JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2002, p. 3093–3097 Vol. 40, No. 8
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.8.3093–3097.2002

Genotyping of Methicillin-Resistant *Staphylococcus aureus* by Assaying for the Presence of Variable Elements Associated with *mecA*

Flavia Huygens,[1] Graeme R. Nimmo,[2] Jacqueline Schooneveldt,[2] Wendy J. Munckhof,[3] and Philip M. Giffard[1]*

Cooperative Research Centre for Diagnostics, Queensland University of Technology, Brisbane, Queensland 4001,[1] and Microbiology Department, Queensland Health Pathology Service,[2] and Infection Management Services Southern Queensland,[3] Princess Alexandra Hospital, Woolloongabba, Queensland 4102, Australia

Received 9 October 2001/Returned for modification 5 December 2001/Accepted 13 May 2002

The region surrounding *mecA* in methicillin-resistant *Staphylococcus aureus* (MRSA) is highly variable. We describe an approach for the rapid genotyping of MRSA by assaying for the presence or absence of variable or mobile elements previously shown to be associated with the *mecA* region.

DNA-based assays provide a rapid method for the detection and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) (2). The most widely used molecular typing method for the study of the local and global epidemiologies of MRSA is pulsed-field gel electrophoresis (PFGE) of large restriction fragments (1, 21). This method has proved to be very successful for the investigation of nosocomial outbreaks (4, 8, 11, 19) and has also been used to identify epidemic MRSA (EMRSA) clones that have a particular ability to cause major outbreaks and to spread nationally and internationally (6, 7, 16, 20). However, PFGE is time consuming and is best suited to large-scale epidemiological investigations rather than rapid identification procedures in the clinical or pathology laboratory environment. Methicillin resistance in *S. aureus* is encoded by the *mecA* gene that is located within a large section (32 to 60 kb) of chromosomally inserted DNA (14). The polymorphic *mecA* gene region has been used as an epidemiological marker and has also been the basis of studies concerning the evolutionary origin of methicillin resistance in *S. aureus* (17). Most of the polymorphisms observed in the *mecA* region reflect variation in DNA downstream of *mecA* (3, 7, 12, 13, 17).

We have hypothesized that the mobile elements found downstream of *mecA* are a useful resource for the rapid typing of MRSA with simple and rapid gene detection procedures. The rationale for adopting this approach is that a gene detection-based method would be particularly suitable for automation with, e.g., a microtiter plate-based PCR assay, a hybridization array, or a real-time PCR device. We have developed such a method and compared it with PFGE by using a variety of MRSA strains from southeast Queensland, Australia. Southeast Queensland recently experienced an epidemic of gentamicin-susceptible community-acquired MRSA in a background of endemic gentamicin-resistant health care facility-acquired MRSA infection, and it was of particular interest to determine whether this approach could be used to distinguish community-acquired and health care facility-acquired isolates.

* Corresponding author. Mailing address: Cooperative Research Centre for Diagnostics, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland 4001, Australia. Phone: 61 7 3864 2015. Fax: 61 7 3864 1534. E-mail: p.giffard@qut.edu.au.

Sixty-five *S. aureus* isolates were included in the study (Table 1). They were obtained between October 1997 and February 2001 in southeast Queensland. The classification of infections as community acquired or nosocomial (hospital or nursing home) was done in accordance with the definitions of the Centers for Disease Control and Prevention (15). Primers were designed to amplify fragments of mobile elements previously shown to be associated with the *mecA* region. The primer sequences are listed in Table 2 and were derived from previously published sequences (GenBank database [http://www.ncbi.nlm.nih.gov]) with the following accession numbers: AF142100 (*mecR1* [170 bp] deletion; strain LHH1), AF181950 (*Cla*I::*mecA* downstream vicinity; strain HUC19), M19465 (pUB110), J01764 (pT181), L29436 (pI258), and M18086 (IS*256*). Cell extracts were made by suspending a single colony in 100 μl of sterile distilled H_2O and boiling for 10 min. The 50-μl PCR mixtures consisted of 10 μl of cell lysate, 0.2 mM concentrations of each deoxynucleoside triphosphate (Roche), 0.5 μM concentrations of each primer, 1 U of Platinum *Taq* DNA polymerase (Gibco BRL and Life Technologies), 10× PCR buffer, and 1.5 mM $MgCl_2$. DNA amplification consisted of an initial cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 10 min. PCR products were visualized on 2% agarose gels stained with ethidium bromide.

