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Type V Staphylococcal Cassette Chromosome *mec* in Community Staphylococci from Australia

F. G. O'Brien,[1]* G. W. Coombs,[1,2] J. C. Pearson,[2] K. J. Christiansen,[1,2] and W. B. Grubb[1]

Gram-Positive Bacteria Typing and Research Unit, Molecular Genetics Research Unit, School of Biomedical Sciences, Curtin University of Technology,[1] and Department of Microbiology and Infectious Diseases, Royal Perth Hospital,[2] Perth, Western Australia, Australia

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Twenty Australian community staphylococci harboring the type V staphylococcal cassette chromosome *mec* (SCC*mec*) were found to belong to eight multilocus sequence types. Five were previously unreported novel type V SCC*mec* elements. The *mec* complexes were of two types, based on the polymorphisms in the IS*431* transposase genes. Five isolates were multiresistant.

Community methicillin-resistant *Staphylococcus aureus* (CMRSA) isolates are emerging as significant pathogens in communities. Unlike the health care-associated methicillin-resistant *S. aureus* (HMRSA) isolates, CMRSA isolates are typically resistant to few non-beta-lactam antibiotics. Resistance to methicillin is mediated by the *mec* complex, which is encoded on a genomic resistance island called the staphylococcal cassette chromosome *mec* (SCC*mec*) (6). SCC*mec* is the genetic element most responsible for stable multiple-drug resistance in HMRSA and is characterized according to the class of *mec* complex and the type of cassette chromosome recombinases (*ccr*) that it harbors. Five types of SCC*mec* have been characterized (4, 6). Types I, II, and III are harbored by HMRSA isolates, while type IV occurs mostly in CMRSA isolates (11). In Australia, however, CMRSA isolates also harbor type V SCC*mec*, which encodes the class C2 *mec* complex, and the type 5 *ccr* gene, *ccrC* (4). In class C2 *mec* complex isolates, the *mecA* regulatory genes *mecI* and most of *mecR1* are deleted by the insertion sequence IS*431*. This type of deletion was first described in clinical isolates of the coagulase-negative staphylococci (CoNS) *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* (5). This study has investigated CMRSA and community CoNS isolates from Australia that harbor SCC*mec* elements that encode either the class C2 *mec* complex and *ccrC* (type V) or unusual combinations involving these elements (novel type V).

Twenty staphylococci (17 methicillin-resistant *S. aureus* [MRSA] isolates, 2 *S. haemolyticus* isolates [WBG10504 and WBG10505], and 1 *S. epidermidis* isolate [WBG10506]) isolated from different people in four states of Australia (Western Australia [WA], South Australia, Victoria, and Queensland) were investigated (Table 1). Two of the MRSA isolates (WBG8318 and WBG8404) and the three CoNS isolates were isolated from people in remote communities in WA in 1995. Isolates WCH100, IMVS67, RPH74, and RBH87 were obtained during a national multicenter surveillance study conducted in 2000. The remaining isolates were from WA and included a 2004 isolate from a nasal screening swab (isolate 04-17116) and isolates from clinical samples collected during 2003 and 2004 either from people in the community or from hospital patients who had been in hospital for less than 48 h.

Antimicrobial susceptibility testing was performed according to the CLSI (formerly the NCCLS) recommendations (8). Multiresistance was classified according to previously published guidelines (12). Oxacillin MICs were determined by Etest (AB Biodisk, Solna, Sweden). The *mecA* gene was detected by using the primers mecAup (5′-TGCTATCCACCC TCAAACAGG-3′) and mecAdn (5′-AACGTTGTAACC ACCCCAAG-3′) (5). Multilocus sequence typing (MLST) (2) and contour-clamped homogeneous electric field (CHEF) electrophoresis were performed as described previously (10). The SCC*mec* elements were typed by PCR; for the class C2 *mec* complex, the primers were mA (7) and IS2 (7); and for *ccrC*, the primers were γF and γR (4). The Panton Valentine leukocidin (PVL) determinant was detected by PCR (3), and its presence was confirmed by sequencing.

