Detection of *mecA*, *femA*, and *femB* genes in clinical strains of staphylococci using polymerase chain reaction

N. KOBAYASHI¹, H. WU¹, K. KOJIMA¹, K. TANIGUCHI¹, S. URASAWA¹,
N. UEHARA², Y. OMIZU², Y. KISHI², A. YAGIHASHI²
AND I. KUROKAWA²

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

² Department of Laboratory Diagnosis, School of Medicine, Sapporo Medical
University, S-1, W-16, Chuo-ku, Sapporo 060, Japan

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SUMMARY

MecA, a structural gene located on the chromosome of *Staphylococcus aureus*, characterizes methicillin-resistant *S. aureus* (MRSA), and *femA* and *femB* (*fem*) genes encode proteins which influence the level of methicillin resistance of *S. aureus*. In order to examine effectiveness of detecting *mecA* and *fem* genes in identification of MRSA, the presence of these genes in 237 clinically isolated strains of staphylococci was investigated by polymerase chain reaction (PCR). An amplified *mecA* DNA fragment of 533 base pairs (bp) was detected in 100% of oxacillin-resistant *S. aureus*, in 16.7% of oxacillin-sensitive *S. aureus*, in 81.5% of *S. epidermidis*, and in 58.3% of other coagulase-negative staphylococci (CNS). While the PCR product of *femA* (509 bp) or *femB* (651 bp) was obtained from almost all the *S. aureus* strains except for five oxacillin-resistant strains (2.5%), neither of these genes were detected in CNS. Therefore, the detection of *femA* and *femB* together with *mecA* by PCR was considered to be a more reliable indicator to identify MRSA by differentiating it from *mecA*-positive CNS than single detection of *mecA*.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial bacterial infection in many countries. Coagulase-negative staphylococci (CNS) derived from normal skin flora have also been recognized as nosocomial pathogens, and the emergence of multiply drug-resistant strains, which may mostly be ascribed to the acquisition of extrachromosomal DNA, is a matter of recent concern [1, 2]. It has been established that the production of an additional penicillin-binding protein PBP-2' (PBP-2a), with low-affinity for beta-lactam antibiotics, is mainly involved in the mechanism of methicillin resistance of *S. aureus* [3]. While the PBP-2', which is encoded by a chromosomal structural gene designated as *mecA*, is usually induced by beta-lactam antibiotics, it is known to be constitutively produced in some MRSA [4, 5]. Further

epidemiological studies revealed that *mecA* genes are also distributed widely among CNS, and are associated with methicillin-resistance [6–8].

Recently two chromosomal *mec* regulator genes *mecR1* and *mecI* have been identified [9, 10]. Surveys of the distribution of *mec* regulator genes among clinical isolates of methicillin-resistant staphylococci indicated that *mecI* encodes the repressor protein of the *mecA* gene and it is deleted or mutated in methicillin-resistant strains [11]. Although the mechanism of regulation of the *mecA* gene has not been completely elucidated, the presence of the *mecA* gene in staphylococci has been considered recently as a molecular basis for the identification of MRSA or methicillin-resistant CNS, even though the strain appears methicillin-sensitive by the measurement of minimum inhibitory concentration (MIC) [12]. On the basis of these findings, attempts have been made to identify MRSA by polymerase chain reaction (PCR) amplification of *mecA* gene fragments derived not only from isolated strains but also from clinical specimens directly [13–15]. However, it has also been recognized that detection of a certain marker which is specific for *S. aureus* is needed to distinguish MRSA from methicillin-resistant CNS, in addition to demonstrating the *mecA* gene by PCR.

Besides the *mec* regulator genes, *femA* and *femB* genes on the chromosome have been shown to encode proteins which considerably affect the level of methicillin resistance of *S. aureus* [16]. Although *fem* genes were suggested to be specific for *S. aureus* [17], distribution of these genes in staphylococci has not been fully established. The first aim of this study was to develop a system that differentiated MRSA and *mecA*-positive CNS by the detection of *fem* genes together with the *mecA* gene by PCR. Secondly, to determine whether or not the presence of *fem* and *mecA* genes in clinical isolates were associated with a high level of drug resistance as observed for certain *S. aureus* strains [16].

