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Diversity among Community Isolates of Methicillin-Resistant *Staphylococcus aureus* in Australia

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Community methicillin-resistant *Staphylococcus aureus* (CMRSA) strains are being isolated with increasing frequency around the world. In Western Australia CMRSA are endemic in geographically remote communities and have been found to belong to five different contour-clamped homogeneous electric field (CHEF) electrophoretic patterns. Representatives of each of these CHEF patterns have been compared to CMRSA representative of CHEF patterns from other Australian states and New Zealand. With one exception, all of the isolates were nonmultiresistant and were not resistant to many antimicrobial agents other than the β-lactams. With one exception, which is not believed to be a CMRSA, all of the isolates harbored a β-lactamase plasmid. Erythromycin resistance was associated with a 2-kb plasmid. One of the β-lactamase plasmids was found to be able to acquire additional resistance determinants to become a multiple resistance plasmid. There were 10 multilocus sequence types belonging to eight distantly related clonal complexes of *S. aureus*. One new sequence type was found. Although most of the CMRSA harbored the type IVa SCC*mec*, a type IV structural variant was found and two new SCC*mec* types were identified. Protein A gene (*spa*) typing revealed two new *spa* types and, with two exceptions, corresponded to multilocus sequence typing. In contrast to other reports on CMRSA, most of the CMRSA strains studied here did not contain the Panton-Valentine leukocidin genes. The results also demonstrate that nonmultiresistant hospital strains such as UK EMRSA-15 may be able to circulate in the community and could be mistaken for CMRSA based on their resistance profiles.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen that has been historically associated with hospitals and health care facilities (22). However, there are increasing reports of MRSA being isolated from the community (10, 27). Two types of MRSA have been isolated from communities: multiresistant (2, 47) and nonmultiresistant (29) MRSA. The former appear to have been transferred from hospitals or health care facilities by patients or health care workers and spread to close contacts (4), whereas the latter have been isolated from people who have had little or no contact with health care facilities or workers (43). We consider these isolates to be true community MRSA (CMRSA). The earliest CMRSA were isolated from people belonging to minority groups and living in disadvantaged communities (8, 15, 25, 56). These strains have since been isolated from the general community, including children (16, 17, 45) and members of close contact sporting groups (6) and from a prison population (7).

In Western Australia (WA) all MRSA that have been isolated since 1982 have been referred to a reference center for epidemiological typing and storage. A report of the first Australian CMRSA was published in 1993. These strains were isolated from people in the remote and sparsely populated Kimberley region of WA, which is located ca. 2,000 km north of the capital city of Perth. These people have little or no contact with urban communities or health facilities. The strains became collectively known as WA MRSA (35, 56). Initially, the Kimberley region had the highest notification rate for WA MRSA, but these organisms are now endemic in most remote communities throughout the state (38). Surveillance data has shown that the isolation of these strains has increased dramatically since 1991 (39).

Like other CMRSA, WA MRSA are typically nonmultiresistant. However, some WA MRSA isolates obtained from people living in the Kimberley region were found to be multiresistant due to the acquisition of a 41.4-kb plasmid that encoded β-lactamase (Bla) production and resistance to mupirocin, tetracycline, trimethoprim, and cadmium (35, 55). The number of WA MRSA that were mupirocin-resistant peaked at 18% of WA MRSA isolates (37) until guidelines restricting the use of mupirocin lowered the rate to 0.3% 3 years later (49).

Nonmultiresistant MRSA have also been reported in the Northern Territory (NT) of Australia, and it was suggested that they might have been WA MRSA that had spread across the border from WA (24). However, the NT strains have been reported to be genetically unrelated to the WA MRSA (21).

CMRSA are not generally known to spread in hospitals. However, in 1995 a WA MRSA caused a single-strain outbreak in a large metropolitan hospital in WA. The index strain was isolated from a patient from a remote community and was indistinguishable from the predominant strain found in two

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isolated communities in WA where 42% of people in one community and 24% in the other were colonized with this strain (29). There have been few other reports of CMRSA involved in a hospital outbreak (3, 42).

