JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2006, p. 236–238 Vol. 44, No. 1
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.1.236–238.2006

Novel PCR-Restriction Fragment Length Polymorphism Analysis for Rapid Typing of Staphylococcal Cassette Chromosome *mec* Elements

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Received 4 September 2005/Returned for modification 13 September 2005/Accepted 14 October 2005

We developed a novel PCR-restriction fragment length polymorphism test for the *ccrB* gene by using HinfI and BsmI for rapid typing of staphylococcal cassette chromosome *mec* (SCC*mec*). When tested with reference strains and methicillin-resistant *Staphylococcus aureus* isolates, the method proved to be valid and useful for rapid identification of four SCC*mec* types, especially type IV.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a common worldwide hospital pathogen. In recent years, MRSA has also emerged as a significant community pathogen, especially in previously healthy children with no recognizable risk factors, and is predominantly associated with skin and soft-tissue infections (2). Some strains of community-acquired MRSA (CA-MRSA) are particularly virulent and capable of causing life-threatening diseases (14). Clinicians should consider MRSA as a potential pathogen in patients with suspected *S. aureus* infections in a community setting, should obtain appropriate material for bacterial culture, and should follow up on the results of susceptibility testing of all *S. aureus* isolates (2, 4). In addition, development of efficient molecular typing methods is needed for local monitoring of the prevalence of CA-MRSA infection or propagation of CA-MRSA strains and infection control.

The genetic basis of the methicillin resistance of MRSA is the presence of *mecA*, a gene coding for the low-affinity penicillin-binding protein PBP2′ (15, 18), carried by a mobile genetic element designated staphylococcal cassette chromosome *mec* (SCC*mec*) (7, 9, 11). The SCC*mec* element is characterized by the presence of terminal inverted and direct repeats, two site-specific recombinases (*ccrA* and *ccrB*), and the *mecA* gene complex (7, 11). Elucidation of the structure of SCC*mec* has disclosed four structural types of MRSA (9). Three types are typically found among hospital-acquired MRSA (HA-MRSA) isolates. Type I (34 kb) was identified in a 1960s isolate (strain NCTC10442); type II (53 kb) was identified in a 1982 isolate (strain N315) which is ubiquitous in Japan, Korea, and the United States; and type III (67 kb) was identified in a 1985 isolate (strain 85/2082) which is prevalent in Germany, Austria, India, and other South Asian and Pacific areas (6, 12). Type IV (20 to 24 kb) is generally carried by CA-MRSA isolates (9, 12), and at least four subtypes have been reported (9). In 2004, type V was reported in an isolate of CA-MRSA, in which the only difference was the presence of a restriction-modification system composed of the *ccrC* gene and the surrounding open reading frames (10).

Conventional multiplex PCR (13, 17) or quantitative PCR (qPCR) (3) assays for rapid SCC*mec* typing have been developed, based on sequence variations in the *mecA* complex and/or the *ccr* gene complex. These methods have proved useful as tools for rapid tentative identification (17) or molecular epidemiological screening of the SCC*mec* types (3). However, conventional PCR assays using five to nine primer pairs in a single-tube assay can give various sensitivities and a chance of contamination, requiring further probe hybridization for the target genes. The qPCR assay requires expensive reagents and instruments, limiting its use in many microbiology laboratories. Thus, the development of an alternative rapid and cost-effective confirmatory test would be of value.

In this study, we developed a novel rapid SCC*mec* typing method by PCR amplification of the *ccrB* gene in combination with restriction fragment length polymorphism (RFLP) with restriction endonucleases HinfI and BsmI. The *ccr* gene complex, *ccrA* and *ccrB*, encodes products responsible for integration and excision of the SCC*mec* element (8). The *ccrB* gene was chosen as the target gene to be amplified because it has highly conserved sequences compared to the *ccrA* gene. To design primers, the *ccrB* genes of SCC*mec* types I (GenBank accession number AB033763), II (GenBank accession number D86934), III (GenBank accession number AB037671), and IV (GenBank accession numbers AB097677, AB096217, AB063172, and AB063173) were aligned with ClustalW to localize homologous regions. The forward primer was 5′-GGC TAT TAT CAA GGC AAT TTA CC-3′, and the reverse primer was 5′-ACT TTA TCA CTT TTG ACT ATT TCG-3′ (PrimerExpress 2.0 software; PE Biosystems, Foster City, Calif.) (Fig. 1). Chromosomal DNA from MRSA was extracted with the Wizard genomic DNA preparation kit (Promega, Madison, Wis.) by using lysostaphin (0.5 mg/ml) and RNase (0.3 mg/ml); 1 μg of each sample was used as template DNA. Thermal cycling was set at 30 cycles of 30 s for denaturation at 94°C, 1 min for annealing at 50°C, and 2 min for elongation at 72°C. Purified PCR products were digested with HinfI and BsmI (New En-