The results are summarized in Table 1. All the staphylococci contained *mecA* and, except for *S. haemolyticus* WBG10504, expressed oxacillin resistance. The MICs for oxacillin ranged from 0.25 mg/liter to >256 mg/liter. Five of the CMRSA isolates (03-16672, 03-17796, 04-17451, 04-16419, and 04-17116) were multiply resistant (12) (Table 1).

The PVL determinant was present only in the three clonally related sequence type 59 (ST59) isolates.

Sixteen staphylococci harbored SCC*mec* type V, and there were four novel type V SCC*mec* elements. Isolates 04-17489, RBH87, WBG10198 and WBG8404, harbored the type 5 *ccrC* determinant with *mec* complexes of classes B, B, E, and B1 (7), respectively, and 04-16419 encoded a class C2 *mec* complex with a type 2 *ccr*. This suggests that genetic recombination involving the *mec* complex has occurred in these CMRSA isolates.

The type V elements were found in eight unrelated MLST genetic lineages, two unrelated strains of *S. haemolyticus* (CHEF pattern similarity of 59%), and one *S. epidermidis* strain. This widespread dissemination of the type V elements across genetic backgrounds and species barriers supports the concept that they are mobile (13). ST5, ST8, ST129, ST30, and

* Corresponding author. Mailing address: Curtin University of Technology, GPO Box U1987 Perth, Western Australia 6845, Australia. Phone: 61 8 92240302. Fax: 61 8 92240303. E-mail: F.G.O'Brien@curtin.edu.au.

TABLE 1. Phenotypic and genotypic properties of the community methicillin-resistant staphylococci investigated in this study[a]

Isolate	Site	Resistance	Ox MIC (mg/liter)	CHEF type	MLST		SCC*mec*				PVL
					ST, allelic profile	CC	Type	*ccr* type	*mec* complex	C2 subgroup	
Community methicillin-resistant *S. aureus*											
03-16918	Wound	Ox, Tp	32	WA10	573, 1-1-1-1-12-1-1	1	V	5	C2	2	Neg
WCH100	Wound	Ox, G	8	Unique	152, 46-75-49-44-13-68-60	S	V	5	C2	2	Neg
03-16672	Wound	Ox, E, T, C	8	WA9	59, 19-23-15-2-19-20-15	59	V	5	C2	2	Pos
04-16811	Wound	Ox, E, T	4	WA9	59, 19-23-15-2-19-20-15	59	V	5	C2	2	Pos
04-17021	Skin	Ox, E, T	4	WA9	59, 19-23-15-2-19-20-15	59	V	5	C2	2	Pos
03-17833	Knee fluid	Ox, G	4	WA11	5, 1-4-1-4-12-1-10	5	V	5	C2	2	Neg
03-17796	Wound	Ox, T, F, R, Tp	16	WA14	5, 1-4-1-4-12-1-10	5	V	5	C2	2	Neg
04-17451	Wound	Ox, G, E, T, Tp	32	WA14	5, 1-4-1-4-12-1-10	5	V	5	C2	2	Neg
*04-17116	Nose	Ox, T, Tp, F	32	WA14	5, 1-4-1-4-12-1-10	5	V	5	C2	2	Neg
IMVS67	Urine	Ox, T	>256	WA12	8, 3-3-1-1-4-4-3	8	V	5	C2	2	Neg
RPH74	Bite on hand	Ox	4	WA4	45, 10-14-8-6-10-3-2	45	V	5	C2	1	Neg
*WBG8318	Skin	Ox	4	WA4	45, 10-14-8-6-10-3-2	45	V	5	C2	1	Neg
04-17489	Wound	Ox	32	WA15	59, 19-23-15-2-19-20-15	59	Novel	5	B	NA	Neg
04-16419	Ulcer	Ox, G, E, T, Tp	32	WA20	5, 1-4-1-4-12-1-10	5	Novel	2	C2	2	Neg
RBH87	Sputum	Ox, E, Cp	16	WA2	129, 22-1-14-41-12-53-31	78	Novel	5	B	NA	Neg
WBG10198	Unknown	Ox, C	2	WSPP	30, 2-2-2-2-6-3-2	30	Novel	5	E	NA	Neg
*WBG8404	Skin	Ox	4	WA4	45, 10-14-8-6-10-3-2	45	Novel	5	B1	NA	Neg
Coagulase-negative staphylococci											
*WBG10504	Skin	Ox, Tp	0.25	SH-A	NA	NA	V	C2	5	2	NA
*WBG10505	Skin	Ox, T	>256	SH-B	NA	NA	V	C2	5	2	NA
*WBG10506	Skin	Ox	32	SE-A	NA	NA	V	C2	5	2	NA

