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Genetic Lineages of Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Kuwait Hospitals[▽]

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Twenty-six community-associated methicillin-resistant *Staphylococcus aureus* (CAMSRA) isolates were characterized by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) and screened for accessory gene regulator (agr), capsular polysaccharide (cap), and Panton-Valentine leucocidin (PVL) genes. They exhibited five PFGE patterns (types A to E). The majority were PFGE type A (12 isolates) or type B (8 isolates). MLST showed that PFGE type A isolates belonged to sequence type 80 (ST80), while the PFGE type B isolates were ST30. The ST80 and ST30 clones contained agr allotype 3, cap type 8, and PVL. The results showed that two internationally recognized CAMRSA clones are dominant in Kuwait hospitals.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially associated with large health care facilities such as teaching hospitals, nursing homes, and long-term care facilities (1) but have now appeared in community settings (3, 9, 10, 12, 14, 15). These MRSA strains, described as community-acquired or community-associated MRSA (CAMRSA), were initially isolated from individuals residing in remote communities with no access to health care centers (9, 12, 14). However, they are now increasingly isolated from patients in health care environments (9, 10, 12). CAMRSA strains are characterized by their susceptibility to a wide range of non-beta-lactam antibiotics, low methicillin MICs, and the carriage of the type IV or V staphylococcal cassette chromosome *mec* (SCC*mec*) element. In contrast health care-associated MRSA strains are usually multiresistant to non-beta-lactam antibiotics and contain type I, II, or III SCC*mec* elements (1, 13).

The genotype distribution of CAMRSA differs in different geographical regions. Recent reports show that, while the USA300 CAMRSA clone (sequence type 8 [ST8] MRSA, SCC*mec* type IV [ST8-SCCmec-IV]) is dominant in the United States (10), the ST80-SCC*mec*-IV and ST30-SCC*mec*-IV clones are dominant in Europe (3, 15) and the ST30-SCC*mec*-IV clone is dominant in New Zealand, the southwest Pacific islands, and Singapore (4, 11).

CAMRSA isolates obtained in Kuwait hospitals were previously characterized by antibiotic resistance patterns and SCC*mec* typing (13), but their sequence types were not determined. Consequently, it was not possible to ascertain whether the CAMRSA strains isolated in Kuwait hospitals constituted indigenous or imported internationally recognized clones. The aim of this study was to determine the genetic lineages and the accessory gene regulator (agr) and capsular polysaccharide (cap) types of CAMRSA strains obtained from patients in Kuwait hospitals to ascertain their relatedness to CAMRSA clones isolated elsewhere. This is important because the population of Kuwait includes a large component of expatriate workers from different countries, which consequently provides opportunities for the dissemination of international bacterial clones.

A total of 26 CAMRSA isolates previously characterized on the basis of SCC*mec* typing were selected for this study. They were among 1,457 MRSA isolates submitted to the MRSA Reference Laboratory for typing and were isolated between July 2001 and October 2003. They were from 7 outpatients and 19 inpatients in seven hospitals (Table 1). Only one patient was an expatriate worker from the Philippines. The rest were Kuwait nationals. They represented five pulsed-field gel electrophoresis (PFGE) patterns commonly encountered among CAMRSA strains in these hospitals (Fig. 1). Their susceptibility to antibiotics and SCC*mec* types were described previously (13). The fusidic acid-resistant isolates were investigated for the carriage of the fusidic acid resistance determinant *far-1* by PCR as described previously (15), and fusidic acid MICs were determined using the Etest. SCC*mec* subtyping was performed by the method of Zhang et al. (16) and included WBG8318 (8) as a control for SCC*mec* type V. The subtypes were assigned based on the

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TABLE 1. Characteristics of CAMRSA isolates

