

Genetic Diversity among Community Methicillin-Resistant *Staphylococcus aureus* Strains Causing Outpatient Infections in Australia

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Increasing reports of the appearance of novel nonmultiresistant methicillin-resistant *Staphylococcus aureus* MRSA (MRSA) strains in the community and of the spread of hospital MRSA strains into the community are cause for public health concern. We conducted two national surveys of unique isolates of *S. aureus* from clinical specimens collected from nonhospitalized patients commencing in 2000 and 2002, respectively. A total of 11.7% of 2,498 isolates from 2000 and 15.4% of 2,486 isolates from 2002 were MRSA. Approximately 54% of the MRSA isolates were nonmultiresistant (resistant to less than three of nine antibiotics) in both surveys. The majority of multiresistant MRSA isolates in both surveys belonged to two strains (strains AUS-2 and AUS-3), as determined by pulsed-field gel electrophoresis (PFGE) and resistogram typing. The 3 AUS-2 isolates and 10 of the 11 AUS-3 isolates selected for multilocus sequence typing (MLST) and staphylococcal chromosomal cassette *mec* (SCC*mec*) analysis were ST239-MRSA-III (where ST is the sequence type) and thus belonged to the same clone as the eastern Australian MRSA strain of the 1980s, which spread internationally. Four predominant clones of novel nonmultiresistant MRSA were identified by PFGE, MLST, and SCC*mec* analysis: ST22-MRSA-IV (strain EMRSA-15), ST1-MRSA-IV (strain WA-1), ST30-MRSA-IV (strain SWP), and ST93-MRSA-IV (strain Queensland). The last three clones are associated with community acquisition. A total of 14 STs were identified in the surveys, including six unique clones of novel nonmultiresistant MRSA, namely, STs 73, 93, 129, 75, and 80slv and a new ST. SCC*mec* types IV and V were present in diverse genetic backgrounds. These findings provide support for the acquisition of SCC*mec* by multiple lineages of *S. aureus*. They also confirm that both hospital and community strains of MRSA are now common in nonhospitalized patients throughout Australia.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in Australia in 1968 (50). The emergence of MRSA strains resistant to gentamicin was first noted in eastern Australia in 1976, and outbreaks of hospital infection due to multiresistant MRSA (mMRSA; see Materials and Methods for the definition) occurred in the state of Victoria in the late 1970s and early 1980s (42, 46). mMRSA became endemic in hospitals in the eastern Australian states in the late 1980s and 1990s, with some spread to hospitals in South Australia, the Northern Territory, and Tasmania (33, 56, 57). However, these strains did not become established in Western Australian hospitals due to active screening and infection control policies (33, 44).

In the early 1990s nonmultiresistant MRSA (nmMRSA; see

Materials and Methods for the definition) were observed in Western Australia, initially from indigenous people in remote communities (58) but subsequently also in Perth, the state capital. They became known as “WA-MRSA” (where WA represents Western Australia), and although nmMRSA strains from the community do not readily spread in hospitals, one of the WA-MRSA strains has been responsible for a documented outbreak of hospital-acquired infection (36, 49). An increase in the proportion of nmMRSA strains was also seen in other Australian states in the 1990s (33). Community-acquired nmMRSA strains were observed in the eastern states, and studies in Queensland and New South Wales showed a strong association between community-acquired infection with nmMRSA and Polynesian ethnicity. Isolates causing these infections were identical by phage typing and pulsed-field gel electrophoresis (PFGE) to those previously reported in Auckland, New Zealand (15, 34). Subsequently, a second strain has been shown to be associated with community-acquired infection in Caucasians in Queensland (28).

The emergence of community-acquired nmMRSA has been

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reported in other parts of the world, including Canada (10), the United States (16, 27, 31), France and Switzerland (59), Denmark (13), and Finland (51). However, other nmMRSA strains, such as EMRSA-15 and EMRSA-16, have been associated with health care settings and have spread internationally (9, 26). nmMRSA strains are generally believed to have originated in the community environment, but they may also include nonmultiresistant sporadic hospital strains that have been taken into the community (3). In addition, the spread of strains associated with the health care setting into the community has been documented (9, 19, 60). Both the emergence of novel community-acquired strains and the spread of health care setting-associated strains of MRSA to the community are causes for public health concern. The prevalence of MRSA in the community must be taken into account in the formulation of health care policy in areas such as antibiotic prescription and infection control. The aim of this study was to establish the proportion of MRSA strains among clinically significant outpatient *S. aureus* isolates in all Australian states and territories, to determine the genetic backgrounds of the MRSA strains, and to determine if any previously undescribed strains are causing infections in outpatients.