S. aureus ATCC 49476 (*mecA* positive) was used as a control for the amplification of the *mecA*-associated regions. Organisms used as negative controls were *mecA*-negative *S. aureus* strains ATCC 29213 (β-lactamase positive), ATCC 25923, and NCTC 6571, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, and *Serratia marcescens* ATCC 8100. PFGE of chromosomal DNA and analysis of the banding patterns were performed as described previously by Nimmo et al. (15). Multilocus sequence typing (MLST) was carried out on selected strains as specified by Enright et al. (9). The sequences obtained were compared with the sequences found at the MLST website (http://www.mlst.net/).

Previously published PFGE results (15) for 31 isolates

TABLE 1. MRSA strains, their mode of acquisition, host ethnicity, and PFGE and *mec* region types

Isolate	Acquisition	Ethnicity	PFGE type	*mec* region type
K703484[a]	Hospital	Caucasian	G1	3
B8-31[a]	Pathcentre		K	6
J710566[a]	Nursing home	Caucasian	C	6
A803355[a]	Community	Polynesian	A0	2
A806533[a]	Community	Polynesian	A0	2
A823547[a]	Community	Aboriginal	A1	2
A830538[a]	Community	Caucasian	A0	2
B826559[a]	Community	Polynesian	A0	2
C801535[a]	Hospital	Caucasian	D	8
B827549[a]	Nursing home	Caucasian	E	4
C810534[a]	Community	Caucasian	A1	2
D808118[a]	Hospital	Caucasian	L	4
D817541[a]	Community	Caucasian	A0	2
D821522[a]	Community	Polynesian	A2	2
D828354[a]	Hospital	Caucasian	E	4
D828570[a]	Community	Polynesian	A0	1
E802537[a]	Community	Polynesian	A3	1
E803543[a]	Community	Polynesian	A0	1
E804531[a]	Hospital	Caucasian	I	1
E822547[a]	Community	Polynesian	A0	1
E822485[a]	Hospital	Caucasian	B	13
F810539[a]	Community	Caucasian	A0	1
F829549[a]	Community	Caucasian	D	1
G821561[a]	Community	Polynesian	A1	1
G823530[a]	Community	Polynesian	A0	2
F809715[a]	Community	Polynesian	A0	1
F809718[a]	Community	Polynesian	A0	1
H823537[a]	Community	Polynesian	A0	2
I823541[a]	Hospital	Caucasian	G2	3
I802552[a]	Hospital	Polynesian	A4	2
I816601[a]	Community	Polynesian	A0	2
K705613[a]	Hospital (GR)[c]	Caucasian	F2	5
K711532[a]	Hospital (GR)	Caucasian	F3	5
K714372[a]	Hospital (GR)	Caucasian	F4	5
E812560[a]	Hospital	Caucasian	J	3
66460/98	Community	New Zealander	A0	7
67043/98	Community	New Zealander	A0	4
IP00M3616	Community	Polynesian	A7	7
IP00M11030	Community	Polynesian	A0	6
IP00M11247	Community	Polynesian	A0	1
IP00M16156	Community	Polynesian	A6	7
IP00M16287	Hospital	Polynesian	A5	2
IP00M17427	Community	Polynesian	A0	2
IP01M2090	Community	New Zealander	A5	1
66593/98	Community	Caucasian	A0	1
IP00M3393	Community	Caucasian	A0	1
IP00M3360	Hospital	Caucasian	A0	1
IP00M15446	Community	Caucasian	R	1
IP00M16759	Community	Caucasian	A5	1
IP00M17753	Community	Torres Strait Islander	R1	1
IP01M430	Community	Caucasian	R	1
IP01M1984	Hospital	Caucasian	A0	1
68284/98	Community	Polynesian	A5	3
73742/99	Community	New Zealander	A5	1
IP00M11998	Community	Caucasian	R	1
IP00M13517	Hospital	Caucasian	S2	9
IP01M1081	Hospital	Caucasian	Q	2
IP01M2046	Hospital	Caucasian	P1	1
IP00M14848	Hospital	Caucasian	S1	3
IP01M81	Hospital	Caucasian	S	10
IP00M14235	Hospital	Caucasian	O	3
IP00M17006	Hospital	Caucasian	S	12
PA01M18489	Hospital (EMRSA-15)	Caucasian	—[b]	11

[a] Previously described strains (14).
[b] The pulsotype of this isolate was essentially identical to that of the EMRSA-15 strain described by O'Neill et al. (18).
[c] GR, gentamicin resistant (15).

