ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1998, p. 717–720 Vol. 42, No. 3
0066-4804/98/$04.00+0

Analysis of Diversity of Mutations in the *mecI* Gene and *mecA* Promoter/Operator Region of Methicillin-Resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*

NOBUMICHI KOBAYASHI,* KOKI TANIGUCHI, AND SHOZO URASAWA

Department of Hygiene, Sapporo Medical University School of Medicine, S-1, W-17, Chuo-ku, Sapporo, 060, Japan

Received 23 June 1997/Returned for modification 22 October 1997/Accepted 4 December 1997

Genomic diversity of mutation in the *mecI* gene or *mecA* promoter/operator region was analyzed for clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE). In most MRSA strains, a single base substitution was detected in either the *mecI* (three different positions) or the *mecA* promoter (two different positions), while a 28-base deletion in *mecI* was found in one strain. In contrast, no mutation was detected in these gene sequences of MRSE strains.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is defined by the production of a specific penicillin-binding protein (PBP), PBP-2a, that has a reduced binding affinity for beta-lactam compounds (4, 25). PBP-2a functions as a transpeptidase in cell wall synthesis in MRSA at high concentrations of beta-lactam antibiotics that inhibit the growth of methicillin-susceptible strains with normal PBPs. This additional PBP is encoded by the structural gene *mecA* on the chromosome (18), which has also been detected in methicillin-resistant strains of other staphylococcal species (10, 16, 19, 24). The *mecA* gene is a component of a large DNA fragment designated *mec* DNA, which is located at the specific site of the *S. aureus* chromosome and has been suggested to be transmitted from other bacterial species (1–3, 7, 27). The acquisition of *mec* DNA is considered to be the first genetic requisite for methicillin resistance of staphylococci.

Expression of PBP-2a is controlled by two regulator genes on *mec* DNA, *mecI* and *mecR1*, located upstream of *mecA*, which encode *mecA* repressor protein and signal transducer protein, respectively (5, 14, 21). An MRSA carrying intact *mecI* and *mecR1* together with *mecA* has been called pre-MRSA, which is represented by prototype *S. aureus* strain N315 (6). Since intact *mecI* product strongly represses the expression of PBP-2a, the pre-MRSA is apparently methicillin susceptible (6, 14). Hence, it is hypothesized that removal of the repressor function for *mecA* is a prerequisite for constitutive expression of methicillin resistance in *S. aureus* with *mec* DNA. Indeed, the deletion of *mecI* or point mutations in the *mecI* gene has been found in a number of methicillin-resistant staphylococcal isolates (6, 8, 12, 20). In some strains, point mutations were detected in the *mecA* promoter region corresponding to a presumptive operator of *mecA*, i.e., the binding site of the repressor protein. Furthermore, genetic alteration on the chromosome which causes high methicillin resistance was presented as another mechanism of evolution of MRSA, although the details are not known (6).

TABLE 1. Properties of MRSA strains analyzed in this study

Year of isolation	Strain	Coagulase type[a]	RFLP pattern of coagulase gene[b]	MIC (μg/ml) of oxacillin[c]	RFLP pattern of *mecI* gene[d]
1993	SH1	II	A	256	1
	SH12	II	A	256	1
	SH13	II	A	256	3
	SH19651	IV	C	256	2
	SH15	IV	C	1,024	2
	SH20	II	A	1,024	1
	SH22	VII	B	1,024	1
	SH24	II	A	256	1
	SH27	II	A	256	1
1994	SH153	II	A	256	1
	SH155	II	A	512	1
	SH158	II	A	256	1
	SH165	II	A	512	1
	SH212	II	A	512	2
1995	SH219	II	A	256	1
	SH320	II	A	256	1
	SH321	II	A	256	1
	SH324	II	A	512	1
	SH326	II	A	512	1
	SH327	II	A	512	1

[a] Coagulase type (I to VIII) was determined with coagulase type-specific antisera.
[b] RFLP patterns based on the staphylocoagulase gene were classified as described previously (11).
[c] Susceptibility to oxacillin was measured by a broth microdilution assay with cation-supplemented Mueller-Hinton broth (BBL) containing 2% NaCl as recommended by the National Committee for Clinical Laboratory Standards (15).
[d] Differentiation of the RFLP patterns to detect mutation in the *mecI* gene was performed as described previously (12; see text).