MATERIALS AND METHODS

Bacterial isolates. Isolates were collected from patients attending primary care clinics, outpatient clinics, emergency departments, or other outpatient settings or residing in long-term-care residential facilities. Twenty teaching hospital laboratories (THLs) and five private pathology laboratories (PPLs) in eight Australian cities participated in the study. The locations of the laboratories were as follows: two THLs and one PPL in Brisbane, Queensland; six THLs and one PPL in Sydney, New South Wales; one THL in Canberra, Australian Capital Territory; three THLs and one PPL in Melbourne, Victoria; one THL in Hobart, Tasmania; three THLs and one PPL in Adelaide, South Australia; three THLs and one PPL in Perth, Western Australia; and one THL in Darwin, Northern Territory. Up to 100 consecutive clinical isolates of *S. aureus* were collected at all laboratories for each of two surveys, the first of which was conducted from 1 June 2000 to 30 April 2001 (the 2000 survey) and the second of which was conducted from 1 October 2002 to 16 March 2003 (the 2002 survey). Isolates from infection control screening specimens were excluded, as were duplicate clinical isolates, as determined by their antimicrobial susceptibility phenotypes.

Identification and susceptibility tests. *S. aureus* was identified by positive results by at least two of three tests: the slide coagulase or latex agglutination, tube coagulase, and heat-stable nuclease tests (21). Urease production was tested by using Christensen's urea slopes incubated for 24 h at 37°C (6). Susceptibility testing was performed by agar dilution by the NCCLS methodology (32), except that a single breakpoint concentration of each antimicrobial was used. Briefly, the following antimicrobials were obtained from the manufacturers as reference powders of assayed potency or in tablet form and were incorporated into agar plates at the indicated concentrations: penicillin G, 0.125 mg/liter; oxacillin, 2 mg/liter; vancomycin, 2 mg/liter; teicoplanin, 2 mg/liter; rifampin, 1 mg/liter; fusidic acid, 1 mg/liter; gentamicin, 4 mg/liter; chloramphenicol, 8 mg/liter; erythromycin, 0.5 mg/liter; clindamycin, 0.5 mg/liter; tetracycline, 4 mg/liter; trimethoprim, 8 mg/liter; ciprofloxacin, 1 mg/liter; and mupirocin, 1 mg/liter. An antibiotic-free control plate was included in each batch. The following control strains were included in each batch: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Escherichia coli* ATCC 25922, and *Enterobacter cloacae* ATCC 13047. Resistance was defined as growth on the concentration tested; a fine haze was ignored for chloramphenicol, tetracycline, and trimethoprim.

MRSA isolates were divided into two heterogeneous groups on the basis of resistance to nine antibiotics: erythromycin, tetracycline, trimethoprim, gentamicin, rifampin, fusidic acid, ciprofloxacin, chloramphenicol, and mupirocin. Isolates which were resistant to three or more of the antibiotics listed above were defined as mMRSA, and those resistant to less than three were defined as nmMRSA. The sources of the patient isolates were recorded in most cases.

Resistogram typing. Resistance testing was performed by disk diffusion against a panel of six chemicals and dyes (54, 55): cadmium acetate (10 mM), sodium

arsenate (0.2 μM), ethidium bromide (15 mM), propamidine isothionate (2% [wt/vol]), mercuric chloride (HgCl₂; 0.4 μM), and phenylmercuric acetate (PMA; 5 mM).

Bacteriophage typing. Bacteriophage typing was performed by the method of Blair and Williams (7) by using (i) the basic international set of 23 phages for *S. aureus* (group I, phages 29, 52, 52A, 79, and 80; group II, phages 3A, 3C, 55, and 71; group III, phages 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; group V, phages 94 and 96; and miscellaneous, 81 and 95) (40), (ii) 3 experimental phages (phages 88, 90, and 187) (4), and (iii) the international MRSA set of 10 phages (phages MR8, MR12, MR25, 30, 33, 38, M3, M5, 56B, and 622) (47). All phages were used at 100 times the routine test dilution.

Coagulase gene typing. Coagulase gene restriction fragment length polymorphism (RFLP) typing was performed as described previously (14).