CMRSA have also been isolated in Auckland, New Zealand (NZ), with the majority of isolates coming from people from Western Samoa (1, 28, 54). The isolates have two phage patterns and are referred to as Western Samoan Phage Pattern 1 and 2 (WSPP-1 and -2) (1, 25). The WSPP clone has been reported to have spread to New South Wales (NSW) (9, 14) and Queensland (28) on the eastern seaboard of Australia. A second CMRSA strain, designated the Queensland clone, has also been reported from Queensland (57).

Since 1995 five different contour-clamped homogeneous electric field (CHEF) electrophoresis patterns have been found among WA MRSA, and a recent investigation of some CMRSA from Australia and NZ identified several CHEF electrophoretic patterns that were mainly confined to specific geographical areas (21).

In an effort to better understand the epidemiology of CMRSA in Australia, CMRSA isolates representing the different CHEF patterns were compared by a number of methods. The overall relatedness of isolates has been compared by using multilocus sequence typing (MLST) (11), protein A gene (*spa*) typing (46), antibiograms, and resistograms. However, isolates with the same overall genetic background can differ as a result of acquiring additional genetic information in the form of cassettes, transposons, and plasmids. The gene for methicillin resistance, *mecA*, is contained within a mobile element known as the *mec* region or staphylococcal cassette chromosome *mec* (SCC*mec*). The SCC*mec*s vary depending on variations in the *mecA* regulatory region (*mec* complex), the type of cassette chromosome recombinases (*ccr* genes) they have, and the resistance determinants they have acquired due to the integration of plasmids and transposons (18). It has been reported that the Panton-Valentine leukocidin (PVL) determinant is common in CMRSA but rare among hospital MRSA and therefore may be a useful marker for the rapid identification of CMRSA (57). Consequently, all of these approaches have been used to compare CMRSA isolates from Australia.

MATERIALS AND METHODS

Strains. The CMRSA isolates used (Table 1) were selected as representative of the CHEF pattern types present in a collection of 425 CMRSA isolates from WA, 32 CMRSA isolates from South Australia (SA), 34 CMRSA isolates from the NT, 36 CMRSA isolates from NSW, and 5 CMRSA isolates from Victoria (VIC). The WA isolates WBG8366, WBG8378, and WBG8404 were nasal carriage isolates from inhabitants of remote WA communities; all of the other isolates were from cases of infection. The criteria for designating the isolates as CMRSA were that they were nonmultiresistant MRSA that were isolated from people from the community. The WA MRSA belonged to five different CHEF patterns. For comparison, the type strains for the NZ WSPP-1 and WSPP-2 isolates were also included. In addition, the plasmids in two isolates belonging to the same CHEF group as WBG7583 were studied in order to elucidate the evolution of multiresistance in these isolates. The recipient for mixed-culture transfer (MCT) of plasmids was WBG1876 (51) and the molecular weight standards were *S. aureus* WBG4483 for plasmids (51) and *S. aureus* NCTC8325 for SmaI-digested chromosomal DNA (33).

Antimicrobial susceptibility. Antibiograms were determined by using the National Committee for Clinical Laboratory Standards guidelines for disk diffusion (26) with Oxoid disks (Oxoid, Basingstoke, England) for gentamicin, kanamycin, streptomycin, neomycin, erythromycin, lincomycin, chloramphenicol, minocycline, tetracycline, trimethoprim, sulfamethoxazole, rifampin, novobiocin, vancomycin, mupirocin, ciprofloxacin, and oxacillin. Resistance to fusidic acid was determined at the same time by using a 10-μg disk (Oxoid). Methicillin resistance was confirmed by PCR detection of *mecA* (21). β-Lactamase (Bla) production was detected by using Cefinase disks (Becton Dickinson, Cockeysville, Md.) according to the instructions of the manufacturer. Resistograms for cadmium acetate, mercuric chloride, phenol mercuric acetate, ethidium bromide, and propamidine isethionate were performed as previously described (52, 53).

Population analysis of methicillin resistance. Population analyses were performed by microdilution as previously reported (36).

DNA isolation procedures. Plasmid (50) and chromosomal (21) DNA was isolated as previously described.