Strain	Hospital[a]	Source	MIC[b] (μg/ml)	SCC*mec* type	ST	CC[c]	agr type	cap type	PVL	PFGE type	Resistant to[d]:
K1814	ADH	Groin	8	IV	80	80	3	8	+	A	Km, Tc, Fd
K1839	JH	Wound	24	IVa	5	5	2	5	−	D	Em, Cd
K1847	AMH	Abscess	32	IVc	30	30	3	8	+	B1	Cd
K1938	MAK	Wound	48	IV	30	30	3	8	+	B	Cd
K1943	ARH	Wound	16	IV	728	80	3	8	+	A	Km, Tc, Fd, Cd
K2005	MAK	Groin	32	IV	30	30	3	8	+	B	Cd
K2076	ASH	Wound	32	IVa	8	8	1	5	−	C2	Cd
K2086	ASH	Wound	24	IV	80	80	3	8	+	A	Km, Fd, Cd
K2116	MAK	Wound	32	IV	30	30	3	8	+	B	Cd
K2129	ASH	Skin	8	IV	80	80	3	8	+	A2	Tc, Fd, Cd
K2141	MAK	Wound	32	IV	30	30	3	8	+	B	Cd
K2220	AMH	Wound	32	IV	30	30	3	8	+	B	Cd
K2402	AMH	Wound	8	IV	80	80	3	8	+	A3	Tc, Fd, Cd
K2408	ADH	Wound	8	IV	30	30	3	8	+	B	Cd
K2442	AMH	Wound	16	IV	30	30	3	8	+	B	Cd
K2499	ADH	Skin	16	IV	80	80	3	8	+	A	Km, Tc, Fd, Cd
K2565	AMH	Wound	24	IV	80	80	3	8	+	A	Km, Tc, Fd, Cd
K2600	ASH	Nose	48	IV	8	8	1	5	−	C	Cd
K2656	ARH	Wound	24	IV	361	UD	1	5	−	E	Km, Cd
K2700	ADH	Eye	8	IV	728	80	3	8	+	A1	Km, Fd, Cd
K2827	ASH	Wound	8	IV	80	80	3	8	+	A	Tc, Fd, Cd
K2917	ARH	Wound	32	IV	80	80	3	8	+	A	Km, Tc, Fd, Cd
K2945	MAK	Groin	16	IV	80	80	3	8	+	A	Km, Fd, Cd
K2979	ISH	Nose	16	IV	5	5	2	5	−	D	Cd
K3290	ADH	Skin	24	IV	80	80	3	8	+	A	Km, Tc, Fd, Cd
K3471	ADH	Skin	16	IV	6	5	2	5	−	C1	Em, Tc, Tp, Cd

[a] ADH, Adan Hospital; ASH, Al-Sabah Hospital; AMH, Amiri Hospital; ARH, Al-Razi Hospital; JH, Jahra Hospital; MAK, Mubarak Hospital.
[b] Methicillin MIC.
[c] CC, clonal complex.
[d] Cd, cadmium acetate; Km, kanamycin; Em, erythromycin; Tc, tetracycline; Tp, trimethoprim; Fd, fusidic acid.

sizes of the amplified products (16). The *agr* (7) and *cap* (6) types were determined by PCR using primers and protocols described previously. The detection of genes for *agr* types I, II, III, and IV was performed by PCR using the primers and conditions described by Lina et al. (6). The Panton-Valentine leucocidin (PVL) genes *lukS-PV* and *lukF-PV* were detected by PCR using primers described previously (5), with WBG10049 (8) as a positive control. Multilocus sequence typing (MLST) was done as described previously (2).

All 26 isolates expressed low-level methicillin resistance (MIC: 8 to 32 μg/ml) (13) and were resistant to the agents shown in Table 1. The fusidic acid MIC for all fusidic acid-resistant isolates was 8 mg/liter, and each of these isolates yielded a 900-kb amplified product in PCR experiments, consistent with carriage of the *far-1* gene (15). All 26 isolates belonged to SCC*mec* type IV (13). However, subtyping using the method of Zhang et al. (16) indicated that two isolates were SCC*mec* type IVa and one isolate was SCC*mec* type IVc (Table 1). None was SCC*mec* type V. All PFGE type A isolates were SCC*mec* type IV, whereas those with SCC*mec* subtypes IVa and IVc had PFGE pattern B or C. All PFGE type A and B isolates were agr type III and cap type 8. The rest exhibited different agr and cap types.

MLST revealed seven sequence types. Ten (38.5%) isolates were ST80, and 8 isolates (30.8%) were ST30. The rest belonged to ST8 (2 isolates, 7.7%), ST5 (2 isolates, (7.7%), ST728 (2 isolates, 7.7%), or ST6 or ST361 (1 isolate each) (Table 1).