PFGE. Pulsed-field gel electrophoresis (PFGE) of chromosomal DNA was performed as described previously (36) with a CHEF DR III system (Bio-Rad Laboratories Pty. Ltd.). Chromosomal DNA patterns were examined visually, scanned with a Fluor-S Multimager, and digitally analyzed by using Multi-Analyst/PC software (Bio-Rad Laboratories). PFGE patterns were grouped according to the criteria of Tenover et al. (52) and by using a dendrogram similarity of 80% or greater to assign strain relatedness. *S. aureus* NCTC 8325 was used as the size marker (41).

Chromosomal DNA preparation. Multilocus sequence typing (MLST) with the chromosomal DNA of the isolates from the 2000 survey was performed as described by Huygens et al. (17). Chromosomal DNA for MLST and staphylococcal chromosomal cassette *mec* (SCC*mec*) typing of isolates from the 2002 survey was prepared either as described previously (23) or with a DNeasy Tissue kit (Qiagen Pty. Ltd., Clifton Hill, Victoria, Australia).

MLST. MLST was performed with selected isolates as specified by Enright et al. (11). The sequences obtained were compared with the sequences at the MLST website (<http://www.mlst.net/>) to assign a sequence type (ST).

SCC*mec* typing. SCC*mec* was typed by PCRs that determined the class of *mec* complex and the type of cassette chromosome recombinase (*ccr*) harbored by the elements. Primers were used as described previously (23). Additional primers included those that detected the class C2 *mec* complex (20) and *ccrC* (18) of the type V SCC*mec* and forward primer mA (23) with reverse primer mI4 (5'-TG GACTCCAGTCCCTTTGTC-3'; this study) or mI2 (22) for investigation of the novel SCC*mec* of isolate CH39.

RESULTS

A total of 2,498 isolates were tested in the 2000 survey, of which 293 (11.7%) were resistant to oxacillin. In the 2002 survey, 2,486 isolates were tested, of which 384 (15.4%) were resistant to oxacillin. The levels of resistance to individual antibiotics were similar for methicillin-susceptible *S. aureus* and MRSA strains in both surveys, with the exception that the rates of resistance to both ciprofloxacin and clindamycin increased by approximately 5% among MRSA isolates in 2002. In 2000 all nmMRSA isolates were gentamicin susceptible, while in 2002 four isolates (1%) were gentamicin resistant. The proportions of specimens from various types of facilities were similar in both surveys. Overall, 14% were from primary care facilities, 47% were from emergency departments, 19% were from outpatient clinics, 7% were from long-term residential care facilities, and 13% were from other or unknown outpatient sources. The overall proportions of MRSA strains that were nmMRSA were similar for the two survey periods. However, there was considerable variation in the proportions of MRSA and nmMRSA isolates throughout the country (Table 1).

Two hundred eighty MRSA isolates from the 2000 survey (124 mMRSA isolates and 156 nmMRSA isolates) and 367 MRSA isolates from the 2002 survey (168 mMRSA isolates and 199 nmMRSA isolates) were further characterized by resistogram testing, urease determination, bacteriophage susceptibility, coagulase RFLP analysis, and PFGE. The mMRSA

TABLE 1. Distribution of MRSA and proportion of nmMRSA isolates in Australian cities

City	No. (%) of isolates					
	2000 survey			2002 survey		
	Total	MRSA	nmMRSA	Total	MRSA	nmMRSA
Brisbane	300	23 (7.7)	17 (73.9)	300	37 (12.3)	25 (67.6)
Sydney	700	139 (19.9)	64 (46.0)	689	175 (25.4)	76 (43.4)
Canberra	100	5 (5.0)	5 (100)	100	8 (8.0)	3 (37.5)
Melbourne	400	42 (10.5)	4 (9.5)	399	46 (11.5)	6 (13.0)
Hobart	100	2 (2.0)	2 (100)	100	6 (6.1)	5 (83.3)
Adelaide	399	29 (7.3)	17 (58.6)	400	36 (9.0)	29 (80.6)
Perth	400	46 (11.5)	43 (93.5)	398	55 (13.8)	51 (92.7)
Darwin	99	7 (7.1)	6 (85.7)	100	21 (21.0)	10 (47.6)
National	2,498	293 (11.7)	158 (53.9)	2,486	384 (15.4)	205 (53.4)

isolates were made up of two major strains, designated AUS-2 and AUS-3 (Table 2). The characteristics of one isolate from the 2000 survey were consistent with the published characteristics of the Irish-2 strain (5), and the characteristics of three isolates from the 2002 survey were consistent with those of the previously described Irish-1 strain. The nmMRSA isolates were grouped into 10 distinctive clones. Two were international epidemic clones, EMRSA-15 and EMRSA-16, which have been associated with hospital outbreaks (26). Two were previously described community-associated clones, the southwest Pacific (SWP) clone or the Western Samoan phage pattern (WSPP) clone and WA-MRSA-1 (37, 59). The remainder of the isolates were designated nmMRSA A to F (Table 2). The distributions of the various strains in the two surveys at the national level and for individual cities are shown in Table 3.