Plasmid analysis. Plasmids were transferred to WBG1876 by MCT (51), and plasmid curing was performed at 43.5°C (53). Colonies showing plasmid transfer or loss of resistance were tested for plasmids, and their antibiograms and resistograms were determined. Strains were further confirmed to have lost plasmids by comparing their CHEF patterns with the parent strains. The restriction fragment length polymorphisms (RFLPs) of the plasmids were compared by EcoRI digestion according to the recommendations of the manufacturer (Promega Corp., Madison, Wis.), followed by gel electrophoresis (44) with a 1-Kb Plus DNA Ladder (Invitrogen, Carlsbad, Calif.) as the size standard.

CHEF electrophoresis of chromosomal DNA. CHEF electrophoresis was performed as previously described (29) by using the CHEF DR III System (Bio-Rad Laboratories, Inc., Hercules, Calif.). Chromosomal patterns were examined visually, as well as by scanning with a Fluor-S MultiImager and analyzing them digitally with Multi-Analyst/PC (Bio-Rad). CHEF patterns were grouped according to the criteria of Tenover et al. (48).

MLST typing. This was performed on either a LI-COR Long Reader 4200 sequencer (LI-COR, Inc., Lincoln, Nebr.) at the Curtin University of Technology, WA, or on an ABI 3700 capillary sequencer (Applied Biosystems, Foster City, Calif.) at the University of Bath, England, with primers and PCR protocols previously established (11). LI-COR sequences were collected with Base Imager software (LI-COR) and assembled and manipulated with the AssemblyLIGN program (Accelrys, Cambridge, England). The sequences were then submitted to an internet database (http://www.mlst.net), where an allelic profile was generated and the sequence type (ST) was determined.

***spa* typing.** DNA sequence analysis of the polymorphic repeat region of the protein A gene (*spa* typing) was performed as previously described (46). Briefly, the *spa* gene was amplified with the following primers: SpaF (5′-GACGATCC TTCAGTGAGCAAAG-3′) and SpaR (5′-GCAGCAATTTTGTCAGCAGTA G-3′). PCR products were purified with the Millipore PCR_{96} Cleanup plates according to the manufacturer's instructions (Millipore Corp., Bedford, Mass.), and the amplicon was sequenced by using the Beckman Coulter CEQ 8000 genetic analysis system (Fullerton, Calif.).

Detection of PVL. The presence of PVL was determined by Southern blotting (45), followed by hybridization with *lukF*, which was amplified by using the primers LukS-PV (5′-GGCCTTTCCAATACAATATTGG-3′) and LukF-PV (5′-CCCAATCAACTTCATAAATTG-3′). The amplicons were labeled by using an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.) and used to blot overnight ClaI-digested DNA that had been separated by electrophoresis and transferred to a Hybond-N^+ membrane (Amersham) by using a VacuGene XL blotting system (Pharmacia). The presence of PVL was confirmed by using a molecular beacon of the *lukF* component.

SCC*mec* typing. The SCC*mec* was typed as previously reported (21) and also by using multiplex primers as described previously (32), with one modification. The *pls* gene was amplified by using the primers PlsF (5′-GGGGTGGTTAAT GGTATGAATAAA-3′) and PlsR (5′-CGGAATGTTGCTCTTGGTTGTGCG TTTTC-3′) (GenBank accession number AF115379). Extra primers were utilized to differentiate SCC*mec* types IVa and IVb (31).

RESULTS

Antimicrobial susceptibilities. All isolates were resistant to methicillin and produced Bla. In population analyses, WBG8287, WBG9087, and WBG9093 expressed heterogeneous resistance to methicillin (not shown). In WA a panel of eight antimicrobial agents (gentamicin, erythromycin, tetracycline, trimethoprim, rifampin, fusidic acid, ciprofloxacin, and mupirocin) is used to provisionally distinguish between multiresistant and nonmultiresistant MRSA (29). Strains resistant less than three of the antibiotics are regarded as nonmultire-

sistant, and strains resistant to three or more of the antibiotics are regarded as multiresistant. All isolates in the study, except WBG8873 were nonmultiresistant MRSA. Two were resistant to fusidic acid (WBG8287 and WBG8873), three were resistant to tetracycline (WBG8873, WBG7583, and WSPP-1a), one was resistant to ciprofloxacin (WBG8897), and seven had inducible resistance to erythromycin (WBG8287, WBG8873, WBG8366, WBG8900, WBG8378, WBG9087, and WBG9093). All isolates were resistant to cadmium acetate, two were resistant to sodium arsenate (WBG8404 and WBG10198), and one was resistant to ethidium bromide (WSPP-1a).