TABLE 2. Oligonucleotide primers

Primer name	Sequence[a]
mecI1	5′-AATGGCGAAAAAGCACAACA-3′
mecI2	5′-GACTTGATTGTTTCCTCTGTT-3′
mecI3	5′-GCACAACAAATTTCTGAGCG-3′
mecI4	5′-TGGACTCCAGTCCTTTTGC-3′
mecI5	5′-CTTGTAGAAGAAAGTGATAT-3′
Pr-mecA	5′-CCTGTATTGGCCAATTCCAC-3′
Pr-mecR	5′-AATGGAATTAACGTGGAGAC-3′

[a] The location of each primer on *mec* DNA is shown in Fig. 1.

* Corresponding author. Mailing address: Department of Hygiene, Sapporo Medical University School of Medicine, S-1, W-17, Chuo-ku, Sapporo, 060, Japan. Phone: 81-11-611-2111. Fax: 81-11-612-1660. E-mail: nkobayas@sapmed.ac.jp.

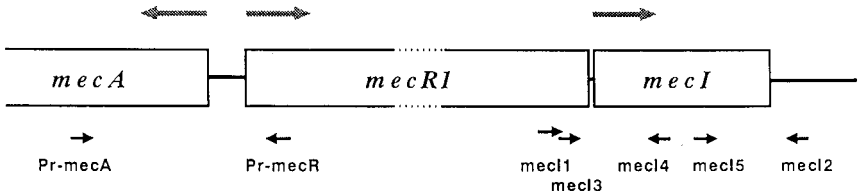

FIG. 1. Schematic representation of the *mecA*, *mecR1*, and *mecI* genes and locations of primers (Table 1) used in this study. The direction of the nucleotide extension reaction (5′ to 3′) of each primer is shown by a solid arrow. The shaded arrows indicate the directions of transcription of the structural genes.

We previously studied the presence of *mec* regulator genes in a number of clinical isolates of MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Most strains were found to possess *mecI* and *mecR1* genes, and the possibility of mutation in the *mecI* or *mecA* promoter region was suggested (12). In the present study, we analyzed the genetic diversity of mutations in the *mecI* and *mecA* promoters of 20 MRSA and 11 MRSE strains isolated at Sapporo Medical University Hospital in Japan between 1993 and 1995. The presence of the *mecI* and *mecR1* genes in these strains has been confirmed previously (12). Oxacillin MICs for the MRSA and MRSE strains ranged from 256 to 1,024 μg/ml and from 16 to 256 μg/ml, respectively. Table 1 lists some characteristics of MRSA strains. Most of the MRSA strains belonged to coagulase type II.

Two point mutations have been most frequently detected in the *mecI* gene (base substitutions C to T at position 202 and T to A at position 260) (6, 20), yielding an additional *Mse*I site (5′T ↓ TAA3′) in the *mecI* sequence. Digestion of PCR product containing the complete open reading frame of the *mecI* gene with *Mse*I enabled us to differentiate three patterns of restriction fragment length polymorphism (RFLP) described in the previous study (12), with pattern 1 representing a prototype of the *mecI* gene, pattern 2 indicating the presence of mutation at position 202, and pattern 3 having a smaller fragment than that in pattern 1, suggesting a deletion of nucleotides. As shown in Table 1, the *mecI* gene of most MRSA isolates was assigned to RFLP pattern 1, while three strains and one strain were classified as RFLP patterns 2 and 3, respectively. All 11 MRSE strains analyzed in the present study exhibited *mecI* RFLP pattern 1.

DNA samples for PCR were prepared with achromopeptidase as described previously (10). Either of two primer pairs, mecI1 and mecI2 or mecI3 and mecI2, was used to amplify DNA fragments containing the *mecI* gene, and another primer pair, Pr-mecA and Pr-mecR, was used to amplify the promoter regions of *mecA* and *mecR1*. Sequences of these primers are shown in Table 2, and the locations of the primers in *mec* DNA are depicted in Fig. 1. DNA amplification was performed with a thermal cycler as described previously (10). The presence of amplified PCR product (481 or 469 bp for the *mecI* gene and 748 bp for the *mecA* promoter region) was ascertained by electrophoresis on a 1% agarose gel and staining with ethidium bromide. With the PCR-amplified DNA fragments as templates, nucleotide sequences of the *mecI* gene (369 bases) and the promoter regions of *mecA* and *mecR1* (99 bases) were determined by the dideoxynucleotide chain termination method with the Sequenase kit, version 2.0 (United States Biochemical Corp., Cleveland, Ohio). Primers listed in Table 2 were used for DNA sequencing.

