ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1990, p. 170–172
0066-4804/90/010170-03$02.00/0

Vol. 34, No. 1

Homology of *mecA* Gene in Methicillin-Resistant *Staphylococcus haemolyticus* and *Staphylococcus simulans* to That of *Staphylococcus aureus*

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Received 21 June 1989/Accepted 18 October 1989

A penicillin-binding protein of molecular weight 76,000 inducible by β-lactams was detected in methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus simulans*. DNA from these strains hybridized to the *mecA* gene from *Staphylococcus aureus*; however, the chromosomal *Hin*dIII fragments containing the *mecA* genes were 3.4 kilobases in *S. haemolyticus* and 4.3 kilobases in *S. simulans*.

The mechanism of methicillin resistance (Mc^r) in *Staphylococcus aureus* has been considered to be the production of a low-affinity penicillin-binding protein (PBP) variously termed PBP 2′ (8, 16, 17), PBP 2a (3, 4, 9), or MRSA PBP (10, 11). The gene encoding PBP 2′, designated *mecA*, was cloned from the chromosomal DNA of Mc^r *S. aureus* (MRSA) in *Escherichia coli* (7) and sequenced (11).

Recently, we recloned the *mecA* gene to Mc^s *S. aureus* and confirmed that expression of PBP 2′ encoded by the gene results in Mc^r (15). Moreover, it has been demonstrated that a similar PBP was present in Mc^r *Staphylococcus epidermidis* (1, 16) and was encoded by a gene identical to that from MRSA (13). In Mc^r *Staphylococcus haemolyticus*, DNA hybridizing with a probe prepared from MRSA has also been reported (2). These findings suggest that *mecA* genes are found widely in different species of staphylococci.

In this paper, we describe the *mecA* gene contained in Mc^r *S. haemolyticus* and *Staphylococcus simulans*.

Table 1 shows the bacterial strains used in these experiments and the MICs of β-lactam antibiotics for the strains. These strains were clinical isolates from inpatients at Teikyo University Hospital in 1986, and species identification of the strains was performed with the API STAPH system (API SYSTEM S.A., Montalieu-Vercieu, France).

To detect PBPs in each strain, the following cell cultures were used: tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 0.5 μg of cefmetazole per ml as an inducer and broth without inducer. Since these strains also produce a penicillinase, [^{14}C]cefuzonam (47.5 mCi/mmol), provided by Lederle Japan Co., Ltd., Shiki, Japan, was substituted for [^{14}C]benzylpenicillin. Preparations of membrane fractions from each strain, the binding reaction of [^{14}C]cefuzonam to PBPs, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed as described previously (16). Purification of total DNA (7) and Southern blot hybridization were also done by previously described methods (6, 12). The DNA fragment used as a probe was obtained from a cloned 4.3-kilobase (kb) *Hin*dIII DNA fragment containing a *mecA* gene from MRSA strain TK784 (15) and was used after being labeled with [α-^{35}S]dCTP (410 Ci/mmol; Amersham Japan Co., Tokyo, Japan).

TABLE 1. Susceptibilities of *S. haemolyticus* and *S. simulans* to β-lactam antibiotics

Species and strain	MIC (μg/ml)[a]						
	Penicillin G	Methicillin	Cloxacillin	Cefazolin	Cefmetazole	Ceftizoxime	Flomoxef
S. haemolyticus							
TK2384	50	25	12.5	12.5	25	≥100	12.5
TK1350	25	50	25	12.5	25	≥100	12.5
S. simulans							
TK1284	12.5	12.5	6.3	3.1	12.5	100	6.3
TK1286	12.5	25	12.5	6.3	25	100	12.5

[a] β-Lactam susceptibility was determined by the dilution method using sensitivity disk agar plates (Eiken Co., Ltd., Tokyo, Japan) after incubation at 32°C for 48 h. Bacteria cultured overnight were inoculated at 10^4 CFU per spot onto the agar.

