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NOTES

Identification of a Methicillin-Resistant Strain of *Staphylococcus caprae* from a Human Clinical Specimen

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The analysis of gel banding patterns of penicillin-binding proteins was used to identify two clinical isolates of a coagulase-negative *Staphylococcus* species as *Staphylococcus caprae*, a species originally isolated from goat's milk. One of the isolates was further shown to carry *mecA*, the structural gene for methicillin resistance.

Coagulase-negative staphylococci, although historically regarded as less pathogenic than their coagulase-positive counterpart *Staphylococcus aureus*, have become more and more recognized as important nosocomial pathogens (1, 8, 11). Recently, clinical coagulase-negative staphylococcus isolates of various species have been shown to be resistant to methicillin. So far, such species of coagulase-negative staphylococci as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus simulans* were proven to carry the methicillin resistance gene *mecA* (12–14) or to produce its product, PBP 2′ (3). While studying the distribution of *mecA* among coagulase-negative staphylococcus strains isolated at Juntendo University Hospital, we noticed two coagulase-negative staphylococcus strains whose taxonomic positions could not be identified by conventional biochemical tests. Using analysis of the gel electrophoretic banding pattern of penicillin-binding proteins (PBP profile; 2, 10), we identified these isolates as *Staphylococcus caprae*, a species the original members of which were isolated from goat's milk (4). One of the isolates was further shown to be methicillin resistant, and the *mecA* gene was identified in its genome.

MICs of β-lactam antibiotics for eight clinical coagulase-negative staphylococcus strains isolated in 1989 at Juntendo University Hospital are presented in Table 1. MICs were determined by the plate dilution method as described previously (15). Six were methicillin resistant (JA5, JA6, JA51, JA177, JA178, and JA187), and two were methicillin susceptible (JA3 and JA21). All produced β-lactamase, as tested by cefinase disk assay (9). Initial identification was performed by the API Staph micromethod (API System S.A., Montalieu-Vercieu, France). Two strains, JA21 and JA187, could not be identified by this test. JA21 was isolated from the pus of a patient with dermatitis in the outpatient clinic, and JA187 was isolated from the urine of a patient hospitalized with a urinary tract infection. We tried to identify them by analyzing their PBP profiles (Fig. 1). Analysis of PBP profiles has been proposed to be useful for identification of coagulase-negative staphylococcus species and has recently been successfully used to survey clinical coagulase-negative staphylococcus strains (10). Each of 16 coagulase-negative staphylococcus type strains had a characteristic PBP profile (Fig. 1a and b). The PBPs of the clinical strains were run side

TABLE 1. MICs of β-lactam antibiotics for eight clinical coagulase-negative staphylococcus strains

Strain	Species[a]	MIC[b] (μg/ml)				
		DMPPC	CEZ	CMZ	CZON	IPM
JA3	*S. haemolyticus*	<3.13	0.2	0.78	0.2	<0.05
JA5	*S. haemolyticus*	25	50	3.13	6.25	<0.05
JA6	*S. haemolyticus*	25	25	3.13	6.25	0.1
JA21	NI	<3.13	0.2	0.78	0.1	<0.05
JA51	*S. saprophyticus*	6.25	0.39	3.13	0.39	<0.05
JA177	*S. epidermidis*	25	12.5	50	100	6.25
JA178	*S. haemolyticus*	25	0.78	6.25	0.78	<0.05
JA187	NI	>100	12.5	25	50	6.25

[a] Determined by API Staph micromethod. NI, Not identified by API Staph micromethod. All strains produced β-lactamase.
[b] Abbreviations: DMPPC, methicillin; CEZ, cefazolin; CMZ, cefmetazole; CZON, cefuzonam; IPM, imipenem.

