	coa-RFLP	PFGE	SCCmec	PVL	ST
Neonates								
N1	UVC tip	Gm, Km, Fa	48	104	A	V	—	97
N1	ETT tip	Gm, Km, Fa	48	104	A	V	—	
N2	UAC tip	Gm, Km, Fa	48	104	A	V	—	
N2	UVC tip	Gm, Km, Fa	48	104	A	V	—	
N3	Eye discharge	Gm, Km, Fa	48	104	A	V	—	
N4	Blood	Gm, Km, Fa	48	104	A	V	—	
N4	Blood	Gm, Km, Fa	48	104	A	V	—	97
N5	Nose	Gm, Km, Fa	48	104	A	V	—	
N6	Umbilicus	Gm, Km, Fa	48	104	A	V	—	
N7	Nose	Gm, Km, Fa	48	104	A	V	—	
N7	Umbilicus	Gm, Km, Fa	48	104	A	V	—	
N8	Nose	Gm, Km, Fa	48	104	A	V	—	
N9	Axilla	Gm, Km, Fa	48	104	A	V	—	
N10	Groin	Gm, Km, Fa	48	104	A	V	—	
N11	Nose	Gm, Km, Fa	48	104	A	V	—	
N11	Pustule	Gm, Km, Fa	48	104	A	V	—	
N12	Groin	Gm, Km, Fa	48	104	A	V	—	
N13	Nose	Gm, Km, Fa	48	104	A	V	—	97
N13	Umbilicus	Gm, Km, Fa	48	104	A	V	—	
N14	Umbilicus	Gm, Km, Fa	48	104	A	V	—	
N15	Umbilicus	Gm, Km, Fa	48	104	A	V	—	
HCWs‡								
SN1	Nose	Cip	64	20	B	IVa	—	22
MD1	Nose	—	64	16	C	IVa	—	1
CL	Nose	—	64	16	C	IVa	—	1
Patients in other wards								
Pt 1	Nose	Km, Sm, Em, Clin, Tet	>256	36	D	IVa	—	5
Pt 2	Wound	Km, Sm, Tet, Fa	48	768	E	IV	+	80
Pt 3	Blood	Gm, Km, Sm, Em, Clin, Tet, Fa, Cip	>256	68	F	III	—	239
Pt 3	Nose	Gm, Km, Sm, Em, Clin, Tet, Fa, Cip	>256	68	F	III	—	239
Pt 4	Groin	Gm, Km, Sm, Em, Clin, Cm, Tet, Fa, Cip	>256	68	F	III	—	239

*ETT, Endotracheal tube; UAC, umbilical arterial catheter; UVC, umbilical venous catheter.

†Cip, ciprofloxacin; Clin, clindamycin; Cm, chloramphenicol; Em, erythromycin; Fa, fusidic acid; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Tet, tetracycline.

‡SN, Staff nurse; MD, clinician; CL, cleaner.

(Schuenck *et al.*, 2009) and, recently, Lebanon (Tokajian *et al.*, 2010). These ST97-CA-MRSA isolates differed phenotypically and genetically from our isolates. Phenotypically, whereas the isolates in this study were resistant to gentamicin, kanamycin and fusidic acid, the USA isolates were resistant to clindamycin and tetracycline while isolates from the UK were resistant to trimethoprim and ciprofloxacin. The isolate from Brazil was resistant to rifampicin. No resistance was reported for the isolate from Lebanon. Genetically, while the ST97 isolates obtained in the USA, Brazil and Sweden contained the type IV SCCmec element, the isolates obtained in this study, the UK and Lebanon carried the type V SCCmec element. However, all ST97 isolates reported so far have lacked genes for PVL. The only PVL-gene-positive isolate in this study was obtained from a

patient in a different ward and was an ST80 clone which has been reported previously in Kuwait hospitals (Udo *et al.*, 2008; Udo & Sarkhoo, 2010). Whereas the ST97-MRSA-SCCmec-V isolates reported in the UK contained the ACME genes (Ellington *et al.*, 2008), those reported in this study were negative.

The source of the ST97 isolates could not be identified. No ST97 isolate was obtained from patients in other areas of the hospital before or after this episode. Although HCWs have contributed to the nosocomial transmission of MRSA elsewhere (Ben-David *et al.*, 2008; Eveillard *et al.*, 2004), the results of this study could not associate the HCWs screened directly with the current outbreak. MRSA carried by the HCWs was unrelated to the ST97 isolates.

Furthermore, our results could not associate the environment with the transmission of the outbreak strain. We could not rule out maternal transmission since none of the mothers were screened for MRSA carriage. Transmission of MRSA from parents to their infants in the NICU has been reported previously (Al-Tawfiq, 2006).

Interestingly, ST97-MRSA has also been isolated from cows, where it caused bovine mastitis (Smyth *et al.*, 2009; Sung *et al.*, 2008), suggesting that it may be a livestock-associated MRSA clone.

In conclusion, our results suggest a nosocomial transmission of a gentamicin-resistant, PVL-negative ST97 CA-MRSA clone with a SCCmec type V genetic element among 15 babies in a NICU and SBCU. However, rapid intervention with adherence to hand hygiene, proper contact precautions, repeated surveillance cultures, successful decolonization of babies and direct observations of infection control practices were crucial factors for rapid containment of the outbreak.

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