The nucleotide sequences of the *mecI* gene and the promoter regions of *mecA* and *mecR1* of MRSA strains employed in this study were compared with those of *S. aureus* N315 (5), a prototype strain possessing intact *mec* regulator genes. In all the MRSA strains, a point mutation or a deletion was detected in one of the two gene sequences, except in one strain (SH212) which had mutations in both sequences. As shown in Table 3, the mutations detected in MRSA strains were classified into seven groups (M1 to M7); five of these mutations, M1, M2, M4, M5, and M7, were identified for the first time in the present study. In contrast, no mutation was found in the *mecI*

TABLE 3. Mutations detected in *mecI* gene and *mecA* promoter region of MRSA strains

Mutation group	MRSA strain(s)	Mutation in *mecI* gene		Mutation in *mecA* promoter region[a]
		Nucleotide position change	Codon change	
M1	SH20	43 (G→T)	Val15→Phe	None
M2	SH22	163 (A→T)	Lys55→stop codon	None
M3	SH15, SH19651	202 (C→T)	Gln68→stop codon	None
M4	SH212	202 (C→T)	Gln68→stop codon	C→A
M5	SH13	Deletion of 28 bases (28–55 or 29–56)[b]	Stop codon after the 11th amino acid	None
M6	SH1, SH12, SH24, SH27, SH153, SH158, SH165, SH219, SH320, SH321, SH324, SH326, SH327	None	None	C→A
M7	SH155	None	None	G→A

[a] Positions of nucleotide substitutions are shown in Fig. 2B.
[b] Presumptive deleted sequence is indicated in Fig. 2A.

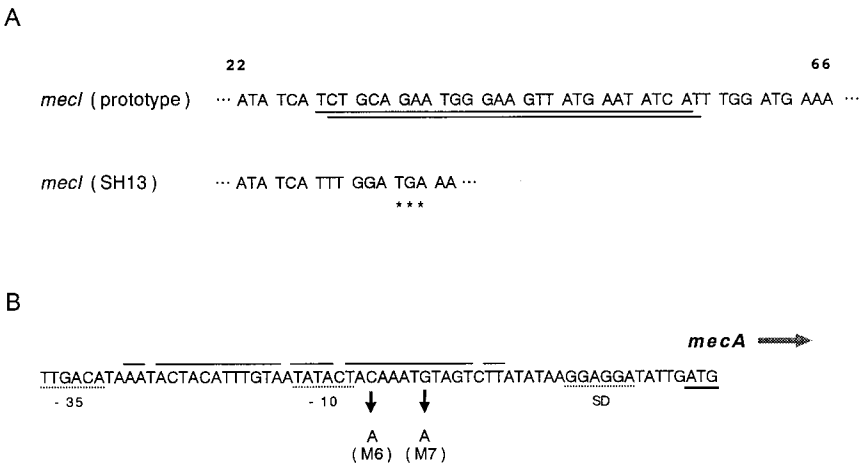

FIG. 2. (A) Partial nucleotide sequences of the *mecI* gene from prototype *S. aureus* N315 (5) and MRSA SH13. Presumptive nucleotide sequences which were deleted in the SH13 *mecI* gene are underlined. Triple asterisks under the *mecI* gene sequence of strain SH13 denote a putative stop codon caused by the deletion. (B) Nucleotide sequence of the *mecA* promoter region. Positions of base substitution (M6 and M7) are shown by solid arrows. Putative −35 and −10 promoter sequences and Shine-Dalgarno (SD) sequences are shown by dotted lines, and a pair of palindrome sequences are indicated by solid lines above the sequence. The direction of *mecA* gene transcription is shown by a shaded arrow, with the initiation codon indicated by solid underlining.

or *mecA* promoter region of the MRSE strains or in the *mecR1* promoter regions of the MRSA or MRSE strains.