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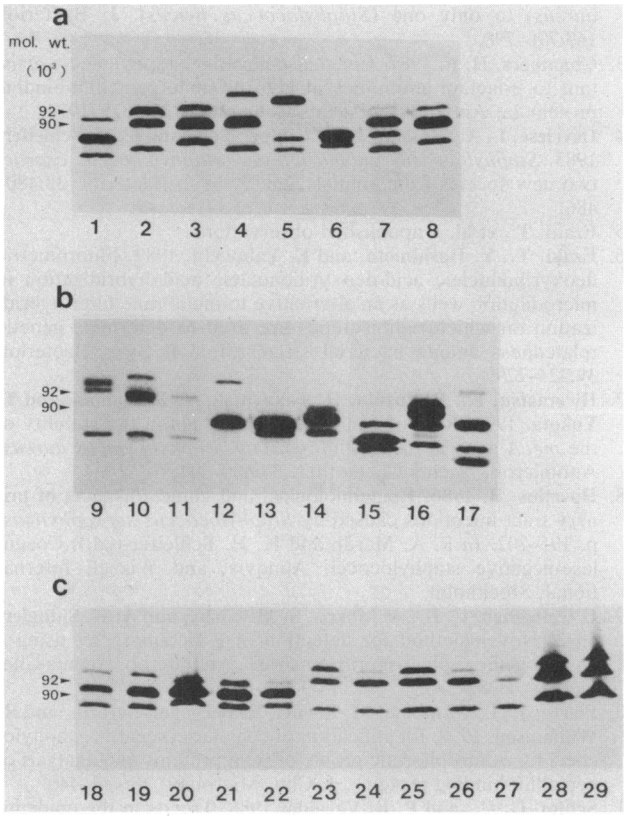

FIG. 1. PBP profiles of coagulase-negative staphylococcus type and clinical strains. The method of PBP profile analysis has been described previously (7). A total of 30 μl of each bacterial membrane suspension adjusted to 10 mg of protein per ml was mixed with 3 μl of a solution of potassium 6-phenyl[1-^{14}C]acetamidopenicillanate (Amersham International plc.; 374 μg/ml, with specific activity of 54 μCi/mmol) and incubated for 10 min at 37°C. The sample was subjected to 8% discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (7). The exposure time for fluorography was 14 days at −70°C. (a and b) PBP profiles of coagulase-negative staphylococcus type strains. Lanes: 1, *S. epidermidis* ATCC 12228; 2, *S. haemolyticus* ATCC 29970; 3, *S. saprophyticus*; 4, *S. xylosus* ATCC 29971; 5, *S. hominis* ATCC 27844; 6, *S. simulans* ATCC 27848; 7, *S. warneri* ATCC 27836; 8, *S. capitis* ATCC 27840; 9, *S. auricularis* ATCC 33753; 10, *S. gallinarum* ATCC 35539; 11, *S. sciuri* ATCC 29062; 12, *S. cohnii* ATCC 29974; 13, *S. hyicus* ATCC 11249; 14, *S. caprae* ATCC 35538; 15, *S. aureus* 209P; 16, *S. saccharolyticus* ATCC 14953; 17, *S. caseolyticus* ATCC 13458. (c) PBP profiles of type and clinical strains. Lanes: 18, *S. epidermidis* (type strain): 19, JA177; 20, *S. caprae* (type strain); 21, JA21; 22, JA187; 23, *S. haemolyticus* (type strain); 24, JA3; 25, JA5; 26, JA6; 27, JA178; 28, *S. saprophyticus* (type strain); 29, JA51. PBPs of *Escherichia coli* were used as molecular markers, which are indicated on the left.

by side with those of type strains for identification (Fig. 1c). The identifications of six strains based on the PBP profiles coincided with those based on the API Staph method, whereas the PBP profiles of JA21 and JA187 were found to correspond to that of *S. caprae* (Fig. 1c, lanes 20–22). This finding was unexpected because the species had not previously been isolated from human sources, and both patients from whom these strains were isolated had no history of contact with goats. To confirm this identification, DNA-DNA hybridization was performed as described previously (6). Chromosomal DNA extracted from strains JA21 and

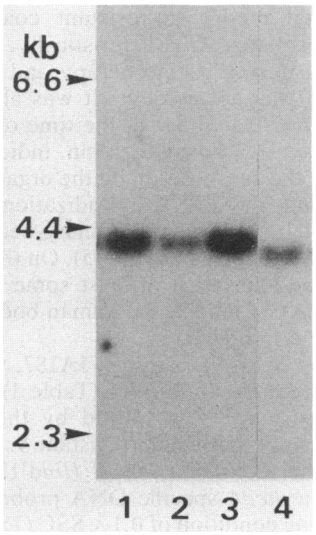

FIG. 2. Detection of *mecA* gene in the genome of JA187. Southern hybridization was performed as described previously (7). Cellular DNA was extracted from each strain of bacteria and digested with *Hin*dIII. Digested DNA was run in a 0.8% agarose gel, transferred to a nitrocellulose membrane, and then hybridized with a *mecA* gene probe from pMR111 (7). Lanes: 1, *S. epidermidis* JA188; 2, *S. caprae* JA187; 3, *S. hominis* JA186; 4, *S. aureus* MR108.