MATERIALS AND METHODS

Bacterial strains and antimicrobial susceptibility to antibiotics

A total of 237 staphylococci (198 *S. aureus*, 27 *S. epidermidis*, 3 *S. capitis*, 2 *S. haemolyticus*, and 7 unidentified CNS) were employed in this study. These bacterial strains were isolated from clinical specimens of 194 patients admitted to a hospital in Sapporo during the period between January 1993 and June 1993. Staphyslide-test (bioMérieux, Inc., France) was used to examine coagulase production of each strain. Coagulase type of 50 *S. aureus* strains isolated in an early stage of the present study was determined by using coagulase type-specific antiserum; they were type II (68%), VII (22%), IV (6%), III (2%), and V (2%). Identification of bacterial species and determination of MIC were performed by the use of MicroScan WalkAway[®]-96 (Baxter Diagnostics, Inc., West Sacramento, U.S.A.). MIC of the following antibiotics was measured; oxacillin (MIPIC), ampicillin (ABPC), piperacillin (PIPC), cefazolin (CEZ), cefmetazole (CMZ), cefotiam (CTM), imipenem (IPM), gentamicin (GM), minocycline (MINO), erythromycin (EM), clindamycin (CLDM), sulfamethoxazole-trimethoprim (ST), ofloxacin (OFLX), and vancomycin (VCM). Although MRSA is also defined by measuring MIC of oxacillin ($\geq 4 \mu\text{g/ml}$) [18], such strains were described as oxacillin-RSA (oxacillin-resistant *S. aureus*) in this study in order to distinguish it from the term 'MRSA' which indicated *S. aureus* that possessed the *mecA* gene.

Table 1. Sequences of oligonucleotide primers and their location in the *mecA*, *femA* and *femB* genes

Target gene	Primer name	Nucleotide sequence (5'-3')	Product length (base pairs)	Location (nucleotide nos)
<i>mecA</i>	Mec-A1	(+) AAAATCGATGGTAAAGGTTGGC	533	1282-1303
	Mec-A2	(-) AGTTCTGCAGTACCGGATTTGC		1739-1814
<i>femA</i>	Fem-A1	(+) AGACAAATAGGAGTAATGAT	509	595-614
	Fem-A2	(-) AAATCTAACACTGAGTGATA		1084-1103
<i>femB</i>	Fem-B1	(+) TTACAGAGTTAACTGTTACC	651	1904-1923
	Fem-B2	(-) ATACAAATCCAGCACGCTCT		2535-2554

Preparation of bacterial DNA samples

A bacterial colony was suspended in TNE buffer (10 mM Tris-HCl, 0.1 M-NaCl, 1 mM-EDTA, pH 7.5). After centrifugation, the pellet was resuspended in 10 μ l of achromopeptidase (10000 U/ml; Wako Pure Chemical Industries) and incubated at 37 °C for 10 min. Then 50 μ l of 0.5 M-KOH was added to lyse the bacterial cells and incubated for 5 min, followed by neutralization with 50 μ l of 1 M-Tris-HCl (pH 6.76). The supernatant obtained after centrifugation was used as the DNA sample for PCR.

Polymerase chain reaction

Based on the nucleotide sequences of *mecA*, *femA* and *femB* genes [4, 16], the oligonucleotides listed in Table 1 were synthesized and employed as PCR primers. Using 10 μ l of template DNA prepared as described above, DNA amplification was performed in 100 μ l of reaction mixture that contained 200 μ M each of dATP, dCTP, dGTP, and dTTP, 100 pM each of a pair of primers, 1.0 unit of Replitherm[®] Thermostable DNA polymerase (Bokusui Brown Inc.), 10 mM-Tris-HCl (pH 8.3), 50 mM-KCl, and 1.5 mM-MgCl₂. The reactions were allowed to proceed with 25 cycles of denaturation (94 °C, 1 min), annealing (57 °C, 1 min), and primer extension (72 °C, 2 min). The amplification product (10 μ l) was analysed by electrophoresis on 1 % agarose gel and visualized with u.v. light after staining with ethidium bromide.

RESULTS

A total of 198 clinical isolates of *S. aureus* employed in this study were classified into 156 (78.8 %) oxacillin-RSA and 42 (21.2 %) oxacillin-sensitive *S. aureus* (oxacillin-SSA). Figure 1 shows electrophoretic patterns of the DNA products after PCR using three representative oxacillin-RSA strains. The DNA fragments of 533, 509 and 651 bp were amplified from *mecA*, *femA*, and *femB* genes, respectively. The detection rate of each amplified gene is summarized in Table 2. *MecA* was detected in 100 % of oxacillin-RSA, in 16.7 % of oxacillin-SSA, and in 81.5 % of *S. epidermidis*. Among other CNS, 2 *S. haemolyticus* (100 %), 1 *S. capitis* (33.3 %), and 4 unidentified CNS (57.1 %) possessed *mecA*. Detection rates of *femA* and *femB* in *S. aureus* were 89.4 % and 97.0 %, respectively, but neither of these genes were found in CNS.