MLST was performed with 26 isolates from the 2000 survey and 7 isolates from the 2002 survey (Table 4). The isolates chosen from the 2000 survey represented strains with more than one isolate, while in 2002 unique or sporadic isolates were

chosen along with one isolate each of the Irish-1 strain and nmMRSA F. The results for clones described previously were in accordance with those presented in previous publications (12, 37, 59). All AUS-2 and AUS-3 isolates with the exception of isolate AH1 were ST239; isolate AH1 was ST128, a single-locus variant of ST239 with a *gmk* polymorphism. nmMRSA A was indistinguishable from the previously described pulsotype R or Queensland (QLD) clone, both of which are ST93 (28, 59).

SCCmec characterization was performed with all isolates from the 2000 and 2002 surveys selected for MLST (Table 4). Six SCCmec types, including two novel types, were identified among the 14 STs. Of the 17 mMRSA isolates, 15 harbored the type III SCCmec, and these comprised the ST239 isolates and the related ST128 isolate. Of the two multiresistant ST8 isolates, isolate RPH2 had the genetic background of the international epidemic Irish-2 clone (unpublished observations) and harbored the pediatric type IV SCCmec (35). The other isolate, isolate CH39, harbored a novel SCCmec that contained

TABLE 2. Summary of phenotypic and genotypic characteristics of mMRSA and nmMRSA strains in Australia

PFGE pattern	Typical resistance pattern ^a	Urease test result	Typical phage susceptibility	Coagulase gene PCR-RFLP pattern
Multiresistant				
22 (AUS-2)	TET, ERY, TMP, GEN, CIP	+	85, 88, 90; 47T, 87M; MR8, MR12, M3	24
24 (AUS-3)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	87M/M3	24
Irish-2	ERY, TMP, CIP	-	85, 90; 90A, 87M	18
Irish-1	ERY, TMP, GEN, CIP	+	Non typeable	18
Sporadic	Variable	+	variable	Variable
Nonmultiresistant				
EMRSA-15	ERY, CIP	-	Nontypeable	22
EMRSA-16	ERY, CIP	+	29; MR12	18
SWP	Nil	+	WSPP1 (29, 52, 52A, 80, 3A, 54, 77, 81), WSPP2 (6, 42E, 47, 54, 77, 81; 67R)	24
WA-MRSA-1	ERY, FUS	+	1A (nontypeable), 1B, 52A, 55, 6, 42E, 47, 53, 54, 77, 83A, 85, 88, 90	20
nmMRSA A	Nil	+	Nontypeable	32
nm MRSA B	ERY	+	Nontypeable	258
nmMRSA C	ERY	+	Variable	Variable
nmMRSA D	TET	+	Variable	18
nmMRSA E	Nil	+	Variable	Did not cut
nmMRSA F	Nil	+	Variable	No product
Sporadic	Variable	+	Variable	Variable

^a TET, tetracycline; ERY, erythromycin; TMP, trimethoprim; GEN, gentamicin; CIP, ciprofloxacin; FUS, fusidic acid.

TABLE 3. Distribution of MRSA isolates typed by PFGE in Australian cities in 2000 and 2002