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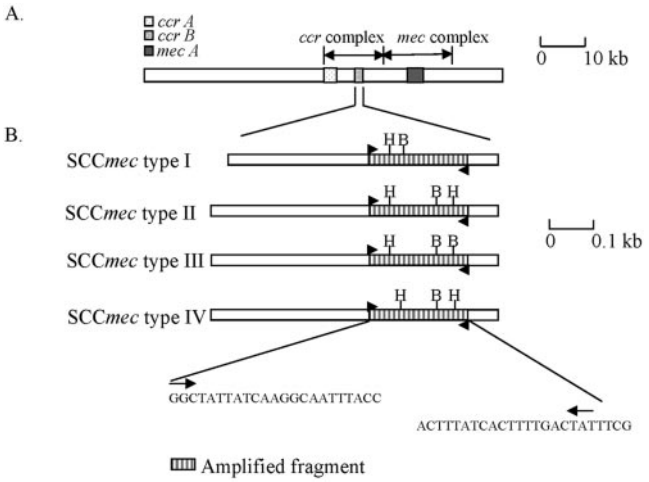

FIG. 1. Physical map of SCC*mec* structure showing the *ccrB* gene and restriction sites for PCR-RFLP analysis. (A) Genomic structure of SCC*mec*. Locations of the *mec* complex and *ccr* complex are illustrated. (B) Sequence of the *ccrB* gene amplified by PCR in this study. Thick arrows indicate the primers. H, HinfI; B, BsmI.

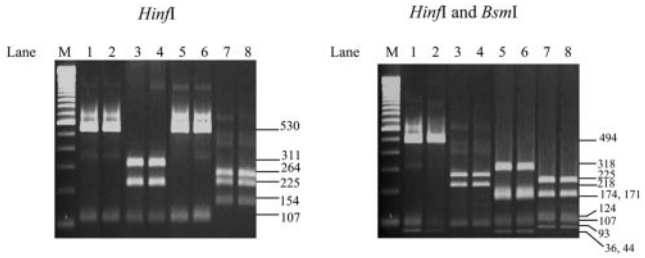

FIG. 2. Agarose gel electrophoresis showing PCR-RFLP patterns of the 643-bp amplified *ccrB* gene fragment from reference MRSA strains of four SCC*mec* types digested by HinfI (left) and by HinfI and BsmI (right). Lanes: 1, HPV107; 2, NCTC10442; 3, BK2464; 4, N315; 5, ANS46; 6, 85/2082; 7, MW2; 8, JCSC4044; M, 100-bp ladder marker.

gland Biolabs Inc.) and analyzed by 2% agarose gel electrophoresis.

The PCR-RFLP method was validated with two sets of representative strains of SCC*mec* types I to IV (HPV107, BK2464, ANS46, MW2, NCTC10442, N315, 85/2082, and JCSC4044). As shown in Fig. 2, four distinctive RFLP patterns were observed. We then applied the PCR-RFLP SCC*mec* typing method to 42 MRSA isolates obtained from clinical cultures during a 6-month period; they were selected on the basis of antibiogram results. Results showed 9 isolates of SCC*mec* type IV (21%), 22 of type II (53%), and 11 of type III (26%). These results matched exactly those obtained by the multiplex PCR assay (18; data not shown). Interestingly, two out of nine MRSA-SCC*mec* type IV isolates were from patients with cellulitis or necrotizing fasciitis in a community setting. The other MRSA-SCC*mec* type IV isolates were from clinical samples in a hospital setting, suggesting that CA- and HA-MRSA are likely to coexist in our hospital (Table 1). A similar situation has been previously reported in hospitals in the United States (1). The genetic background of nine MRSA-SCC*mec* type IV isolates was further analyzed by multilocus sequence typing and pulsed-field gel electrophoresis (PFGE). All SCC*mec* type IV MRSA isolates from the hospital setting showed PFGE type A (sequence type 72 [ST72], 1-4-1-8-4-4-3), except for one isolate (PFGE type C; ST239, 2-3-1-1-4-4-3), while isolates from the community setting revealed PFGE type B (ST1, 1-1-1-1-1-1-1) or PFGE type D (ST375, 19-23-15-2-19-20-42). This suggests that the PFGE type A strain may propagate in our hospital environment.