MLST and CHEF analysis. MLST identified 10 STs that belong to eight clonal complexes (CCs), seven of which have been described previously, and one, designated new1, that has not been previously described (Table 1). Except for ST75, all of the STs have been found in other countries (determined at http://www.mlst.net).

Except for WBG9087 and WBG9093, the CHEF pattern types belonged to the same MLST CCs. WBG9087 and WBG9093 belong to different CHEF groups, F and G, respectively, but have related STs, ST75, and ST258, respectively, that differ at the MLST *gmk* allele (Table 1), indicating that they belong to the same CC but have undergone genetic changes.

***spa* typing.** In all but two instances isolates with the same *spa* type belonged to the same CC (Table 1). In CC30, WBG10198 and the WSPP isolates had different *spa* types of 33 and 19, respectively. Also, WBG9087 and WBG9093 had new and different *spa* types of 401 and 400, respectively, and, although they both belonged to the same CC, they had slightly different STs of ST75 and ST258, respectively. This, together with the fact that they had different CHEF patterns, suggests that they have undergone genetic changes in the community. Overall, these results suggest that *spa* typing may be a useful sequencing technique for quickly detecting particular types of MRSA.

SCC*mec* typing. Thirteen of the CMRSA harbored the type IVa SCC*mec* (23, 31) (Table 1). WBG9093 harbored type 2 *ccr* genes and class B *mec* complex and was classified as having a type IV SCC*mec*. However, it did not amplify with primers for either the a or b types of the cassette, indicating that it harbors a difference in the J1 region (31). Two isolates, WBG8404 and WBG10198, harbored previously undescribed SCC*mec* elements; both encoded the type 5 *ccr* gene (19) but harbored different *mec*-complexes of classes B1 and E, respectively (21). WBG8404 did not amplify with either the IVa or IVb primers, but WBG10198 amplified weakly with the IVa primers, indicating that it has some similarities with the type IVa SCC*mec* in the J1 region.

The multiplex PCR protocol gave the same results as the primers previously used (21) for all of the CMRSA except WBG10198 which gave the type III pattern, indicating that it was also structurally similar to the type III SCC*mec* (32) but contained different *ccr* genes and *mec* complex.

Plasmid content and characterization. Plasmid sizes are presented in Table 1. Except WBG8897 all CMRSA studied harbored plasmids. All plasmid-containing isolates had one large plasmid that varied in size depending on the isolate. Seven isolates—WBG8287, WBG8873, WBG8366, WBG8900, WBG8378, WBG9087, and WBG9093—harbored an additional small plasmid of 2 kb and one isolate (WSPP-1a) harbored three plasmids. MCT was performed on all of the

TABLE 1. Genetic and phenotypic properties of CMRSA isolates from Australia and NZ[a]