Strain	No. (%) of isolates							
	National totals		Brisbane		Sydney		Canberra	
	2000 (n = 280)	2002 (n = 367)	2000 (n = 23)	2002 (n = 33)	2000 (n = 129)	2002 (n = 169)	2000 (n = 5)	2002 (n = 8)
Nonmultiresistant								
EMRSA-15	30 (10.7)	42 (11.4)	1 (4.3)	1 (3.0)	23 (17.8)	28 (16.6)		
SWP	33 (11.8)	26 (7.1)	9 (39.1)	9 (27.3)	20 (15.5)	6 (3.6)	2 (40.0)	2 (25.0)
WA-MRSA-1	45 (16.1)	59 (16.1)	3 (13.0)	6 (18.2)	4 (3.1)	10 (5.9)		
nmMRSA A	13 (4.6)	36 (9.8)	1 (4.3)	3 (9.1)	9 (7.0)	26 (15.4)	1 (20.0)	1 (12.5)
nmMRSA B	14 (4.9)	18 (4.9)	1 (4.3)	2 (6.1)		1 (0.6)	1 (20.0)	
nmMRSA C	4 (1.4)	10 (2.7)			2 (1.6)	3 (1.8)		
nmMRSA D	2 (0.7)							
nmMRSA E	3 (1.1)	1 (0.3)						
nmMRSA F	2 (0.7)	2 (0.5)						
UK EMRSA-16		1 (0.3)						
Sporadic	10 (3.6)	4 (1.1)	2 (8.7)		3 (2.3)	1 (0.6)	1 (20.0)	
Subtotal	156 (55.7)	199 (54.2)	17 (73.9)	21 (63.6)	61 (47.3)	75 (44.4)	5 (100)	3 (37.5)
Multiresistant								
AUS-2	90 (32.1)	123 (33.5)	5 (21.7)	9 (27.3)	61 (47.3)	88 (52.1)		5 (62.5)
AUS-3	18 (6.3)	39 (10.6)		3 (9.1)	1 (0.8)	2 (1.2)		
Irish-2	1 (0.4)							
Irish-1		3 (0.8)				2 (1.2)		
Sporadic	15 (5.4)	3 (0.8)	1 (4.3)		6 (4.7)	2 (1.2)		
Subtotal	124 (44.3)	168 (45.8)	6 (26.1)	12 (36.4)	68 (52.8)	94 (55.6)	0	5 (62.5)

type 2 *ccr* genes and a *mec* complex that contained intact *mecA* and *mecRI* genes but a truncated *mecI* gene in which only the 5' end was detected. Of the 16 nmMRSA strains tested, 12 harbored the type IV SCC*mec* that is found predominantly in MRSA strains of community origin (34). Type IV SCC*mec* was found in eight different genetic backgrounds (ST22, ST30, ST1, ST93, ST73, ST8, ST80slv, and STnew). ST8, ST45, and ST152 isolates IMVS67, RPH74, and WCH100, respectively, harbored the type V SCC*mec* that has recently been described (18) and reported in Australian community MRSA isolates (35). A second novel SCC*mec* that harbored a class B *mec* complex and the type5 *ccrC* gene was found in ST8 isolate RBH87.

DISCUSSION

The increasing prevalence of MRSA infections in nonhospitalized patients due to both the spread of health care setting-associated strains into the community and the emergence of unique community-associated strains is now a global problem. The substantial and increasing proportions of MRSA isolates in outpatient settings identified in these surveys are of clinical concern. Approximately half of the MRSA isolates in the 2000 survey were due to known hospital-associated clones of ST239- and ST128-MRSA-III (strains AUS-2 and AUS-3) and ST22-MRSA-IV strain (EMRSA-15), with the prevalence of both of these types of isolates slightly increasing in 2002. Of the remainder, the majority were accounted for by nmMRSA clones ST30 (SWP), ST1 (WA-MRSA-1), and ST93 (QLD), all of which have been associated with well-documented community acquisition (28, 34, 49).

The epidemic of health care setting-associated multiresistant MRSA in eastern Australia began in Victoria in the late 1970s (42). The epidemic strain, known as EA MRSA, spread to the

United Kingdom, where it was designated EMRSA-1 (53). The earliest isolate of EA MRSA characterized by MLST, ANS46 (RM4), which was isolated in Melbourne in 1982, belongs to ST239-MRSA-III (25, 38). As described above, the strains prevalent in Australia at present, AUS-2 and AUS-3, belong to the same lineage. ST239-MRSA-III is possibly the most successful international epidemic clone of MRSA and is now present in Australia, Europe, North America, South America, and eastern Asia (2, 12, 39).

EMRSA-15 (ST22-MRSA-IV), an international clone of nmMRSA associated with hospital infection, was first documented in Perth, Australia, in 1997, where it was detected during the preemployment screening of health care workers coming from the United Kingdom, Ireland, and eastern Australia. It was responsible for outbreaks in four hospitals (45). It is apparent from the 2000 and 2002 surveys that this clone is now well established in Brisbane, Adelaide, Sydney, and Perth.