Detection patterns of *mecA*, *femA*, and *femB* in *S. aureus* are summarized in Table 3. In 135 (86.5 %) strains of oxacillin-RSA and 7 (16.7 %) strains of

Fig. 1. Agarose gel electrophoresis of PCR product amplified from *mecA*, *femA*, and *femB* genes. These genes from three oxacillin-resistant *S. aureus* strains, SH-102 (lanes 1-3), SH-104 (lanes 4-6), and SH-109 (lanes 7-9) are shown. Lanes 1, 4 and 7 are *mecA* fragment; lanes 2, 5 and 8, *femA* fragment; and lanes 3, 6 and 9, *femB* fragment. M, DNA marker fragments.

Table 2. Detection of *mecA*, *femA* and *femB* genes by PCR

Species	Total no. of isolates	PCR positive strains (%)		
		<i>mecA</i>	<i>femA</i>	<i>femB</i>
<i>S. aureus</i> (total)	198	163 (82.3)	177 (89.4)	192 (97.0)
oxacillin-RSA	156	156 (100.0)	136 (87.2)	150 (96.1)
oxacillin-SSA	42	7 (16.7)	41 (97.6)	42 (100.0)
<i>S. epidermidis</i>	27	22 (81.5)	0	0
Other CNS*	12	7 (58.3)	0	0

* Three strains of *S. capitis*, two strains of *S. haemolyticus*, and seven unidentified strains of coagulase-negative *Staphylococci* are included.

Table 3. Detection pattern of *mecA*, *femA* and *femB* genes in *S. aureus*

Species	Detection of PCR product			No. of isolates
	<i>mecA</i>	<i>femA</i>	<i>femB</i>	
Oxacillin-resistant <i>S. aureus</i>	+	+	+	135
	+	+	—	1
	+	—	+	15
	+	—	—	5
Oxacillin-sensitive <i>S. aureus</i>	+	+	+	7
	—	—	+	1
	—	+	+	34

Table 4. Antibiotic resistance of clinical isolates of staphylococci

Species	Total no. isolated	Rate of antibiotic resistance												
		MIPIC	ABPC	PIPC	CEZ	CMZ	CTM	IPM	GM	MINO	EM	CLDM	ST	OFLX
<i>S. aureus</i> oxacillin-RSA (<i>mecA</i> -positive, <i>femA</i> and/or <i>femB</i> -positive)	151	100-0	100-0	100-0	100-0	98-7	99-3	90-7	82-8	35-8	100-0	99-3	2-0	76-8
oxacillin-RSA (<i>mecA</i> -positive, <i>femA</i> , <i>femB</i> -negative)	5	100-0	100-0	100-0	100-0	100-0	100-0	100-0	80-0	20-0	100-0	100-0	0-0	80-0
oxacillin-SSA (<i>mecA</i> -positive)	7	0-0	85-7	71-4	28-6*	28-6*	28-6*	28-6*	57-1*	14-3	71-4	42-9†	0-0	28-6
oxacillin-SSA (<i>mecA</i> -negative)	35	0-0†	74-2†	51-4†	0-0†	0-0†	0-0†	0-0†	14-3†	0-0†	31-4†	2-9†	0-0	5-7†
<i>S. epidermidis</i> (<i>mecA</i> -positive)	22	59-1	90-9*	81-8†	68-2*	63-6*	68-2*	63-6*	77-3†	0-0	54-5	59-0	45-5	50-0
<i>S. epidermidis</i> (<i>mecA</i> -negative)	5	0-0	40-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
Other CNS (<i>mecA</i> -positive)	7	100-0	100-0	100-0	100-0	100-0	100-0	100-0	57-1	57-1	0-0	28-6	0-0	71-4
Other CNS (<i>mecA</i> -negative)	5	40-0	60-0	60-0	40-0	40-0	40-0	40-0	0-0	0-0	0-0	20-0	0-0	0-0

The breakpoints of MIC for antibiotic resistances were as follows: MIPIC, ≥ 4 µg/ml; ABPC, > 8 µg/ml; PIPC, > 8 µg/ml; PIPC, > 64 µg/ml; CEZ, > 16 µg/ml; CMZ, > 32 µg/ml; CTM, > 16 µg/ml; IPM, > 8 µg/ml; GM, > 8 µg/ml; MINO, > 4 µg/ml; EM, > 4 µg/ml; CLDM, > 2 µg/ml; ST, > 2 µg/ml; OFLX, > 4 µg/ml; VCM, > 16 µg/ml.

, † Statistically significant difference (, $P < 0.05$; † $P < 0.01$) between *mecA*-positive and *mecA*-negative strains of oxacillin-SSA, *S. epidermidis*, and other CNS.