Isolate	Antimicrobial properties	Plasmid profile[b]	CHEF pattern	ST	MLST allelic profile	CC	*ccr* gene complex	*mec* complex	SCC*mec* type	Presence (+) or absence (−) of *pvl* genes	*spa* type	*spa* motif	Origin (reference)
WBG8287	Bla, E^{I}, F, C	19.6, 2	B1	1	1-1-1-1-1-1-1	1	2	B	IVa	−	35	U1-J1-F1-K1-B1-P1-E1	WA (29)
WBG8873	Bla, E^{I}, T, F, C	19.6, 2	B2	1	1-1-1-1-1-1-1	1	2	B	IVa	−	35	U1-J1-F1-K1-B1-P1-E1	SA (21)
WBG10200	Bla, F, C	19.6	B3	1	1-1-1-1-1-1-1	1	2	B	IVa	−	35	U1-J1-F1-K1-B1-P1-E1	VIC (21)
WBG8366	Bla, E^{I}, C	19.6, 2	C1	255	22-3-14-23-12-53-31	298	2	B	IVa	−	9	U1-G1-F1-M1-E1-E1-B1-P1-B1	WA (29)
WBG8900	Bla, E^{I}, C	19.6, 2	C2	257	22-1-14-19-12-53-31	298	2	B	IVa	−	ND		SA (21)
WBG8378	Bla, E^{I}, C	21.6, 2	D1	5	1-4-1-4-12-1-10	5	2	B	IVa	−	2	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	WA (29)
WBG10201	Bla, C	32	D2	5	1-4-1-4-12-1-10	5	2	B	IVa	−	2	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	VIC (21)
WBG8404	Bla, C, As	24.5	E	45	10-14-8-6-10-3-2	45	5	B1	New	−	6	A2-A1-K1-B1-E1-K1-B1-K1-B1	WA (21, 29)
WBG7583	Bla, T, C	28.6	A	8	3-3-1-1-4-4-3	8	2	B	IVa	−	1	Y1-H1-G1-F1-M1-B1-Q1-B1-L1-O1	WA (56)
WBG8897	Bla, Ci, C	None	H	22	7-6-1-5-8-6	22	2	B	IVa	−	382	T1-J1-J1-E1-J1-N1-F2-M1-N1-F2-M1-O1-M1-O1-K1-R1	SA (21)
WBG9087	Bla, E^{I}, C	25.6, 2	F	75	36-3-43-34-39-52-49	new1	2	B	IVa	−	401	Q2-N1-M1-M1-J1-M1-M1-J1-M1-L1	NT (21)
WBG9093	Bla, E^{I}, C	19.6, 2	G	258	36-3-43-1-39-52-49	new1	2	B	IV	−	400	Q2-J1-J1-M1-M1-M1-J1-J1-J1-M1-K1	NT (21)
WSPP-1b	Bla, C	25	J1	30	2-2-2-2-6-3-2	30	2	B	IVa	+	19	X1-K1-A1-K1-A1-O1-M1-Q1	NSW (9)
WSPP-1a	Bla, T, C, Eb	43, 8, 4.4	J1	30	2-2-2-2-6-3-2	30	2	B	IVa	+	ND		NZ (1)
WSPP-2	Bla, C	25	J2	30	2-2-2-2-6-3-2	30	2	B	IVa	+	19	X1-K1-A1-K1-A1-O1-M1-Q1	NZ (1)
WBG10198	Bla, C, As	36	J3	30	2-2-2-2-6-3-2	30	5	E	New	−	33	W1-G1-K1-A1-K1-A1-O1-M1-Q1-O1	VIC (21)

[a] Abbreviations: As, arsenate resistance; Bla, Bla production; C, cadmium resistance; Ci, ciprofloxacin resistance; E^{I}, inducible erythromycin resistance; Eb, ethidium bromide resistance; F, fusidic acid resistance; Hg, mercury resistance; ND, not determined; T, tetracycline resistance.

[b] Plasmid sizes, when given, are in kilobases.

TABLE 2. Characterization of plasmids from CMRSA isolates in Australia[a]

Strain	CC	Plasmid size (kb)	Phenotype	EcoRI fragment size(s) (kb)
WBG8287	1	19.6	Bla, C	14.6, 4.0, 1.0
		2.0	E^I	ND
WBG8873	1	19.6	Bla, C	14.6, 4.0, 1.0
		2.0	E^I	ND
WBG10200	1	19.6	NA	14.6, 4.0, 1.0
WBG8900	78	19.6	Bla, C	14.6, 4.0, 1.0
WBG8404	45	24.5	Bla, C, As	10.2, 5.0, 4.3, 3.6, 1.4
WBG9087	75	25.6	Bla, C	23.0, 2.6
WBG9093	75	19.6	Bla, C	14.6, 4.0, 1.0
WBG7583	8	28.6	Bla, C	11.5, 10.3, 2.6, 2.3, 1.9
WBG8320	8	32.1	Bla, C, T	14.3, 11.0, 2.6, 2.3, 1.9
WBG7569	8	41.4	Bla, C, T, Mup, Tp	14.3, 11.5, 3.7, 3.0, 2.6, 2.3, 2.1, 1.9
WBG10198	30	36	NA	20.0, 11.0, 3.2, 1.2, 0.6
WBG10201	30	32.0	NA	19, 13.0
WSPP-1b	30	25.0	NA	17.3, 5.0, 2.7
WSPP-2	30	25.0	NA	17.3, 5.0, 2.7