In Australia, community-associated nmMRSA strains were first noted in remote communities in Western Australia in the late 1980s (24, 49). The predominant clone, WA-MRSA-1 (ST1-MRSA-IV) (35), has since spread east throughout the country and was detected in both surveys in all cities except Canberra. Two other community-associated clones have become established more recently, both of them initially in the eastern part of the continent. An epidemic of community-acquired nmMRSA associated with the Polynesian population was noted in all eastern states and the Australian Capital Territory in 1997 (8). This was subsequently shown to be due to the SWP clone (ST30-MRSA-IV), which was initially detected in New Zealand, where it was identified by phage patterns WSP-1 and WSP-2 (15, 34, 48, 59). The 2000 survey confirmed its presence in all eastern capital cities (Brisbane, Sydney, Canberra, Melbourne, and Hobart); the 2002 survey

TABLE 3—Continued

No. (%) of isolates									
Melbourne		Hobart		Adelaide		Perth		Darwin	
2000 (n = 40)	2002 (n = 39)	2000 (n = 2)	2002 (n = 6)	2000 (n = 28)	2002 (n = 36)	2000 (n = 46)	2002 (n = 55)	2000 (n = 7)	2002 (n = 21)
				3 (10.7)	5 (13.9)	3 (6.5)	8 (14.5)		
2 (5.0)	1 (2.6)				2 (5.6)		1 (1.8)		5 (23.8)
1 (2.5)	2 (5.1)	2 (100)	3 (50.0)	5 (17.9)	14 (38.9)	27 (58.7)	22 (40.0)	3 (42.9)	2 (9.5)
1 (2.5)			2 (33.3)	1 (3.6)	3 (8.3)		1 (1.8)		
	1 (2.6)			1 (3.6)	1 (2.8)	11 (23.9)	13 (23.6)		
				2 (7.1)	2 (5.6)	11 (23.9)	13 (23.6)		
				2 (7.1)		5 (9.1)			
				1 (3.6)	1 (2.8)	2 (4.3)			
								2 (28.6)	2 (9.5)
							1 (1.8)		
4 (10.0)	1 (2.6)			2 (7.1)	1 (2.8)	1 (2.2)		1 (14.3)	1 (4.8)
	5 (12.8)	2 (100)	5 (83.3)	17 (60.7)	29 (80.6)	44 (95.7)	51 (92.7)	6 (85.7)	10 (47.6)
22 (55.0)	12 (30.8)		1 (16.7)	2 (7.1)					8 (38.1)
8 (20.0)	22 (56.4)			8 (28.6)	6 (16.7)		3 (5.5)	1 (14.3)	3 (14.3)
						1 (2.2)			
							1 (1.8)		
6 (15.0)				1 (3.6)	1 (2.8)	1 (2.2)			
36 (90.0)	34 (87.2)	0	1 (16.7)	11 (39.3)	7 (19.4)	2 (4.3)	4 (7.3)	1 (14.3)	11 (52.4)

showed that it had spread westward to Adelaide, Darwin, and Perth. The QLD clone (ST93-MRSA-IV) was first identified as a cause of community-acquired infection in the Caucasian population in Ipswich, Queensland, in 2000 (28, 59). In 2000 it was present in the eastern capitals (except Hobart) and Adelaide. Its prevalence increased in the 2002 survey, and it had also spread to Hobart and Perth.

MLST is particularly suited to the study of lineages of *S. aureus*, including MRSA (11). *S. aureus* is clonal and is not subject to frequent genomic recombination. Relying as it does on the enumeration of the allelic profiles of housekeeping genes spread throughout the core genome, MLST provides a logical nomenclature for *S. aureus* clones: the ST precisely defines a strain as having a unique and unambiguous allelic profile and identifies those isolates that have descended from the same recent common ancestor. MLST provides data that are well suited to studies of the global epidemiology and the population biology of bacterial pathogens (12).

The population structure of *S. aureus* has previously been studied by a variety of techniques, including multilocus enzyme electrophoresis, PFGE (29, 30), and MLST (11). We applied MLST to a representative sample of our MRSA isolates and found that they comprised 14 STs. Twelve STs fell into nine clonal complexes, while two STs were singletons. All of the five major clonal complexes previously associated with MRSA were present (12). Three STs were represented in the group of mMRSA isolates, namely, ST239, ST128, and ST8, all of which belong to clonal complex 8 (CC8). MLST did not discriminate between strains AUS-2 and AUS-3. However, a single-locus variant of ST239 was found in an AUS-3 isolate (isolate AH1, ST128). There was much more diversity among nmMRSA isolates, among which 11 different STs were found, including the four major clones already discussed. Furthermore, six of the STs were unique to Australia, namely, STs 73, 93, 129, 75,

80slv, and new. The ST80slv-MRSA-IV isolates belong to the same clonal complex as the ST80-MRSA-IV clone which has been associated with community-acquired and hospital infections in a number of European countries (1, 13, 59). ST73 is a single-locus variant of ST5, and so the ST73-MRSA-IV strain (strain CH97) has a genetic background similar to that of the pediatric clone; however, the SCCmec element is not of the pediatric type (12). ST129 is a single-locus variant of ST78 and ST150. Methicillin-resistant strains of both of the last two STs are registered on the MLST database at <http://www.mlst.net/>. The remaining three unique STs, STs 75, 93, and new, are singletons and may represent new acquisitions of SCCmec.