[a] Abbreviations: Ox, oxacillin; Tp, trimethoprim; G, gentamicin; E, erythromycin; T, tetracycline; C, chloramphenicol; F, fusidic acid; Cp, ciprofloxacin; NA, not applicable; Neg, negative; Pos, positive; S, singleton; CC, clonal complex. Asterisk indicates carriage isolates.

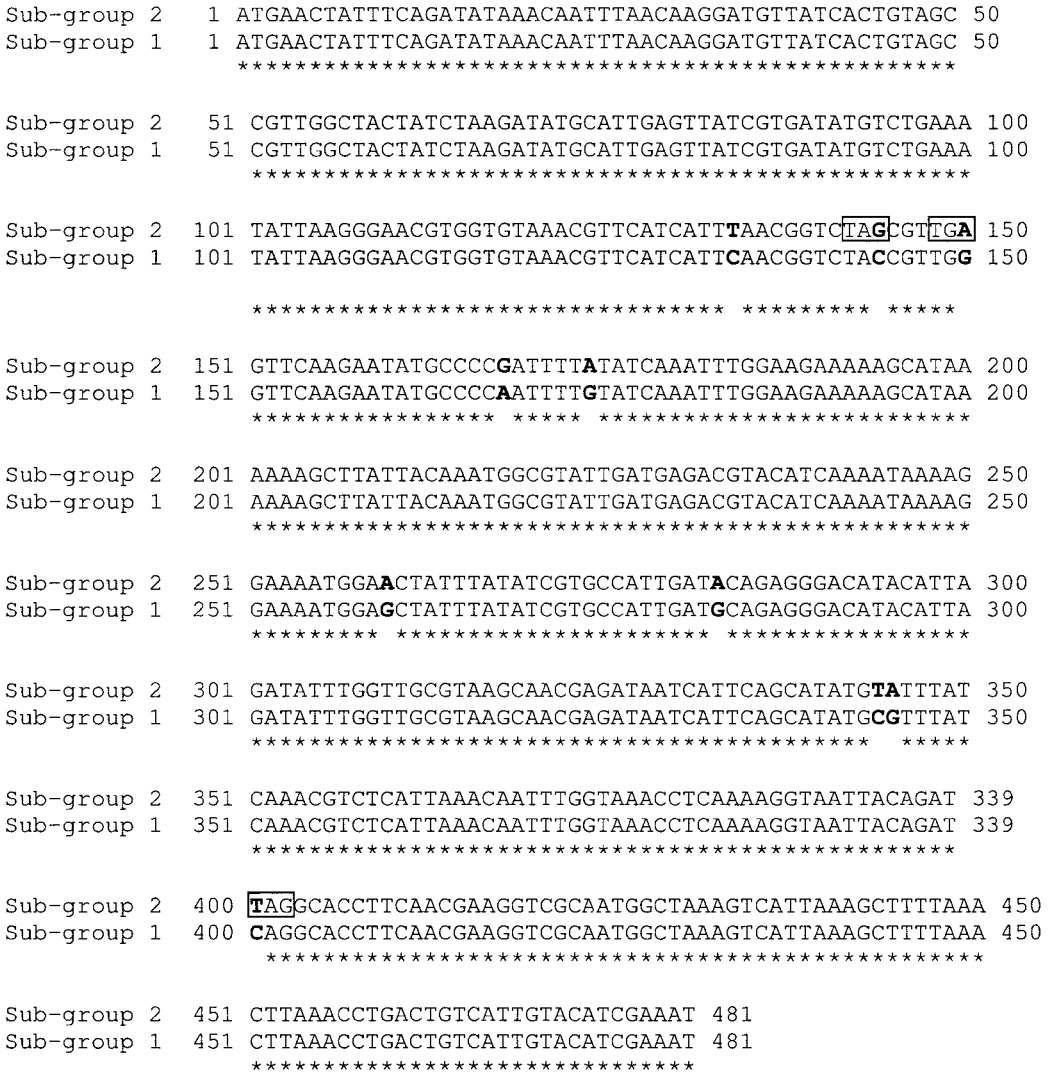

FIG. 1. ClustalW alignment of the 5′ 481 nucleotides of the truncating IS*431* transposase gene. Nucleotide positions are indicated at the ends. Identical nucleotides are indicated by an asterisk; nonidentical nucleotides are in boldface and are indicated by a gap. Stop codons are boxed.

ST45 are well-established lineages of CMRSA in Australia, in which the majority of isolates except for ST45 harbor type IV SCC*mec* elements (1, 9). The presence of SCC*mec* type V elements in these lineages suggests that acquisition and recombination events are involved in the emergence of CMRSA in Australia.

The C2 *mec* complex was amplified with primers for the 5′ terminus of *mecA* and the left-hand end of the IS*431* that has deleted *mecI* and truncated *mecR1*. The resulting amplicons were sequenced. In each of these, *mecR1* was truncated by IS*431* at nucleotide (nt) 93. One nucleotide downstream of the *mecR1* truncation site was a 13-bp imperfect inverted repeat (IR) sequence of nt 94-CaAAATaTTATGT |c|ACATAAgATTTgG-nt 120 (lowercase nucleotides are noncomplementary). This is 1 bp downstream from a previously reported natural truncation site found in class C2 *mec* complexes (5). The close proximity of these sites to the IR suggests that the structure may provide a site where IS*431* truncation of *mecR1* is more likely to occur.

