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Genomic Rearrangement of the *mec* Regulator Region Mediated by Insertion of IS*431* in Methicillin-Resistant Staphylococci

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Genomic diversification of the *mec* regulator region mediated by IS*431* was investigated for clinical isolates of methicillin-resistant staphylococci. A single rearranged form of the *mecR1* gene due to IS*431* insertion was detected in the three staphylococcal species, while another type of *mecR1* truncation with IS*431* and an IS*431* located downstream of *mecI* were found only in *Staphylococcus haemolyticus*. Genetic differentiation of IS*431* and staphylococcal isolates suggested transmission of *mec*DNA with IS*431*-mediated rearrangement among different staphylococcal species.

Methicillin resistance in staphylococci is defined by the presence of the *mecA* gene, which encodes PBP 2a, having low affinity to beta-lactam antibiotics (7, 20). The *mecA* gene in methicillin-resistant (MR) *Staphylococcus aureus* (MRSA) is located on a large genetic element designated *mec*DNA and is suggested to be transmitted from coagulase-negative staphylococci (CNS) (5, 9, 10).

Expression of *mecA* is originally controlled by the *mec* regulator proteins encoded by the *mecR1* and *mecI* genes, which are located upstream of *mecA* (8), and methicillin resistance is induced by the presence of beta-lactams. That is, the *mecI* product (MecI) usually represses *mecA* expression (17), but this function is removed when the bacterial cells are exposed to beta-lactams (10). However, it is known that recent MRSA isolates are rendered constitutively resistant to beta-lactams through mutations generated in *mec* regulator regions and the resultant loss of the repression function of MecI. These mutations are nucleotide substitutions in the *mecI* or *mecA* promoter region or nucleotide deletion in *mecI* (9, 14, 21).

In addition to such genetic changes, truncation of *mecR1* and deletion of *mecI* through insertion of IS*1272* have been identified in some MR staphylococci (1, 16). IS*1272* is prevalent primarily in *Staphylococcus haemolyticus*, but it is considered to have disseminated among other staphylococcal species and is associated with methicillin resistance of staphylococci (2). However, in our previous study (16), IS*1272* was not found in some MR isolates with incomplete *mec* regulator genes, suggesting that the deletion of *mec* regulator regions was generated by a mechanism other than IS*1272* insertion.

IS*431*, a well-known mobile genetic element in staphylococci, is 782 bp long (IS*431*mec) and contains an open reading frame (ORF) of a putative transposase gene and 14- to 22-bp terminal inverted repeats (3, 4). IS*431* is implicated in transfer of a gene(s) or entire plasmid into other replicons or the chromosome, and particularly in transfer of antimicrobial resistance genes, because variable resistance genes are found to be flanked by IS*431* (18, 19). In *mec*DNA, a pair of IS*431* elements flanking a plasmid, pUB110, are located downstream of *mecA* in a prototype MRSA strain (N315) and other MRSA isolates (11).

In the present study, we investigated the rearrangement of the *mec* regulator region mediated by IS*431* insertion. Previously, we examined a total of 118 clinical isolates of MR staphylococci with respect to the presence of *mecR1* and *mecI* through PCR amplification of individual genes, and we found that 80 isolates possessed both *mecR1* and *mecI*, while 23 isolates had an incomplete *mecR1* truncated with IS*1272* (16). However, neither *mec* regulator genes nor IS*1272* was detected in 15 isolates (2 *S. aureus*, 1 *Staphylococcus epidermidis*, and 12 *S. haemolyticus*). These isolates were analyzed in the present study in regard to IS*431* insertion into the *mec* regulator region. In addition to these, 16 staphylococcal isolates having both *mecR1* and *mecI* were examined for the presence of IS*431* downstream of *mecA* or *mecI*. *S. aureus* isolates were classified by coagulase type, coagulase gene type (13), and protein A type (15), and *S. haemolyticus* was discriminated by use of an arbitrarily primed PCR (AP-PCR) with ERIC2 and M13R primers (6, 22).

The presence of IS*431* in *mec*DNA and its orientation were examined by PCR with primers with different directions complementary to *mecA*, *mecR1*, or IS*431* sequences (Fig. 1). Extraction of bacterial DNA and PCR were performed as described previously (12), employing TaKaRa Ex Taq (Takara) as the *Taq* DNA polymerase. Nucleotide sequences around the insertion site and ORF of IS*431* located at different sites were determined directly from PCR products by the dideoxynucleotide chain termination method using a Sequenase version 2 PCR Product Sequencing Kit (United States Biochemical, Cleveland, Ohio).