All of the ST239 isolates and the related ST128 isolate harbored the type III SCCmec, which is the type most frequently found in this pandemic lineage of health care setting-associated mMRSA (11, 38). It is most likely that these strains have been acquired in the hospital environment and taken out into the community by colonized patients. The ST8 Irish-2 strain harbored the pediatric type IV SCCmec that has predominantly been reported in the pandemic ST5 pediatric clone of MRSA (38). We have previously found the novel SCCmec present in strain CH39 to be present in a strain from the epidemic Irish-1 clone. Furthermore, the PFGE patterns of strains CH39 and Irish-1 differ at only two bands (unpublished observations), indicating that EMRSA isolates from the Irish-1 clone are also present in Australia.

The diversity of genetic backgrounds found to harbor the type IV and V SCCmec types in this study suggests that, in Australia, these two types of the cassette are well adapted for the community environment and have probably been acquired by methicillin-susceptible strains of *S. aureus* on multiple occasions. The novel SCCmec harbored by strain RBH87 encodes the class B mec complex that is typical of type IV

TABLE 4. Phenotypic and genotypic characteristics of 33 isolates representative of strains of mMRSA and nmMRSA identified in Australia in the 2000 and 2002 surveys

Isolate (survey yr)	Resistance pattern ^d	Urease test result	Phage susceptibility ^c	Coagulase gene PCR-RFLP pattern ^d	MLST		SCC _{mec} type	Strain designation ^b
					ST, allelic profile	CC		
Multiresistant								
RPAH15 (2000)	TET, ERY, TMP, GEN, CIP	+	88, 85, (90); MR12, MR25, 56B; 56A, 56B, 56C, 67R, 87M	24	239, 2-3-1-1-4-4-3	8	III	AUS-2
RPAH18 (2000)	TET, ERY, TMP, GEN, CIP	+	83A; MR8, MR12, MR25, 622, 56B; 56A, 56B; 56C, 67R, 87M	24	239, 2-3-1-1-4-4-3	8	III	AUS-2
LVH25 (2000)	TET, ERY, TMP, GEN, CIP	+	88, 85, 90; MR8, MR12, 622, 56B; 47T, 56A, 56B; 56C, 90A, 67R, 87M, 13M	24	239, 2-3-1-1-4-4-3	8	III	AUS-2
CH10 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	88; 87M; MR12, MR25, 33, 38, M5	24a	239, 2-3-1-1-4-4-3	8	III	AUS-3
IMVS51 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	85	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
GP35 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	85	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
IMVS20 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	88, 54, 85	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
FMC19 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	Nontypeable	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
RCH66 (2000)	ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	Nontypeable	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
AH41 (2000)	TET, ERY, TMP, GEN, CIP, RIF, HgCl ₂ , PMA	+	Nontypeable	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
RDH81 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	Nontypeable	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
AH1 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	Nontypeable	24	128, 2-3-1-40-4-4-3	8	III	AUS-3
FMC1 (2000)	TET, ERY, TMP, CIP, HgCl ₂ , PMA	+	88, 85	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
AH13 (2000)	TET, ERY, TMP, GEN, CIP, HgCl ₂ , PMA	+	Nontypeable	24	239, 2-3-1-1-4-4-3	8	III	AUS-3
RPH2 (2000)	ERY, CIP, TMP	+	(85), (90), (90A), 13M	18	8, 3-3-1-1-4-4-3	8	IV (pediatric)	Irish-2
LIV79 (2002)	ERY, GEN, CIP	+	Nontypeable	18	239, 2-3-1-1-4-4-3	8	III	sporadic
CH39 (2002)	ERY, TMP, GEN, CIP	+	Nontypeable	Not tested	8, 3-3-1-1-4-4-3	8	Novel (i)	mMRSA G
Nonmultiresistant								
CH16 (2000)	ERY, CIP	-	Nontypeable	22	22, 7-6-1-5-8-8-6	22	IV	EMRSA 15
PAH58 (2000)		+	52, 52A, 80, 55, 54, 77, 84	24	30, 2-2-2-2-6-3-2	30	IV	SWP
PAH1 (2000)	ERY, MVP	+	55, 47, 54, 77, 84	24	30, 2-2-2-2-6-3-2	30	IV	SWP
SJOG30 (2000)	FUS	+	Nontypeable	20	1, 1-1-1-1-1-1-1	1	IV	WAMRSAI
PC8 (2000)	ERY	+	88, 52A, 6, 42E, 47, 53, 54, 83A, 85, 90	20	1, 1-1-1-1-1-1-1	1	IV	WAMRSAI
RBH98 (2000)		+	Nontypeable	32	93, 6-64-44-2-43-55-51	Singleton	IV	QLD
RBH87 (2000)	ERY, CIP	+	Nontypeable	258	129, 22-1-14-41-12-53-31	298	Novel (ii)	nmMRSA B
CH97 (2000)	ERY	+	88, 29, 52A, 79, 47, 53, 54, 90	36	73, 1-4-27-4-12-1-10	5	IV	nmMRSA C
IMVS67 (2000)	TET	+	88, 77, 83A, 84	18	8, 3-3-1-1-4-4-3	8	V	nmMRSA D
RPH74 (2000)		+	54	DNC	45, 10-14-8-6-10-3-2	45	V	nmMRSA E
RDH57 (2000)		+	52A, 79, 53	DNA	75, 36-3-43-34-39-52-49	New	IV	nmMRSA F
RDH29 (2002)	ERY	+	Nontypeable	Not tested	new, 36-new-43-34-new-new-new	Singleton	IV	Sporadic
WCH100 (2002)	GEN	+	Nontypeable	Not tested	152, 46-75-49-44-13-68-60	Singleton	V	Sporadic
RCH88 (2002)	TET, FUS	+	Nontypeable	Not tested	80slv, 1-3-1-14-11-27-10	80	IV	Sporadic
RDH72 (2002)		+	Nontypeable	Not tested	80slv, 1-3-1-14-11-27-10	80	IV	Sporadic