There were 10-nt polymorphisms in the transposase gene (*tnp*) of the IS*431* element truncating the C2 *mec* complexes (Fig. 1). This differentiated the isolates with type V SCC*mec* elements into two subgroups. The ST45 CMRSA isolates were in subgroup 1, with the remainder belonging to subgroup 2 (GenBank accession number DQ171730). Polymorphisms at nucleotide positions 144, 150, and 400 of the subgroup 2 *tnp* sequences have created three in-frame stop codons of TAG, TGA, and TAG, respectively. These polymorphisms would almost certainly stabilize the *mec* complex by ensuring that an active transposase is not produced. The more widespread distribution of SCC*mec* type V, subgroup 2 (five lineages of CMRSA isolates and three unrelated CoNS isolates), supports this hypothesis and also suggests that the subtype is more readily disseminated.

This study has shown not only that there is diversity among the community staphylococci that harbor the SCC*mec* type V and novel type V elements but also that there is structural and genetic diversity within the elements themselves. It is of con-

cern that some of the CMRSA isolates are now multiply resistant. With CMRSA threatening to become a substantial health problem in the general community, where it will be very difficult to control its dissemination, it is now important that health authorities develop surveillance and antibiotic prescribing policies that will restrict both the spread of CMRSA isolates in the community and their acquisition of further resistance determinants.

Nucleotide sequence accession number. The partial sequence of the nonfunctional IS*431* transposase was submitted to GenBank and assigned accession number DQ171730.

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