Figure 1 shows the results of Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (A) and its fluorogram (B) of Mc^r *S. haemolyticus* TK2384 and TK1350 and *S. simulans* TK1284 and TK1286. Newly increased protein, indicated with arrowheads, was detected only in the lanes in which membrane fractions prepared from

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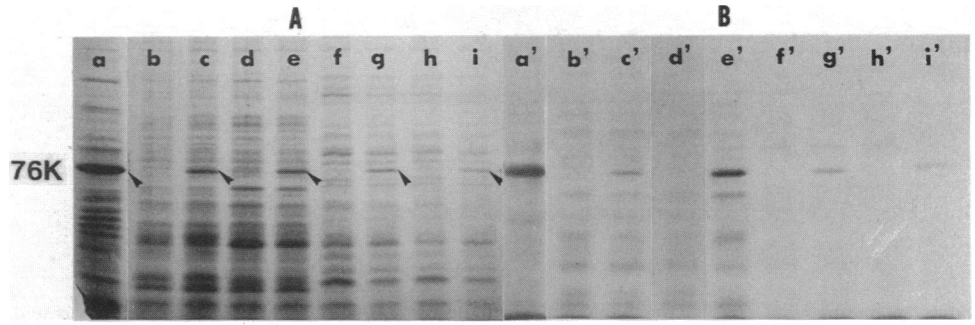

FIG. 1. Coomassie brilliant blue stain (A) and fluorogram (B) of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of membrane fractions isolated from Mc^r staphylococcal cells uninduced or induced by 0.5 μg of cefmetazole per ml. After addition of the antibiotics, cells were cultured until late-logarithmic phase at 32°C. Preparation of the membrane fraction, gel electrophoresis, and reaction with [^{14}C]cefuzonam were performed by methods described previously (16). The samples in each lane are as follows: a and a', MRSA strain TK388E (16); b and b', uninduced *S. haemolyticus* TK2384; c and c', induced TK2384; d and d', uninduced *S. haemolyticus* TK1350; e and e', induced TK1350; f and f', uninduced *S. simulans* TK1284; g and g', induced TK1284; h and h', uninduced *S. simulans* TK1286; i and i', induced TK1286. Arrowheads indicate PBP 2's.

the four strains after induction (c, e, g, and i) were applied. Furthermore, this protein corresponded to a newly appearing PBP (lanes c', e', g', and i'), and the molecular weight was identical to that of the PBP 2' (76,462, calculated from the coding frame of *mecA* [10]) of MRSA strain TK388E (lanes a and a').

Figure 2 shows Southern blot hybridization results for detecting DNA homologous to the *mecA* gene contained in the 4.3-kb *Hin*dIII fragment from MRSA strain TK784. Total DNAs purified from each strain were digested with *Hin*dIII, electrophoresed in a 0.8% agarose gel, and hybridized with the probe. In *S. haemolyticus* TK2384 (b) and TK1350 (c), the hybridizing *Hin*dIII fragments were about 0.9 kb shorter than the probe (a). On the other hand, the hybridizing DNA fragments in *S. simulans* TK1284 (d) and TK1286 (e) had the same length as that of the probe.

The *Hin*dIII fragments exhibiting homology were extracted from an agarose gel and hybridized with the probe after further double digestion by the combination of *Cla*I, *Hin*cII, *Hae*III, and *Pst*I. The resulting restriction maps are shown in Fig. 3. Although a partial lack was observed on the

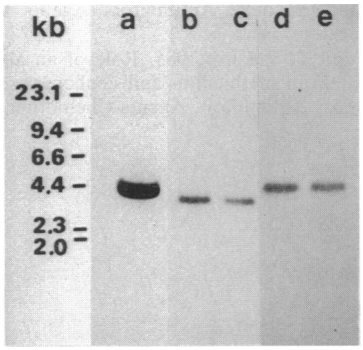

FIG. 2. Southern blot hybridization of *Hin*dIII-digested chromosomal DNA isolated from Mc^r staphylococci. Purification of total DNA from each strain and subsequent hybridization procedures were done by methods described previously (6, 12). The samples in each lane are as follows: a, 4.3-kb probe DNA cloned from MRSA strain TK784; b, *S. haemolyticus* TK2384; c, *S. haemolyticus* TK1350; d, *S. simulans* TK1284; e, *S. simulans* TK1286.