† Statistically significant difference ($P < 0.01$) between *mecA*-positive *S. aureus* (156 oxacillin-RSA and 7 oxacillin-SSA) and *mecA*-negative *S. aureus* strains (35 oxacillin-SSA).

oxacillin-SSA, all the three genes were detected. Although five oxacillin-RSA strains were *fem*-negative despite repeated PCR experiments, all the other *S. aureus* strains were *femA* and/or *femB*-positive.

The relationship between antibiotic resistance of individual *Staphylococcus* species and the presence of *mecA*, *femA*, and *femB* genes was investigated (Table 4). In *S. aureus* and *S. epidermidis*, significant differences in resistance to beta-lactam antibiotics were observed between *mecA*-positive and *mecA*-negative strains. Similarly, in other CNS, all the *mecA*-positive strains were resistant to beta-lactam antibiotics, whereas resistant rate of *mecA*-negative CNS to these antibiotics ranged from 40–60%. OFLX-resistance was significantly more common among *mecA*-positive than *mecA*-negative *S. aureus*. However, there was no significant difference in antimicrobial resistance rates between *fem*-positive and *fem*-negative strains of oxacillin-RSA.

DISCUSSION

The usefulness of the PCR assay for the detection of bacterial and viral pathogens has been established. Although PCR amplification of the *mecA* gene fragment has also been applied to the identification of MRSA, the method proved to be unreliable because the *mecA* gene is also found in some CNS [7, 8]. In the present study, *mecA* was detected not only in *S. aureus* (100% of oxacillin-RSA, 16.7% of oxacillin-SSA) but also in 74.4% of CNS strains. As a means for excluding *mecA*-positive CNS in the identification of MRSA, the simultaneous detection of *femA* and *femB* genes has been proposed previously [17]. In the report, *fem* genes were possessed by almost all MRSA strains, whereas 18 strains (17.8%) of *fem*-negative methicillin-sensitive *S. aureus* and one (1.1%) *femB*-positive *S. epidermidis* were also detected. In our present study, *femA* and/or *femB* genes were detected in all *S. aureus* isolates except five strains (2.5%), but these genes were not found in *S. epidermidis*, *S. haemolyticus*, or *S. capitis* strains. Thus, our data confirmed the usefulness of the simultaneous detection of *mecA* and *fem* genes by PCR for identification of MRSA. However, it should be noted that *fem* genes were not detected in very small number of *S. aureus* and that the detection rate of *femB* (97.0%) was higher than *femA* (89.4%). Further analysis of the distribution of these genes in staphylococci will be needed.

FemA and *femB* genes, which are located distantly from the *mecA* gene on the chromosome, encode proteins of approximately 48 and 47 kDa, respectively [16]. Analysis of the *femA* product indicated that this protein is associated with the expression of high-level methicillin resistance without affecting PBP-2' production. The significance of the *fem* genes in the mechanism of methicillin-resistance was supported by the demonstration that a *S. aureus* strain with *femA* inactivated lost the methicillin-resistance trait, but that transduction of *fem* genes restored the resistance. Another biochemical analysis suggested that *femA* product may be involved in the metabolism of cell wall synthesis [19]. In our study, however, no significant difference in antimicrobial susceptibility was observed between *fem* gene-negative MRSA and *femA* and/or *femB*-positive MRSA. Further, all the oxacillin-SSA strains were found to possess either of these genes. Therefore, the influence of *fem* gene products on methicillin resistance in the

current *S. aureus* strains appears to be slight. Methicillin resistance in *S. aureus* may be significantly regulated by recently identified *mecR1* and *mecI* genes, or other unidentified factors.

In this study, it was of note that 16.7% of the MRSA were judged as oxacillin-sensitive strain by automated MIC analysis. Such strains, namely *mecA*-positive but probably non-PBP-2'-producing strains, have been detected previously and referred to as 'cryptically' methicillin-resistant strains [14]. The clinical problem posed by such strains is that during chemotherapy with beta-lactam antibiotics, production of PBP-2' may be induced, converting them into oxacillin-resistant strains as demonstrated *in vitro* [20]. For this reason, detection of *mecA* gene is also indispensable for precise differentiation of MRSA and the PCR method employed in this study will be a useful technique in clinical laboratories.

Widespread distribution of the *mecA* gene among CNS has recently been regarded as one of the reasons responsible for the increase of multidrug-resistant CNS [6, 7, 14]. Consistent with these observations, approximately 60% of *mecA*-positive *S. epidermidis* and all of the *mecA*-positive other CNS were resistant to beta-lactam antibiotics in our study. Accordingly, detection of the *mecA* gene in CNS may also provide useful information in estimating the potential antimicrobial resistance of the strain. However, the finding that 40% of *mecA*-positive *S. epidermidis* were sensitive to oxacillin, and that some *mecA*-negative CNS were resistant to oxacillin suggest that certain factors other than PBP-2' may be involved in methicillin-resistance in CNS.

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