[a] Abbreviations: As, arsenate resistance; Bla production; C, cadmium resistance; E, erythromycin resistance; Mup, mupirocin resistance; NA, not applicable; ND, not determined; Tp, trimethoprim resistance.

isolates and the large plasmids of seven were successfully transferred. All encoded determinants for Bla and resistance to cadmium. The 24.5-kb plasmid in WBG8404, in addition, encoded resistance to sodium arsenate (Table 2). Curing of the 2-kb plasmids from WBG8287 and WBG8873 was accompanied by the loss of inducible resistance to erythromycin (Table 2).

Only in CC298 did all isolates have the same plasmid content. However, WBG9093 in CCnew1 and the members of CC1, except for WBG10200, which lacked the 2-kb plasmid, had the same plasmid content as the CC298 isolates. In the other CCs there was no uniformity of plasmid content (Table 1).

Transferred plasmids and plasmids from isolates that contained a single large plasmid were further analyzed by digestion with EcoRI. The 19.6-kb plasmids in WBG8287, WBG8873, WBG10200, WBG8900, and WBG9093 gave identical EcoRI RFLPs, as did the 25-kb plasmids in WSPP-1b and WSPP-2. The remaining plasmids had unique EcoRI restriction profiles (Table 2).

The role played by plasmids in the acquisition of antimicrobial resistances in WA CMRSA was demonstrated by the RFLPs of the plasmids present in the CC8 isolates WBG7583, WBG8320, and WBG7569 (Table 2). These isolates carry plasmids of 28.6, 32.1, and 41.4 kb, respectively, which have related EcoRI RFLPs. The differences in their RFLPs are associated with the acquisition of tetracycline resistance in the case of the 32.1-kb plasmid and with the acquisition of tetracycline, mupirocin, and trimethoprim resistance in the case of the 41.4-kb plasmid.

PVL determinant. All Australian CMRSA isolates studied lacked the PVL determinant except for the CC30 WSPP isolate from NSW. This isolate is believed to have originated in the Western Samoan Islands or NZ, whose related CC30 type strains also contained the element (Table 1).

DISCUSSION

It has been reported that CMRSA from different parts of the world have different genetic backgrounds (31). The results presented here demonstrate that this is also the case for Australian CMRSA. These results also demonstrate that there is a close correlation between CHEF pattern type, CC type, and *spa* type and that, generally, CMRSA types are confined to particular geographic areas. WA CMRSA strains, however, are of five distinct types. Four of these, CC1, CC5, CC45, and CC8, belong to four distantly related pandemic genetic lineages (12, 31), and one, CC298, belongs to a smaller clone that includes isolates from Australia, Portugal, and Japan (determined at http://www.mlst.net). CMRSA strains belonging to CC1 and CC298 were found in SA and the neighboring states of WA and VIC. Likewise, CMRSA belonging to CC30 were found in the neighboring eastern states of NSW and VIC. CMRSA isolates belonging to CC5 were found in WA and VIC.

The CC1 strains have an MLST allelic profile and a *spa* type that are the same as the *S. aureus* that is the proposed ancestor of MW2, the CMRSA that was responsible for the deaths of four children in the United States (5, 13, 41). Furthermore, as well as being found in the United States (31), CC1 CMRSA strains have been reported in France (57) and Australia (31), indicating that this is a particularly successful clone.

Despite the diversity of CCs, the CMRSA strains were remarkably uniform in their SCC*mec*s. Of the vast majority of CMRSA strains studied thus far, most harbor the Type IVa SCC*mec* (31), suggesting that, in the Australian community environment, this type is the most-transmissible and best-adapted type of SCC*mec*. The CC45 isolate WBG8404 and the CC30 isolate WBG10198 had been previously reported to harbor classes B1 and E *mec* complexes, respectively (21). The present study has now found that they harbor the type 5 *ccr* gene which has also been described in CMRSA isolates (19). The vastly different structures of the WBG8404 and WBG10198 SCC*mec*s would suggest that they have been acquired from different sources on different occasions. However, how, and from where, the SCC*mec* has been acquired is yet to be determined.