A nucleotide substitution at position 202 (M3) in *mecI* was detected in three strains (SH15, SH19651, and SH212) which had exhibited *mecI* RFLP pattern 2. Other base substitutions, M1 in strain SH20 and M2 in strain SH22, generated an amino acid change and a new termination codon, respectively. The deletion of 28 bases (M5) near the 5′ end of the *mecI* gene was found in MRSA strain SH13, which had shown *mecI* RFLP pattern 3. This base deletion, shown in Fig. 2A, caused a premature termination at position 33 on the *mecI* gene.

Point mutations in the *mecA* promoter region (M6 and M7) were detected in 15 strains, although M7 was found in only one strain (SH155). Both mutations M6 and M7 are located downstream of the *mecA* promoter sequence (−10) on a palindrome structure corresponding to the presumptive operator of the *mecA* gene (Fig. 2B) (6, 18). It was reported previously that a C-to-A substitution (corresponding to M6) caused a decrease in the stacking energy of the stem (6); this also seems to be the case with the G-to-A (M7) nucleotide substitution detected in the present study.

A mutation observed in strain SH212 was assigned to group M4, because a point mutation was found in the *mecI* and the *mecA* promoter regions, coinciding with M3 and M6, respectively. In spite of the presence of double mutations in SH212, no significant difference in the MIC of oxacillin was seen for SH212 and other MRSA strains with a single mutation. The emergence of this peculiar MRSA strain can be explained as follows. Although this strain originally possessed a point mutation only in the *mecI* gene, a mutation in the *mecA* promoter region occurred subsequently and the mutant was selected to escape from the *blaI* repressor protein, which is a plasmid factor controlling *blaZ*, a penicillinase gene (9, 13, 26, 28). This assumption is based on the findings that the *mecA* promoter sequence is quite similar to that of *blaZ* on the plasmid and that expression of PBP-2a is also regulated by the *bla* regulator in some MRSA strains (17, 22, 23).

The finding that all 11 MRSE strains harbored no mutations in the two gene sequences was unexpected. Although the expression of *mecI* and *mecA* has not been confirmed in these strains, some mechanisms may be considered as explanations of the methicillin resistance of these MRSE strains, e.g., genomic alteration in a *mec* regulator gene other than *mecI* or the presence of certain unknown genetic factors controlling *mecA* expression that may suppress regulation by *mecI*. In any case, methicillin resistance in MRSE strains is presumably mediated by a mechanism different from that observed in MRSA strains.

REFERENCES

1. **Archer, G. L., and D. M. Niemeyer.** 1994. Origin and evolution of DNA associated with resistance to methicillin in staphylococci. Trends Microbiol. **2:**343–347.
2. **Archer, G. L., D. M. Niemeyer, J. A. Thanassi, and M. J. Pucci.** 1994. Dissemination among staphylococci of DNA sequences associated with methicillin resistance. Antimicrob. Agents Chemother. **38:**447–454.
3. **Couto, I., H. de Lencastre, E. Severina, W. Kloos, J. A. Webster, R. J. Hubner, I. S. Sanches, and A. Tomasz.** 1996. Ubiquitous presence of a *mecA* homologue in natural isolates of *Staphylococcus sciuri*. Microb. Drug Resist. **2:**377–391.
4. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:**513–516.
5. **Hiramatsu, K., K. Asada, E. Suzuki, K. Okonogi, and T. Yokota.** 1992. Molecular cloning and nucleotide sequence determination of the regulator region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). FEBS Lett. **298:**133–136.
6. **Hiramatsu, K.** 1995. Molecular evolution of MRSA. Microbiol. Immunol. **39:**531–543.
7. **Hiramatsu, K., N. Kondo, and T. Ito.** 1996. Genetic basis for molecular epidemiology of MRSA. J. Infect. Chemother. **2:**117–129.
8. **Hürlimann-Dalel, R. L., C. Ryffel, F. H. Kayser, and B. Berger-Bächi.** 1992. Survey of the methicillin resistance-associated genes *mecA*, *mecR1-mecI*, and *femA-femB* in clinical isolates of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **36:**2617–2621.
9. **Imanaka, T., T. Himeno, and S. Aiba.** 1987. Cloning and nucleotide sequence of the penicillinase antirepressor gene *penJ* of *Bacillus licheniformis*. J. Bacteriol. **169:**3867–3872.
10. **Kobayashi, N., H. Wu, K. Kojima, K. Taniguchi, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, and I. Kurokawa.** 1994. Detection of *mecA*, *femA*, and *femB* genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiol. Infect. **113:**259–266.
11. **Kobayashi, N., K. Taniguchi, K. Kojima, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, and I. Kurokawa.** 1995. Analysis of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* by a molecular typing method based on coagulase gene polymorphisms. Epidemiol. Infect. **115:**419–426.
12. **Kobayashi, N., K. Taniguchi, K. Kojima, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, I. Kurokawa, and N. Watanabe.** 1996. Genomic diversity of *mec* regulator genes in methicillin-resistant *Staphylococcus au-*