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TABLE 4—Continued

Isolate (survey yr)	Resistance pattern ^a	Urease test result	Phage susceptibility	Coagulase gene PCR-RFLP pattern	MLST		SCCmec type	Strain designation ^b
					ST, allelic profile	CC		
CH36 (2002)	TET, ERY	+	52A, 53, 54, 83A, 85, 88, 90; 47T, 90A, 1648, 67R, 13M; MR8, MR12, MR25, 30, 33, 38, M5, 622	Not tested	8, 3-3-1-1-4-4-3	8	IV	Sporadic

^a TET, tetracycline; ERY, erythromycin; TMP, trimethoprim; GEN, gentamicin; CIP, ciprofloxacin; RIF, rifampin; FUS, fusidic acid; MUP, mupirocin.

^b Synonyms for strain AUS-2 or AUS-3 are Eastern Australia; UK EMRSA-1, -4, and -11; Portuguese/Brazilian; and Vienna. A synonym for strain MRSA G is Irish-1. A synonym for EMRSA 15 is Barnim. Synonyms for strain SWP are WSPPI, WSPPI2, and Oceania. A synonym for strain QLD is pulsotype R.

^c Parentheses mean variable reaction.

^d DNC, did not cut; DNA, did not amplify.

SCCmec and the *ccrC* recombinase gene that is typical of type V SCCmec, indicating that it may be a hybrid of the two types.

As a typing method, MLST provides good reproducibility and the results between centers are comparable. The question might be asked, what role does MLST play in epidemiological studies? One application is the comparison of isolates collected over a short period of time in different geographical locations (43). This is the application that we adopted in this study, and we found MLST to be sufficiently discriminatory for all strains tested. Peacock et al. (43) indicated that the levels of discrimination obtained by MLST and PFGE were comparable if the isolates are grouped into PFGE types on the basis of two or three band differences, which corresponds to the "closely related" category by the criteria of Tenover et al. (52). The combination of MLST and SCCmec typing proved particularly useful in describing the variety of MRSA isolates present in Australia. The diverse lineages described included both previously described international clones and new clones not previously associated with methicillin resistance. While SCCmec typing provided no further discrimination of strains, the presence of SCCmec types IV and V in multiple clones argues strongly for their acquisition by community *S. aureus* isolates with genetic backgrounds more diverse than was previously thought. The ability of some of these clones to spread widely in a relatively short time is further cause for public health concern and may require modification of guidelines for the treatment and control of community-acquired infections due to *S. aureus*.

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