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Restriction Maps of the Regions Coding for Methicillin and Tobramycin Resistances on Chromosomal DNA in Methicillin-Resistant Staphylococci

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Chromosomal *Bam*HI DNA fragments containing both the *mecA* gene encoding the penicillin-binding protein responsible for methicillin resistance and the *aadD* gene encoding 4′,4″-adenylyltransferase responsible for tobramycin resistance were cloned from three methicillin- and tobramycin-resistant strains of *Staphylococcus aureus* and one strain of *Staphylococcus epidermidis*. Physical maps of the fragments were similar, suggesting their unique origin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was recently shown to form an additional penicillin-binding protein (PBP), which has been referred to as PBP 2′ (7, 16, 17), PBP 2a (1, 2, 8), or MRSA PBP (9, 10) and which has low affinities for β-lactam antibiotics. This PBP is encoded by the *mecA* gene (10, 14). MRSA TK784, which has been used in our studies (5, 16), is also tobramycin resistant owing to the production of a 4′,4″-adenylyltransferase by its *aadD* gene.

Table 1 shows the strains used in the experiments. MRSA 85/2215, which had been isolated in the United States, was provided by J. F. Richardson of the Division of Hospital Infection, Central Public Health Laboratory, London, United Kingdom. The others were clinical isolates from the Teikyo University Hospital and their derivative strains. TK784E and TK1180E were revertant strains in which methicillin and tobramycin resistances had been eliminated

TABLE 1. Methicillin-resistant and revertant staphylococci used in the experiments

Strain	Relevant phenotype (genotype)	Phage type	Source or reference	Yr of isolation
S. aureus				
TK784	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Tm^r (*aadD*)	Not typable	Clinical isolate from Japan (16)	1983
TK784E[a]	Mc^s Pc^r (*blaZ*) MLS^r Tm^s		16	
TK856	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Tm^r (*aadD*)	54/75	Clinical isolate from Japan	1985
85/2215	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Tm^r (*aadD*)	Not typable	Clinical isolate from the United States	1985
TK2566	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Tm^r (*aadD*)	47/54/77	Clinical isolate from Japan	1986
TK731	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Km^r (*aphA*) Gm^r (*aacA-aphD*)	80	Clinical isolate from Japan (16)	1983
TK731E[b]	Mc^r (*mecA*) MLS^r		16	
TK1015	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Km^r (*aphA*) Gm^r (*aacA-aphD*)	29/52/52A	Clinical isolate from Japan	1986
S. epidermidis				
TK1180	Mc^r (*mecA*) Pc^r (*blaZ*) MLS^r Tm^r (*aadD*)		Clinical isolate from Japan	1986
TK1180E[a]	Mc^s Pc^r (*blaZ*) MLS^r Tm^s		This study	

[a] Methicillin-susceptible strain derived from parent strain after overnight incubation at 43.5°C.
[b] Penicillinase-plasmid-negative strain derived from TK731.

Methicillin- and tobramycin-resistant *Staphylococcus epidermidis* (MRSE) has also been found clinically, and these resistances appeared to be linked, as seen by coordinate elimination with growth at a high temperature (16), as in tobramycin-resistant MRSA. In this paper, we describe the similarity of the chromosomal DNA regions containing *mecA* and *addD* genes among MRSA and MRSE.

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by overnight incubation at 43.5°C (16). *Escherichia coli* TG1 [K-12 Δ(*lac-pro*) *supE thi hsdD5*/F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ* Δ*M15*] and vector plasmid pACYC184 were used for the cloning experiments.

Chromosomal DNA fragments carrying the genetic determinants *mecA* and *aadD* in MRSA and MRSE strains were cloned on vector pACYC184 to give recombinant plasmids containing chromosomal DNA fragments: pSU4 (14) carrying the chromosomal fragment from *S. aureus* TK784, pSU7