reus and *Staphylococcus epidermidis*. Epidemiol. Infect. **117:**289–295.
13. **Kobayashi, T., Y. F. Zhu, N. J. Nicholls, and J. O. Lampen.** 1987. A second regulatory gene, *blaR1*, encoding a potential penicillin-binding protein required for induction of β-lactamase in *Bacillus licheniformis*. J. Bacteriol. **169:**3873–3878.
14. **Kuwahara-Arai, K., N. Kondo, S. Hori, E. Tateda-Suzuki, and K. Hiramatsu.** 1996. Suppression of methicillin resistance in a *mecA*-containing pre-methicillin-resistant *Staphylococcus aureus* strain is caused by the *mecI*-mediated repression of PBP 2′ production. Antimicrob. Agents Chemother. **40:**2680–2685.
15. **National Committee for Clinical Laboratory Standards.** 1993. Approved standard M7-A3. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Ryffel, C., W. Tesch, I. Birch-Machin, P. E. Reynolds, L. Barberis-Maino, F. H. Kayser, and B. Berger-Bächi.** 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gene **94:**137–138.
17. **Ryffel, C., F. H. Kayser, and B. Berger-Bächi.** 1992. Correlation between regulation of *mecA* transcription and expression of methicillin resistance in staphylococci. Antimicrob. Agents Chemother. **36:**25–31.
18. **Song, M. D., M. Wachi, M. Doi, F. Ishino, and M. Matsuhashi.** 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. **221:**167–171.
19. **Suzuki, E., K. Hiramatsu, and T. Yokota.** 1992. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. Antimicrob. Agents Chemother. **36:**429–434.
20. **Suzuki, E., K. Kuwahara-Arai, J. F. Richardson, and K. Hiramatsu.** 1993. Distribution of *mec* regulator genes in methicillin-resistant *Staphylococcus* clinical strains. Antimicrob. Agents Chemother. **37:**1219–1226.
21. **Tesch, W., C. Ryffel, A. Strässle, F. H. Kayser, and B. Berger-Bächi.** 1990. Evidence of a novel staphylococcal *mec*-encoded element (*mecR*) controlling expression of penicillin-binding protein 2′. Antimicrob. Agents Chemother. **34:**1703–1706.
22. **Ubukata, K., N. Yamashita, and M. Konno.** 1985. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **27:**851–857.
23. **Ubukata, K., R. Nonoguchi, M. Matsuhashi, and M. Konno.** 1989. Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. J. Bacteriol. **171:**2882–2885.
24. **Ubukata, K., R. Nonoguchi, M. D. Song, M. Matsuhashi, and M. Konno.** 1990. Homology of *mecA* gene in methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus simulans* to that of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **34:**170–172.
25. **Utsui, Y., and T. Yokota.** 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **28:**397–403.
26. **Wittman, V., and H. C. Wong.** 1988. Regulation of the penicillinase genes of *Bacillus licheniformis*: interaction of the pen repressor with its operators. J. Bacteriol. **170:**3206–3212.
27. **Wu, S., C. Piscitelli, H. de Lencastre, and A. Tomasz.** 1996. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mecA* from a methicillin susceptible strain of *Staphylococcus sciuri*. Microb. Drug Resist. **2:**435–441.
28. **Zhu, Y. F., I. H. A. Curran, B. Joris, J.-M. Ghuysen, and J. O. Lampen.** 1990. Identification of BlaR, the signal transducer for β-lactamase production in *Bacillus licheniformis*, as a penicillin-binding protein with strong homology to the OXA-2 β-lactamase (class D) of *Salmonella typhimurium*. J. Bacteriol. **172:**1137–1141.