TABLE 2. Primers used for *mec* region typing

GenBank accession no.	Target	Primer	Sequence	PCR amplicon size (bp)
AF181950	HVR	HVRPF	TGC AAC ATC TAA CTC CAA CC	300
		HVRP2	TGG AGC TTG GGA CAT AAA TG	
M19465	pUB110	DF4	TAA CAT GCT GTT TTA ACC	331
		MR1	TGA ACG TGG CTC TGA CCG	
AF181950	Ins117	MDVF1	GCT TGG GTA ACT TAT CAT GG	215
		IS117R1	CTA AAT ATA GTA AAT TAC GG	
J01764	pT181	DF1	CAC GAG ATG AAA TGA TTT GG	255
		DR1	GCA TCT GCA TTA TCT TTA CG	
L29436	pI258	DF2	ATA GAA AGG AAA AAA CAT GG	295
		DR2	TTT ATA CGT AAA CCA GTC GG	
L29436	pI258	EF1	CAA AGT GTA AGT AAC CCG	270
		ER1	TAT ACG TAA ACC AGT CGG	
AF142100	*mecR1*	AF1	TGA TAT GGG TAT TTG G	406
		AR1	TTT TTC ACA GTC ATT GTC C	
M18086	IS*256*	DF3	ACT AAT GGA AAA TCA ACG	371
		DR3	TTT TTT TCT GAT AAT AAA CG	

showed that nine pulsotypes (A to E, G, I, J, L) could be distinguished. In the second subset of isolates tested, all of the strains from Polynesian patients and the majority of strains from Caucasian patients fell into pulsotype A subtypes (Table 1). However, there were a small number of isolates that showed unique pulsotypes not found in the original set of isolates (O, P1, P2, Q, R, R1, S, S1, S2). These pulsotypes were mostly found in isolates from Caucasian patients. Each strain was analyzed by PCR for fragments of *mecR1*, the hypervariable region (HVR), pUB110, pI258, pT181, IS*256*, and the junction between the downstream common region and Ins117. Thirteen different patterns were found (Table 3). During optimization of this method, there was essentially complete reproducibility. As a final validation after all isolates had been typed, 42 isolates were partially retyped such that three positive and three negative reactions from each primer pair were checked. The results obtained were completely concordant with the original typing. The majority of the isolates (23 in all) showed pattern 1 (Table 3). The second largest group of strains fell into polymorphic pattern 2. The *mecA*-positive *S. aureus* control strain (ATCC 49476) possesses the HVR, pT181, pI258, *mecR1*, and IS*256* regions. As expected, none of the negative control strains gave positive reactions with any of the primer pairs.

TABLE 3. *mec* region types

No. of isolates	PCR amplicon[a]							*mec* region type
	HVR	pUB110	Ins117	pT181	pI258	*mecR1*	IS*256*	
23	+	−	+	−	−	+	−	1
15	+	−	+	−	−	−	−	2
6	+	+	+	−	−	+	+	3
4	+	−	+	+	−	−	−	4
3	+	−	−	−	+	+	+	5
3	+	−	−	−	−	−	−	6
3	−	−	+	−	−	−	−	7
1	+	−	−	−	−	+	−	8
1	+	+	+	−	−	−	−	9
1	−	−	+	−	−	+	+	10
1	−	−	−	−	−	−	+	11
1	+	+	+	−	−	−	+	12
1	+	+	+	−	−	+	−	13

[a] Presence (+) or absence (−) of the region in the strain.

An important rationale for this study was the development of a simple approach for identifying MRSA clones associated with community acquisition in southeast Queensland, Australia. The data in Table 1 show an apparent association between community isolation and pulsotypes A1-5 pulsotype and *mec* region types 1, 2, and 7. PFGE and *mec* region typing were similarly sensitive in their abilities to detect community-acquired isolates, and the use of both methods together resulted in the identification of all of the community-acquired isolates. The correlation between *mec* region typing and PFGE in a more diverse population was tested by examination of the health care facility-acquired strains. It is evident that there are significant differences between the results from the two procedures, with instances of multiple pulsotypes in single *mec* region types and multiple *mec* region types in single pulsotypes occurring (e.g., *mec* region type 3 was found in six different pulsotypes [O, S1, A5, G1, G2, and J]). Therefore, a background genotyping method, such as PFGE, is necessary if the degrees of relationship between isolates need to be determined accurately. The resolving power of the two techniques in concert clearly exceeded that of either technique in isolation, with virtually all health care facility-derived isolates possessing a unique *mec* region type-pulsotype combination. The only instance of good correlation between the two methods occurred with the three gentamicin-resistant isolates, which were all pulsotype F and *mec* region type 5. This probably reflects a very close relationship among the three isolates that is consistent with their acquisition by cross infection. Two phenomena may contribute to the lack of correlation between PFGE and the *mec* region in the more divergent health care facility-acquired isolates. First, the *mec* region itself may undergo rearrangements with very high frequency. Second, recombination between different lineages within *S. aureus* may transmit particular *mec* regions into different backgrounds. Studies with *S. aureus* and other largely nonclonal organisms have shown that virulent or highly transmissible clones can emerge from nonclonal backgrounds (5). In these instances, dissemination outruns recombination. Consequently, a method that targets

only a small portion of the genome has some ability to detect such clones.