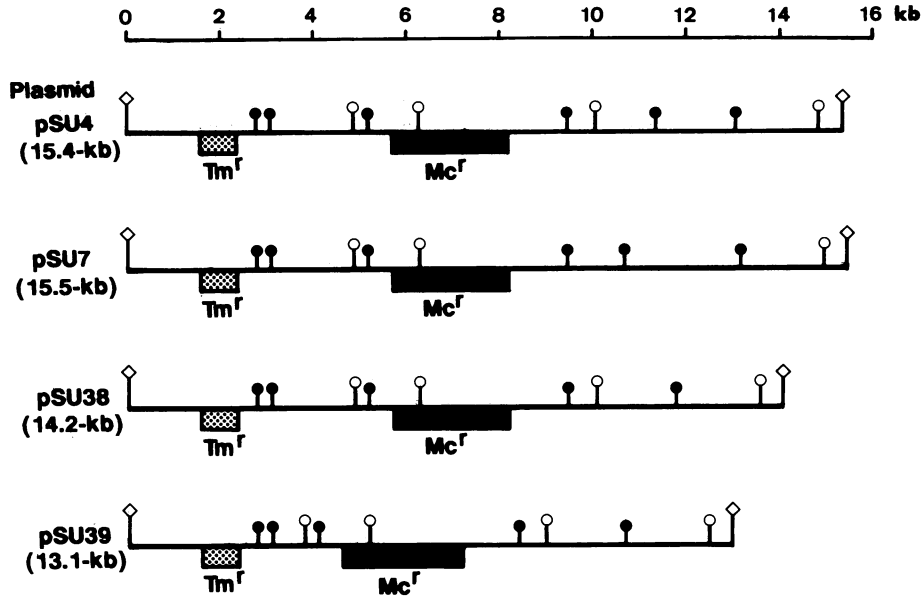

FIG. 1. Restriction cleavage maps of chromosomal DNA fragments encompassing the genes of methicillin and tobramycin resistances cloned from three MRSA strains and one MRSE strain. Recombinant plasmids: pSU4 from *S. aureus* TK784 , pSU7 from *S. aureus* TK856, pSU38 from *S. epidermidis* TK1180, and pSU39 from *S. aureus* 85/2215. The closed box indicates the tentative methicillin resistance (Mc^r) domain which contains the *mecA* gene (10) and the possible regulatory sequence (M. D. Song, M. Matsuhashi, K. Ubukata, and M. Konno, manuscript in preparation). The stippled box indicates the tobramycin resistance (Tm^r) domain which contains the *aadD* gene. Restriction sites: *Bam*HI (◇), *Hin*dIII (●), and *Pst*I (○).

carrying the fragment from TK856, pSU39 carrying the fragment from 85/2215, and pSU38 carrying the fragment from *S. epidermidis* TK1180. Figure 1 shows a restriction map of *Bam*HI DNA fragments of these strains. Plasmids pSU4, pSU7, and pSU38 had spacing regions between *mecA* and *aadD* very similar both in size and in restriction pattern, whereas the spacing region in pSU39 was 1.1 kilobases (kb) shorter. The 5′-flanking region of *mecA* was about 7 kb in pSU4 and pSU7 but was 5.8 kb in pSU38 and pSU39. Restriction sites in this region were similar among four methicillin-resistant staphylococci and identical between pSU38 from MRSE TK1180 and pSU39 from MRSA 85/2215.

A detailed restriction map of the 2.8-kb *Bam*HI-*Hin*dIII region encompassing the tobramycin resistance gene, *aadD*, from plasmid pSU4 is shown in Fig. 2A; and that of the 4.3-kb *Hin*dIII region encompassing the methicillin resistance gene, *mecA*, and the flanking sequences is shown in Fig. 2B. The production of 4′,4″-adenylyltransferase by the transformant cells with the introduced 2.8-kb DNA fragment was confirmed by enzymatic assays (15) and the formation of PBP 2′ by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The *aadD* region of about 1 kb in length was estimated by deduction from the physical map of the known *aadD* gene of plasmid pUB110 (6). These two fragments from four methicillin-resistant staphylococci showed indistinguishable restriction maps as far as they were examined.

Figure 3 shows *Hin*dIII fragments of the chromosomes of seven MRSA, one MRSE, and three derivative strains visualized by Southern hybridization using a 4.3-kb *Hin*dIII DNA fragment from MRSA TK784 as a probe. The *Hin*dIII fragment from the MRSE chromosomal DNA (strain TK1180; lane e) hybridized with the probe, and its size was indistinguishable from those of the four MRSA TK784, TK856, 85/2215, and TK2566 (lanes a, c, d, and g). In contrast, the *Hin*dIII fragments from strains TK1015 (lane

