Epidemiol. Infect. **113** (1994). 259–266

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

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(*Accepted 26 May 1994*)

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 (MPIPC), 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 ($\geqslant 4\,\mu g/ml$) [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.

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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)
mecA	Mec-A1	(+) AAAATCGATGGTAAAGGTTGGC	533	1282–1303
	Mec-A2	(−) AGTTCTGCAGTACCGGATTTGC		1739–1814
femA	Fem-A1	(+) AGACAAATAGGAGTAATGAT	509	595–614
	Fem-A2	(−) AAATCTAACACTGAGTGATA		1084–1103
femB	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 μl of achromopeptidase (10000 U/ml; Wako Pure Chemical Industries) and incubated at 37 °C for 10 min. Then 50 μl of 0·5 M-KOH was added to lyse the bacterial cells and incubated for 5 min, followed by neutralization with 50 μl 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 μl of template DNA prepared as described above, DNA amplification was performed in 100 μl of reaction mixture that contained 200 μM 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-$MgCl_2$. 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 μl) 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

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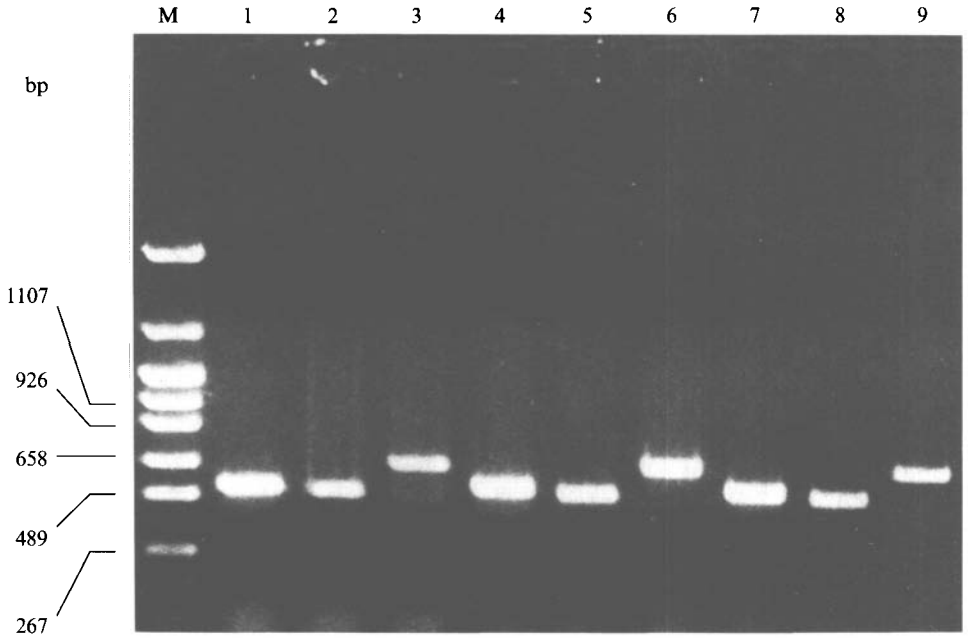

Fig. 1. Agarose gel electrophoresis of PCR product amplified from *mecA*, *femA*, and *femB* genes. These genes from three oxacillin-resistant *S. aureus* strains, SH-102 (lanes 1–3), SH-104 (lanes 4–6), and SH-109 (lanes 7–9) are shown. Lanes 1, 4 and 7 are *mecA* fragment; lanes 2, 5 and 8, *femA* 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 (%)		
		mecA	*femA*	*femB*
S. aureus (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)
S. epidermidis	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
	mecA	*femA*	*femB*	
Oxacillin-resistant *S. aureus*	+	+	+	135
	+	+	−	1
	+	−	+	15
	+	−	−	5
Oxacillin-sensitive *S. aureus*	+	+	+	7
	−	−	+	1
	−	+	+	34

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Table 4. *Antibiotic resistance of clinical isolates of staphylococci*

Species	Total no. isolated	Rate of antibiotic resistance													
		MPIPC	ABPC	PIPC	CEZ	CMZ	CTM	IPM	GM	MINO	EM	CLDM	ST	OFLX	VCM
S. aureus															
oxacillin-RSA (*mecA*-positive, *femA* and/or *femB*-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	0·0
oxacillin-RSA (*mecA*-positive, *femA*, *femB*-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	0·0
oxacillin-SSA (*mecA*-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	0·0
oxacillin-SSA (*mecA*-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‡	0·0
S. epidermidis (*mecA*-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	0·0
S. epidermidis (*mecA*-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	0·0
Other CNS (*mecA*-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	0·0
Other CNS (*mecA*-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	0·0

The breakpoints of MIC for antibiotic resistances were as follows: MPIPC, ≥ 4 μg/ml; ABPC, > 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, > 8 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 (, $P < 0{\cdot}05$; †$P < 0{\cdot}01$) between *mecA*-positive and *mecA*-negative strains of oxacillin-SSA, *S. epidermidis*, and other CNS.