Although the PCR-RFLP method used in this study includes two steps of restriction digestion, with HinfI followed by BsmI, one-step restriction digestion with HinfI is enough for the identification of SCC*mec* type II and IV strains. The second step with BsmI digestion is only necessary for discriminating type I and III strains. Since no SCC*mec* type I strains were found among 42 clinical MRSA isolates tested, identifying SCC*mec* types II to IV by one-step digestion with HinfI seems possible. With one-step digestion, we have successfully discriminated nine isolates of SCC*mec* type IV.

TABLE 1. Results of antibiogram analysis and genotyping of nine MRSA isolates of SCC*mec* type IV identified by PCR-RFLP analysis of the *ccrB* gene

Strain	Origin	Sample	Antibiogram[d] result										MLST[a] (ST[e], allelic profile[f])	PFGE pattern[g]
			CEF	CIP	CLI	ERY	GEN	OXA	PEN	TZP	TET	VAN		
K-1	HA	Sputum	R	S	S	R	S	R	R	R	S	S	72, 1-4-1-8-4-4-3	A
K-2	HA	Wound	I	S	S	R	S	R	R	R	S	S	72, 1-4-1-8-4-4-3	A
K-3	HA	Sputum	S	S	S	R	S	R	R	I	S	S	72, 1-4-1-8-4-4-3	A
K-4	HA	Urine	S	S	S	R	S	R	R	I	S	S	72, 1-4-1-8-4-4-3	A
K-5	CA	Wound[b]	S	S	R	S	S	R	R	R	S	S	375, 19-23-15-2-19-20-42	D
K-6	HA	Ear discharge	S	S	S	R	S	R	R	R	S	S	72, 1-4-1-8-4-4-3	A
K-7	HA	Nasal swab	S	S	S	R	R	R	R	R	S	S	72, 1-4-1-8-4-4-3	A1
K-8	CA	Wound[c]	S	S	S	R	S	R	R	I	S	S	1, 1-1-1-1-1-1-1	B
K-9	HA	Nasal swab	S	S	S	R	I	R	R	I	S	S	239, 2-3-1-1-4-4-3	C

[a] MLST, multilocus sequence typing.
[b] Cellulities.
[c] Necrotizing fasciitis.
[d] Antibiotic abbreviations: CEF, cephalothin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; OXA, oxacillin; PEN, penicillin; TZP, piperacillin-tazobactam; TET, tetracycline; VAN, vancomycin. R, resistant; S, susceptible; I, intermediate.
[e] ST, sequence type.
[f] Allelic profile: *arcC aroE glpF gmk pta tpi yqiL*.
[g] According to criteria of Tenover et al. (19).

This study demonstrates the value of a novel PCR-RFLP analysis of the *ccrB* gene as a rapid typing method for the SCC*mec* elements (types I to IV) in HA- or CA-MRSA strains. Since the *ccrC* gene of type V shows approximately 37.4% homology to the *ccrB* gene of types I to IV at the nucleic acid level, the PCR-RFLP method is not likely to identify type V. We confirmed that the PCR-RFLP method is a highly specific, simple, time-effective alternative to multiplex PCR methods (3, 13, 17). The PCR-RFLP test can be achieved with just one pair of primers and one or two restriction enzymes ($3 to $4 per sample), and more than 100 samples can be processed in a day. The cost and efficiency of the PCR-RFLP method are comparable to those of the qPCR method published previously (3). Especially, it seems to be a very useful tool for rapid identification of SCC*mec* type IV MRSA in clinical laboratories, since serious MRSA infection has been increasingly reported in persons without identified predisposing risk factors, including recent healthcare exposure (5).

We thank Keiichi Hiramatsu (Juntendo University, Tokyo, Japan) and Herminia de Lencastre (Instituto de Technologia Quimica e Biologica, Oeiras, Portugal) for reference strains. We thank Hee Sun Sim for excellent technical assistance.

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