The plasmid content of the CMRSA in CC1 further supports the similarity of the isolates in this CC and suggests that the same strain is found in WA, SA, and VIC, although the state from which it originated cannot be ascertained from these results. Likewise, the plasmid content of the strains in CC298 also indicates that this strain is found in WA and SA. The isolates in CC1, CC298 and the ST258 isolate, WBG9093, contain the same plasmids, suggesting that they have possibly been acquired from the same source. It is interesting that the NSW strain WSPP-1b has the plasmid content of the NZ strain WSPP-2 but the CHEF pattern of WSPP-1a, whereas the VIC isolate in this CC has a different *spa* type, different plasmids and a different SCC*mec*, suggesting that it may have originated independently of the other strains.

The present study has provided some insight into how CMRSA strains have acquired additional resistances. Inducible erythromycin resistance was always accompanied by a 2-kb plasmid (Table 1) that appears to be the same as the 2-kb inducible erythromycin resistance plasmid that was cured from WBG8287 and WBG8873 (Table 2). In two cases, WBG8873 and WBG7583 tetracycline resistance is chromosomal. However, it is not known at this stage whether this is due to the integration of a pT181 type plasmid into SCC*mec*, as has occurred in multiresistant MRSA (18). In the case of WSPP-1a it appears that tetracycline resistance is due to the acquisition of

a 4.4-kb plasmid of the pT181 type. Analysis of the Bla plasmids in WBG7583, WBG8320, and WBG7569 (Table 2) has demonstrated that the 28.6-kb Bla, cadmium resistance plasmid can acquire tetracycline resistance and also mupirocin and trimethoprim resistance. It is known that the acquisition of tetracycline resistance is due to integration of a pT181-type plasmid, trimethoprim resistance is due to the acquisition of Tn*4003*, and mupirocin resistance is due to the acquisition of *mupA* (J. T. Slattery, E. E. Udo, J. W. Pearman, T. V. Riley, and W. B. Grubb, Abstr. Microbiol. Aust. **19:**GP.17, p. 8122, 1998). The demonstration that the control of mupirocin usage could control the spread of mupirocin resistance in CMRSA (49) suggests that monitoring the resistance of isolates and controlling the use of antibiotics is important in controlling the spread of antibiotic resistance in CMRSA.

The SA isolate, WBG8897, is particularly interesting. It was regarded as a CMRSA, and yet it has the same ST as the English epidemic strain, UK EMRSA-15 (12), and has a CHEF pattern that is 92% similar to that of UK EMRSA-15 (21). Like UK EMRSA-15, it is resistant to ciprofloxacin (30). UK EMRSA-15 is known to be present in hospitals in Australia (34), and these results suggest that it is now in the community in SA.

All of the isolates produced Bla and were resistant to cadmium. In every case, except for WBG8897, which is probably UK EMRSA-15, these were plasmid encoded. It is not known why isolates should be resistant to cadmium, but there is a good rationale for why they produce Bla, even though they do not require it for penicillin resistance, since *mecA* mediates resistance to all β-lactam antibiotics. All of the CMRSA strains studied harbored *mec* complexes that have their regulatory genes disrupted (23), leaving *mecA* unregulated. The Bla regulators *blaR1* and *blaI* are better regulators of *mecA* than *mecR1* and *mecI*, and it is possible that they are regulating the expression of *mecA* in the CMRSA (40). Also, it has been suggested that the *blaI* determinant may stabilize *mecA* in *S. aureus* genetic backgrounds (20).

It has been suggested that the PVL genes may be a good marker for detecting CMRSA (57), but its absence in all but one CC of the Australian CMRSA isolates investigated suggests that it would not be useful for this purpose in Australia. PVL has been associated with virulence (57), but there was no correlation between virulence and possession of the determinant in the present study. Three of the WA CMRSA strains were carriage isolates, and all of the other isolates, including a hospital outbreak index strain, WBG8287 (29), were from patients with infections.

The present study emphasizes the diversity of CMRSA found in Australia and the importance of typing in tracing the origin of isolates and in designing antibiotic policies for their control in the community.

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