The downstream primer for the HVR was found to coincide with a 2-bp deletion in the HVR of strain HUC19 (17), which is distinct from the HVR of the *S. aureus* strain described by Ryffel et al. (20). With a new reverse primer (5′-GATTACAAAATGGAGCTTGGG-3′), HVR-negative strains became positive for this region; therefore, by use of this primer, the HVR allele found in the previously described *S. aureus* strain (20) can be distinguished from the HVR allele found in *S. aureus* HUC19 (17). The one strain that remains negative for the HVR is the EMRSA-15 strain, and this fact warrants further investigation.

One example of the ability of *mec* region typing to discriminate between two strains of the same pulsotype is provided by the two pulsotype D strains. One of these strains is *mec* region type 1 (strain F829549) and the other is *mec* region type 8 (strain C801535). In order to determine whether PFGE was failing to discriminate strains that were significantly divergent, these two strains were subjected to MLST. The two isolates were virtually identical, with a single-base difference present at the *tpi* sequence and no differences between the isolates at the other six loci. At the *tpi* locus, F829549 was allele 4 while C801535 had an A-to-C change at position 2. The alleles at the other loci were as follows: *arc* allele 22, *aro* allele 1, *glp* allele 14, *gmk* allele 23, *pta* allele 12, and *yqi* allele 31. It was therefore concluded that these strains are very closely related but may still be discriminated by *mec* region typing. This suggests that variation at the *mec* locus does occur frequently. It was also interesting that both strains were of a novel sequence type and that the allele at the *tpi* locus of isolate C801535 was previously unreported.

In a recent review by Hiramatsu et al. (10), the classification of the staphylococcal cassette chromosome (SCC) *mec* element was described in detail. SCC *mec* types I-IV are defined by the particular combination of two parts, namely, a *ccr* complex (types 1, 2, and 3) plus a *mec* complex (classes A and B). Depending on the arrangement of the *ccr* complex plus the *mec* complex, an SCC *mec* type is defined. The *mec* A complex comprises *mecR1* (upstream of *mecA*) plus *mecA* and IS*431*, while the *mec* B complex differs from the A complex only by the Δ*mecR1* region. Furthermore, type II SCC *mec* contains the pUB110 region, and type III SCC *mec* contains the pT181 region.

In our study, four *mec* region patterns (3, 9, 12, and 13) were found to contain pUB110 regions, and one *mec* region pattern contained pT181. It can therefore be said that four of the *mecA* downstream pattern types resemble SCC *mec* type II and one *mecA* downstream pattern type resembles SCC *mec* type III based on the presence of pUB110 and pT181, respectively.

One final item to note regarding our community-acquired isolates and SCC *mec* types is that Hiramatsu et al. (10) described all community-acquired MRSAs as being SCC *mec* type IV and as not carrying any resistance genes other than *mecA*. In our study, we found one community-acquired MRSA isolate that harbored the pT181 gene and one community-acquired MRSA isolate that contained the pUB110 gene. This is the first description of community-acquired MRSA isolates harboring other resistance genes along with *mecA*.

In conclusion, we have found that information for identifying MRSA clones can be obtained by assaying for the presence or absence of a small number of variable or mobile stretches of DNA that are commonly found in association with the *mecA* gene. This may prove useful in the design of a straightforward, multiplexed, real-time or microplate-based PCR assay for the rapid identification of particular MRSA clones.

This work was supported in part by the Australian Federal Government Program for Cooperative Research Centres and in part by Queensland Health Pathology Services.

We thank Adele Millis for assistance with the PFGE.