‡ Statistically significant difference ($P < 0{\cdot}01$) 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.

REFERENCES

1. Cohen ML, Wong ES, Falkow S. Common R-plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis* during nosocomial *Staphylococcus aureus* outbreak. Antimicrob Agents Chemother 1982; **21**: 210–15.
2. Thore M, Kühn I, Löfdahl S, Burman LG, Drug-resistant coagulase-negative skin staphylococci. Epidemiol Infect 1990; **105**: 95–105.
3. Utsui Y, Yokota T. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 1985; **28**: 397–403.
4. Song MD, Wachi M, Doi M, Ishino F, Matsuhashi M. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett 1987; **221**: 167–71.
5. Ubukata K, Nonoguchi R, Matsuhashi M, Konno, M. Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. J Bacteriol 1990; **171**: 2882–5.
6. Ryffel C, Tesch W, Birch-Machin I, et al. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gene 1990; 137–8.
7. Suzuki E, Hiramatsu K, Yokota T. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. Antimicrob Agents Chemother 1992; **36**; 429–34.
8. Ubukata K, Nonoguchi R, Song MD, Matsuhashi M, Konno M. Homology of *mecA* gene in methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus simulans* to that of *Staphylococcus aureus*. Antimicrob Agents Chemother 1990; **34**: 170–2.
9. Hiramatsu K, Asada K, Suzuki E, Okonogi K, Yokota T. Molecular cloning and nucleotide sequence determination of the regulator region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). FEBS Lett 1992; **298**: 133–6.
10. Tesch W, Ryffel C, Strässle A, Kayser FH, Berger-Bächi B. Evidence of a novel Staphylococcal *mec*-encoded element (*mecR*) controlling expression of penicillin-binding protein 2′. Antimicrob Agents Chemother 1990; **34**: 1703–6.

11. Suzuki E, Kuwahara-Arai K, Richardson JF, Hiramatsu K. Distribution of *mec* regulator genes in methicillin-resistant *Staphylococcus* clinical strains. Antimicrob Agents Chemother 1993; **37**: 1219–26.
12. Hiramatsu K, Kihara H, Yokota T. Analysis of borderline-resistant strains of methicillin-resistant *Staphylococcus aureus* using polymerase chain reaction. Microbiol Immunol 1992; **36**: 445–53.
13. Higashiyama Y, Koga H, Kohno S, Maesaki S, Kaku M, Hara K. Detection of *mecA* gene of methicillin resistant Staphylococci from oral swabs by polymerase chain reaction. J Jpn Assoc Infect Dis 1993; **67**: 12–17.
14. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of Staphylococci by polymerase chain reaction. J Clin Microbiol 1991; **29**: 2240–4.
15. Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) using polymerase chain reaction amplification. Tohoku J Exp Med 1991; **163**: 31–7.
16. Berger-Bächi B, Barberis-Maino L, Strässle A, Kayser FH. *FemA*, a host-mediated factor essential for methicillin resistance in *Staphylococcus aureus*: molecular cloning and characterization. Mol Gen Genet 1989; **219**: 263–9.
17. Yamashita K, Kagawa S. Detection and identification of methicillin-resistant *Staphylococcus aureus* by non-radioactive probes. J Clin Exp Med (IGAKU NO AYUMI) 1992; **162**: 535–9.
18. NCCLS: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard. NCCLS Document M7–A2 (M100–S4), 1992.
19. Maidhof H, Reinicke B, Blümel P, Berger-Bächi B, Labischinski H. *FemA*, which encodes a factor essential for expression of methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J Bacteriol 1991; **173**, 3507–13.
20. Ubukata K, Yamashita N, Konno M. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant *Staphylococci*. Antimicrob Agents Chemother 1985; **27**: 851–7.