JA187 was photobiotinylated and hybridized with DNAs of 27 staphylococcus type strains which were immobilized in microtiter plate wells. The 27 type strains used as references included *S. aureus* subsp. *aureus* ATCC 12600, *S. aureus* subsp. *anaerobius* ATCC 35844, *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, *S. saprophyticus* ATCC 15305, *S. auricularis* ATCC 33753, *S. cohnii* ATCC 29974, *S. hominis* ATCC 27844, *S. simulans* ATCC 27848, *S. caseolyticus* ATCC 29750, *S. saccharolyticus* ATCC 15943, *S. sciuri* ATCC 29062, *S. lentus* ATCC 29070, *S. xylosus* ATCC 29971, *S. lugdunensis* ATCC 43809, *S. schleiferi* ATCC 43808, *S. delphini* DSM 20771, *S. warneri* ATCC 27836, *S. gallinarum* CCM 3572, *S. caprae* CCM 3573, *S. hyicus* ATCC 11247, *S. capitis* ATCC 27840, *S. carnosus* DSM 20501, *S. equorum* DSM 20674, *S. kloosii* DSM 20676, *S. arlettae* DSM 20672, and *S. intermedius* ATCC 29663. The DNAs of JA21 and JA187 were found to hybridize most strongly with the reference DNA of *S. caprae* CCM 3573 (data not shown). Therefore, identification based on PBP profile was supported by DNA-DNA hybridization, and strains JA21 and JA187 were concluded to belong to the species *S. caprae*.

Both strains of *S. caprae* were isolated from patients after continuous use of antibiotics (mostly β-lactams) for more than 3 weeks. There was no evidence that these *S. caprae* strains were responsible for the patients' infections. JA21 was isolated transiently during chemotherapy of dermatitis, and JA187 was also isolated transiently during chemotherapy of a urinary tract infection caused by methicillin-resistant *S. aureus*. Thus, the *S. caprae* strains did not seem to be potent in their pathogenicity and were likely present as a result of superinfection during chemotherapy.

The prevalence of *S. caprae* in clinical coagulase-negative staphylococcus isolates seems to be considerably high. In Juntendo University Hospital, we have so far isolated 13 other strains of *S. caprae* in 1990. These isolates accounted

for 11% of total methicillin-resistant coagulase-negative staphylococcus isolates. Characteristically, they were isolated transiently during chemotherapy and their causative role in infection was not evident. It was also noticed that most strains were isolated not at the time of admission but after at least a week of hospitalization, indicating the nosocomial nature of the colonization by the organism. Recently, Ezaki et al., using DNA-DNA hybridization, observed that more than 6% of 1,500 clinical coagulase-negative staphylococcus isolates belong to *S. caprae* (5). On the basis of these findings, it seems likely that at least some biotypes of the species *S. caprae* can inhabit the human body and are quite widely distributed in hospitals.

One of the two *S. caprae* strains, JA187, was resistant to methicillin and cephem antibiotics (Table 1). To determine whether this resistance was caused by the same genetic mechanism as that of methicillin-resistant *S. aureus* (13), we performed Southern blot analysis of *Hin*dIII-digested DNA of JA187 using a *mecA*-specific DNA probe from pMR111 (7), with a washing condition of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 60 min at 65°C. The result is presented in Fig. 2. As positive controls, three methicillin-resistant staphylococcus strains were analyzed in parallel. With JA187 (Fig. 2, lane 2), a band of about 4.2 kb which had the same migration rate as those of JA186 (*S. hominis*; lane 3) and JA187 (*S. epidermidis*; lane 1) and a slightly slower migration rate than the 4.0-kb band of MR108 (methicillin-resistant *S. aureus*; lane 4) was observed. From this result, we concluded that JA187, a clinical isolate of *S. caprae*, possessed the *mecA* gene. So far, we have observed the *mecA* gene among such coagulase-negative staphylococcus species as *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. hominis*, *S. capitis*, *S. warneri*, and *S. simulans* (unpublished observation). All of these strains are known as habitants of the human body. The presence of the *mecA* gene in one of the *S. caprae* strains indicated a wider distribution of the gene among coagulase-negative staphylococcus species than we had initially expected. We are undertaking a more extensive survey of clinical coagulase-negative staphylococcus isolates for the distribution of *mecA* gene.

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