REFERENCES

1. **Bannerman, R. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
2. **Bergeron, M. G., and M. Ouellette.** 1998. Preventing antibiotic resistance through rapid genotypic identification of bacteria and of their antibiotic resistance genes in the clinical microbiology laboratory. J. Clin. Microbiol. **36:**2169–2172.
3. **Corso, A., I. Santos Sanches, M. Aires de Sousa, A. Rossi, and H. de Lencastre.** 1998. Spread of a methicillin-resistant and multiresistant epidemic clone of *Staphylococcus aureus* in Argentina. Microb. Drug Resist. **4:**277–288.
4. **Cox, R. A., C. Conquest, C. Mallaghan, and R. R. Marples.** 1995. A major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage type (EMRSA-16). J. Hosp. Infect. **29:**87–106.
5. **Day, N. P. J., C. E. Moore, M. C. Enright, A. R. Berendt, J. M. Smith, M. F. Murphy, S. J. Peacock, B. J. Spratt, and E. J. Feil.** 2001. A link between virulence and ecological abundance in natural populations of *Staphylococcus aureus*. Science **292:**114–116.
6. **de Lencastre, H., E. P. Severina, R. B. Roberts, B. N. Kreiswirth, A. Tomasz, et al.** 1996. Testing the efficacy of a molecular surveillance network: methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VREF) genotypes in six hospitals in the metropolitan New York City area. Microb. Drug Resist. **2:**343–351.
7. **de Sousa, M. A., I. S. Sanches, M. L. Ferro, M. J. Vaz, Z. Saraiva, T. Tendeiro, J. Serra, and H. de Lencastre.** 1998. Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. J. Clin. Microbiol. **36:**2590–2596.
8. **Dominguez, M. A., H. de Lencastre, J. Linares, and A. Tomasz.** 1994. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. J. Clin. Microbiol. **32:**2081–2087.
9. **Enright, M. C., N. P. J. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
10. **Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. **9:**486–493.
11. **Jorgensen, M., R. Givney, M. Pegler, A. Vickery, and G. Funnel.** 1996. Typing multidrug-resistant *Staphylococcus aureus*: conflicting epidemiological data produced by genotypic and phenotypic methods clarified by phylogenetic analysis. J. Clin. Microbiol. **34:**398–403.
12. **Kreiswirth, B., J. Kornblum, R. D. Arbeit, W. Eisner, J. N. Maslow, A. McGeer, D. E. Low, and R. P. Novick.** 1993. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science **259:**227–230.
13. **Leski, R., D. Oliveira, K. Trzcinski, I. S. Sanches, M. A. de Sousa, W. Hryniewicz, and H. de Lencastre.** 1998. Clonal distribution of methicillin-resistant *Staphylococcus aureus* in Poland. J. Clin. Microbiol. **36:**3532–3539.
14. **Matsuhashi, M., M. D. Song, F. Ishino, M. Wachi, M. Doi, M. Inoue, K. Ubukata, N. Yamashita, and M. Konno.** 1986. Molecular cloning of the gene of a penicillin binding protein supposed to cause high resistance to β-lactam antibiotics in *Staphylococcus aureus*. J. Bacteriol. **167:**975–980.
15. **Nimmo, G. R., J. Schooneveldt, G. O'Kane, B. McCall, and A. Vickery.** 2000. Community acquisition of gentamicin-sensitive methicillin-resistant *Staphylococcus aureus* in Southeast Queensland, Australia. J. Clin. Microbiol. **38:**3926–3931.
16. **Oliveira, D., I. S. Sanches, M. Tamayo, G. Ribeiro, R. Maro, D. Costa, and H. de Lencastre.** 1998. Virtually all MRSA infections in the largest Portuguese hospital are caused by two internationally spread multiresistant strains: the "Iberian" and the "Brazilian" clones of MRSA. Clin. Microbiol. Infect. **4:**373–384.
17. **Oliveira, D. C., S. W. Wu, and H. de Lencastre.** 2000. Genetic organization of the downstream region of the *mecA* element in methicillin-resistant *Staphylococcus aureus* isolates carrying different polymorphisms of this region. Antimicrob. Agents Chemother. **44:**1906–1910.

18. **O'Neill, G. L., S. Murchan, A. Gil-Setas, and H. M. Aucken.** 2001. Identification and characterization of phage variants of a strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-15). J. Clin. Microbiol. **39:** 1540–1548.
19. **Richardson, J. F., and S. Reith.** 1993. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. J. Hosp. Infect. **25:**45–52.
20. **Roman, R. S., J. Smith, M. Walker, S. Byrne, K. Ramotar, B. Dyck, A. Kabai, and L. E. Nicolle.** 1996. Rapid geographic spread of a methicillin-resistant *Staphylococcus aureus* strain. Clin. Infect. Dis. **25:**698–705.
21. **Ryffel, C., R. Bucher, F. H. Kayser, and B. Berger-Bachi.** 1991. The *Staphylococcus aureus mec* determinant comprises an unusual cluster of direct repeats and codes for a gene product similar to the *Escherichia coli sn*-glycerophosphoryl diester phosphodiesterase. J. Bacteriol. **173:**7416–7422.
22. **Tenover, F., R. Arbeit, R. Goering, P. Mickelsen, B. Murray, D. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal CAN restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2333–2339.