fragment from *S. haemolyticus*, the restriction sites for the endonucleases on these fragments were almost the same as those of the 4.3-kb DNA fragment from MRSA strain TK784 (10). In particular, the restriction pattern of the coding frame believed to contain the gene for the newly increased PBP was equivalent to that of the *mecA* region from strain TK784. The deletion site observed in *S. haemolyticus* DNA presented within the *Hin*dIII-*Hin*cII site at about 0.9 kb, which is outside the coding frame of the gene believed to be identical to *mecA* (11, 15). From these findings, we suggest that Mc^r *S. haemolyticus* and *S. simulans* also have a *mecA* gene encoding a PBP 2'.

Production of a PBP 2', which is a detour enzyme for the essential PBPs in staphylococcal cells, in MRSA and Mc^r *S. epidermidis* has been considered the major, if not the only, cause of Mc^r. Production is always inducible in clinical isolates of these species (1, 9, 16). The characteristic expression of PBP 2' disappeared simultaneously when the penicillinase plasmid was cured, and production was converted to constitutive in the cells (16). These results suggest that the expression of PBP 2' is controlled by a regulatory function of an inducible *blaZ* gene.

Recently, we also demonstrated that expression of PBP 2' was constitutive in staphylococcal cells transformed with a recombinant plasmid containing the *mecA* gene (15). Introduction of an inducible penicillinase plasmid (5) converted these cells from constitutive expression of PBP 2' to inducible production. These findings suggest that Mc^r caused by production of PBP 2' in penicillinase-producing staphylococci is usually an inducible resistance. Especially in clinical isolates of Mc^r coagulase-negative staphylococci, expression of PBP 2' was strongly suppressed under conditions free of β-lactam antibiotics and was only markedly evident when cells were exposed to β-lactam antibiotics. These characteristics of expression of PBP 2' might explain the finding that MICs of β-lactam antibiotics for Mc^r strains were undistinguishable from those for the Mc^s strains but increased markedly when the cells were exposed to β-lactams.

Moreover, results thus far demonstrate that the *mecA* gene encoding PBP 2' is common in Mc^r staphylococci, although a slight variation may exist. The finding that the *mec* gene can be present on a transposon has also been reported (14). In the future, it will be necessary to determine

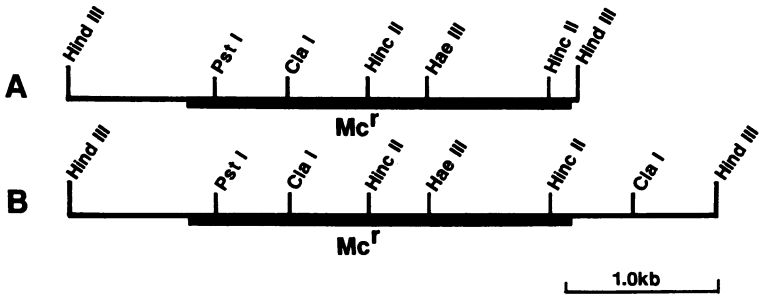

FIG. 3. Restriction cleavage maps of *Hin*dIII fragments hybridizable to probe DNA from Mc^r *S. haemolyticus* and *S. simulans*. (A) A 3.4-kb DNA fragment from *S. haemolyticus* TK2384 and TK1350. (B) A 4.3-kb fragment from *S. simulans* TK1286 and TK1284. Mc^r region marked by a thick line encompasses the *mecA* gene with its possible promoter and repressor-binding regions and an open reading frame believed to encode a repressor protein (M. D. Song, M. Wachi, K. Ubukata, M. Konno, and M. Matsuhashi, manuscript in preparation).

whether the *mecA* gene widely spread among staphylococci is transposon mediated.

This research was supported by funds from Shionogi & Co., Ltd., Osaka, Japan.

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