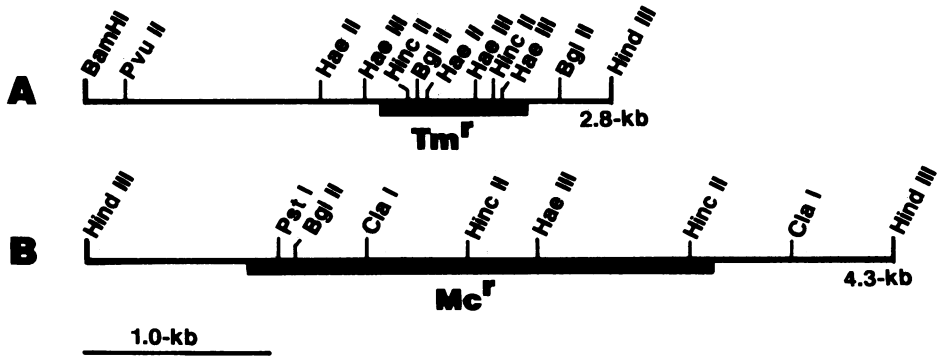

FIG. 2. Detailed restriction cleavage maps of the 2.8-kb *Bam*HI-*Hin*dIII DNA fragment carrying the tobramycin resistance (Tm^r) gene (A) and the 4.3-kb *Hin*dIII fragment carrying the methicillin resistance (Mc^r) gene (B).

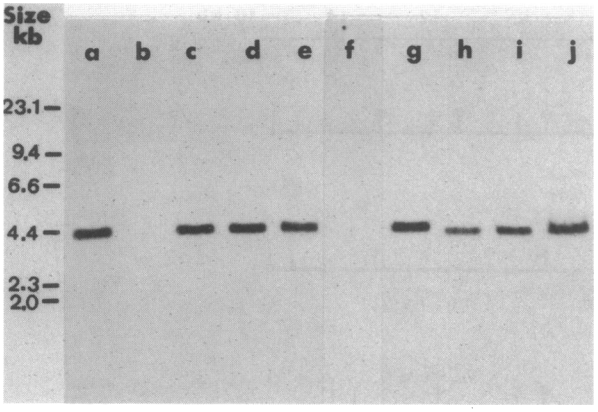

FIG. 3. Southern hybridization analysis of *Hin*dIII-digested chromosomal DNA isolated from methicillin-resistant and revertant staphylococci. The lanes containing DNAs are as follows: a, MRSA TK784; b, methicillin-susceptible *S. aureus* TK784E; c, MRSA TK856; d, MRSA 85/2215; e, MRSE TK1180; f, methicillin-susceptible *S. epidermidis* TK1180E; g, MRSA TK2566; h, MRSA TK1015; i, MRSA TK731; and j, MRSA TK731E (penicillinase plasmid negative).

h), TK731 (lane i), and TK731E (lane j) (in which the penicillinase plasmid of strain TK731 was heat eliminated and only intrinsic methicillin resistance remained) were slightly shorter than that from TK784; and this difference was located in the terminal *Hin*dIII-*Cla*I region (Fig. 2B) of the *Hin*dIII fragment outside the coding frame of *mecA* (data not shown). No *Hin*dIII fragments from methicillin-susceptible strains TK784E (lane b) and TK1180E (lane f) hybridized with the probe.

The *mecA* gene was previously cloned into *E. coli* cells (5) and expressed in *S. aureus* cells (14), carrying the formation of PBP 2′ and the conversion of methicillin-susceptible cells to MRSA. Similar expression of methicillin resistance in staphylococcal transformant cells by the introduction of a chromosomal fragment of MRSA or MRSE was previously reported (3, 12). Two kinds of MRSA strains differing in the length of *Hin*dIII fragments were recognized by Southern hybridization in the present experiment, one containing a 4.3-kb *Hin*dIII fragment and the other containing a 4.0-kb *Hin*dIII fragment. However, tobramycin-resistant MRSA studied so far possessed the longer, 4.3-kb *Hin*dIII fragment, and the occurrence of this type of MRSA has increased rapidly in Japan since its first report in 1983 (4). Most strains of tobramycin-resistant MRSA have been classified as bacteriophage type III or nontypeable and coagulase type II (4); these also produce toxic shock syndrome toxin 1 (manuscript in preparation).

The similarity of the physical maps of the areas containing the genes for methicillin and tobramycin resistances among MRSA and MRSE tested so far suggests a unique origin of this double-resistance area on the staphylococcal chromosome. Methicillin resistance carried on a moving element was described previously (11), and Trees and Iandolo (13) recently proposed that methicillin resistance is present on a transposon. Further investigation is required for concluding whether the DNA fragment containing the linked resistance genes, *mecA* and *aadD*, has been introduced into *S. aureus* and *S. epidermidis* by transposition.

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