The application of MLST to type CAMRSA isolated in Kuwait hospitals has helped define their relationship to isolates obtained in other countries. It identified two sequence types, ST80 and ST30, as the dominant CAMRSA clones in Kuwait hospitals. Both sequence types constituted 76.9% of the isolates from six of the seven hospitals with CAMRSA, establishing their dominance in these hospitals. The other clones were isolated less frequently. Our ST80 isolates contained genes for PVL, belonged to agr type III, and had type 8 capsular polysaccharide. Furthermore, they contained *far-1*-mediated fusidic acid resistance similar to that of the ST80 MRSA IV clone isolated in Germany and other European countries (3, 15). The ST30 MRSA IV clone in this study was similar to the ST30 isolates from Singapore in their susceptibility to non-beta-lactam agents, but, whereas the majority of ST30 isolates from Singapore were SCC*mec* type IVc (4), only one of our ST30 isolates was SCC*mec* type IVc. indicating that internationally recognized clones of CAMRSA are dominant in Kuwait hospitals. These CAMRSA clones could have been introduced into Kuwait hospitals by Kuwaiti nationals following medical treatment abroad or by expatriate workers from countries where these clones are common. Although there were no records of overseas travel for any of the

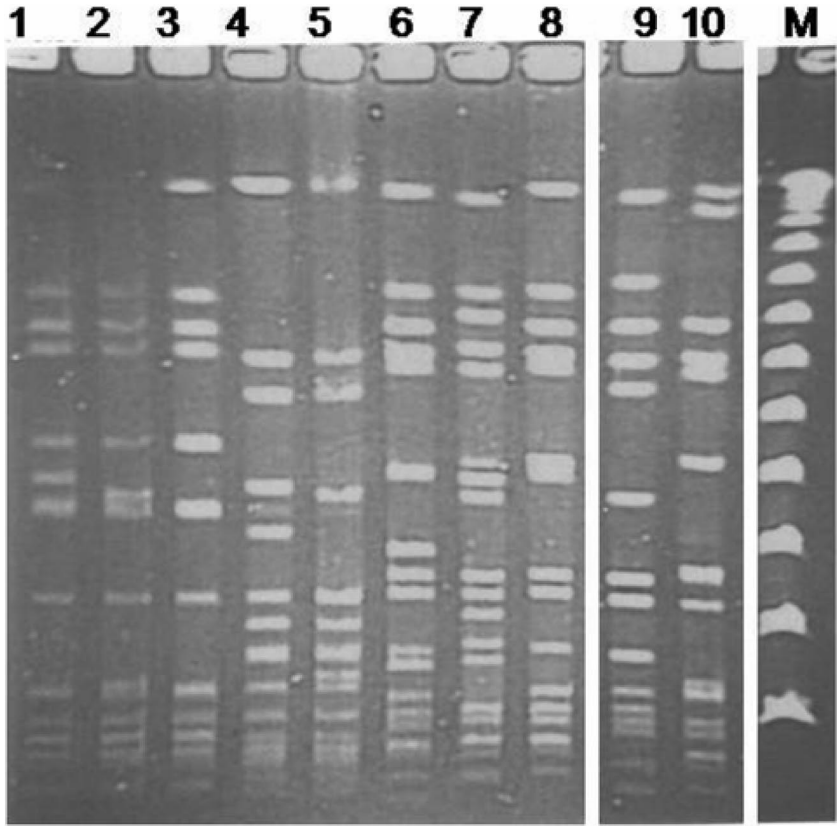

FIG. 1. PFGE of representative CAMRSA isolates. Lane 1, PFGE type A, ST80; lane 2, PFGE type A1, ST80; lane 3, PFGE type A2, ST80; lane 4, PFGE type B, ST30; lane 5, PFGE type B1, ST30; lane 6, PFGE type C, ST8; lane 7, PFGE type C1, ST6; lane 8, PFGE type C2, ST8; lane 9, PFGE type D, ST5; lane 10, PFGE type E, ST361; lane M, molecular size marker.

Kuwait nationals, many Kuwait patients do seek medical treatment abroad.

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