The presence of IS*431* upstream of *mecA* in a single isolate each of *S. aureus* and *S. epidermidis* and in 12 isolates of *S. haemolyticus* was confirmed. In one MRSA isolate, SH220, a PCR product suggesting the presence of IS*431* upstream of *mecA* was not obtained. The IS*431* downstream of *mecI* was detected only in the two *S. haemolyticus* isolates. The presence of an IS*431* located downstream of *mecA* (IS*431*-A) was confirmed for all of the staphylococci examined in this study.

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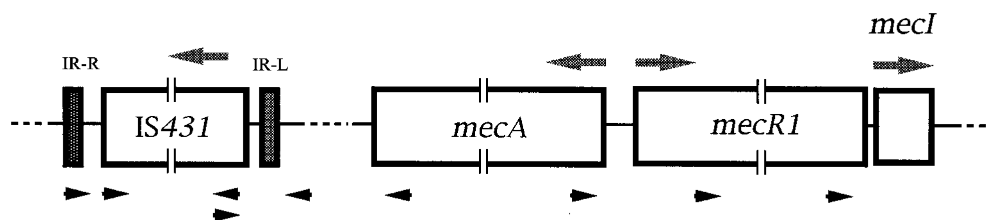

FIG. 1. Schematic representation of *mecA*, *mec* regulator genes, and IS*431* and locations of the primers used in this study. Arrowheads indicate primers, while arrows above ORFs of genes show directions of transcription.

Two distinct types of IS*431* insertion into *mecR1* were clarified by nucleotide sequencing. In the first type (Fig. 2a), an IS*431* (IS*431*-F) was linked with the 5′ 92 bp of the *mecR1* gene (Δ*mecR1*a), and the transcription direction of its ORF was identical to that of *mecR1*. An ORF of the rearranged *mecR1* gene contained the initial 16 bp of IS*431* after the truncation site of *mecR1* and was presumed to encode an extremely short peptide with 36 amino acids. This type of insertion, i.e., Δ*mecR1*a-IS*431*-F, was found in a single isolate each of *S. aureus* and *S. epidermidis* and in 10 *S. haemolyticus* isolates that were divided into at least three genetic groups (i, iii, and iv) by AP-PCR (data not shown) (Table 1). In the second type of insertion, which was detected only in *S. haemolyticus*, an IS*431* (IS*431*-R) with the reverse orientation to that of IS*431*-F was integrated after the 5′ 968 bp of *mecR1* (Δ*mecR1*b) (Fig. 2b). The rearranged ORF containing the partial IS*431* sequence is suggested to encode a product of 332 amino acids. The two isolates having this *mec*DNA were classified into a single AP-PCR type (type ii) which was different from those found in other *S. haemolyticus* isolates with Δ*mecR1*a-IS*431*-F (Table 1). The IS*431* located downstream of *mecI* (IS*431*-I) was inserted after nucleotide 190 from the termination codon of *mecI* (Fig. 2c). The transcription direction of the IS*431*-I ORF was opposite to that of *mecR1*, as seen for IS*431*-R.

By comparison of the nucleotide sequences of the ORFs of the four IS*431* elements (IS*431*-A, IS*431*-F, IS*431*-R, and IS*431*-I) located at different positions, three IS*431* genotypes (A, B, and C) were discriminated (Fig. 3). Genotype A represents the one virtually identical to IS*431*mec, that was reported for MRSA strain BB270 (4). All of the IS*431*-A and IS*431*-R sequences were grouped into genotype A. IS*431*-F sequences were classified in genotype B, which showed sequence divergence of 16 to 20 nucleotides compared with the IS*431*mec sequence. Notably, the nucleotide substitution at position 144 (C to G) generates a new stop codon (Fig. 3); therefore, the IS*431*-F ORF is presumed to encode a short product. Genotype C included only IS*431*-I, which was detected in two *S. haemolyticus* isolates. In this genotype, a 17-bp sequence corresponding to nucleotides 29 to 45 of IS*431*mec was deleted, accompanied by substitution of several nucleotides in other regions (Fig. 3). The IS*431*-I ORF is suggested to be extremely short (69 bp) due to a frameshift caused by the sequence deletion. These findings suggested that IS*431*-R and IS*431*-A were derived from the same origin but were genetically distinct from IS*431*-F and IS*431*-I.

In addition to the genomic rearrangement of the *mec* regulator region via deletion and insertion with IS*1272* (1, 16), our present study indicates that IS*431* also played an important role in the genomic evolution of *mec*DNA and probably in

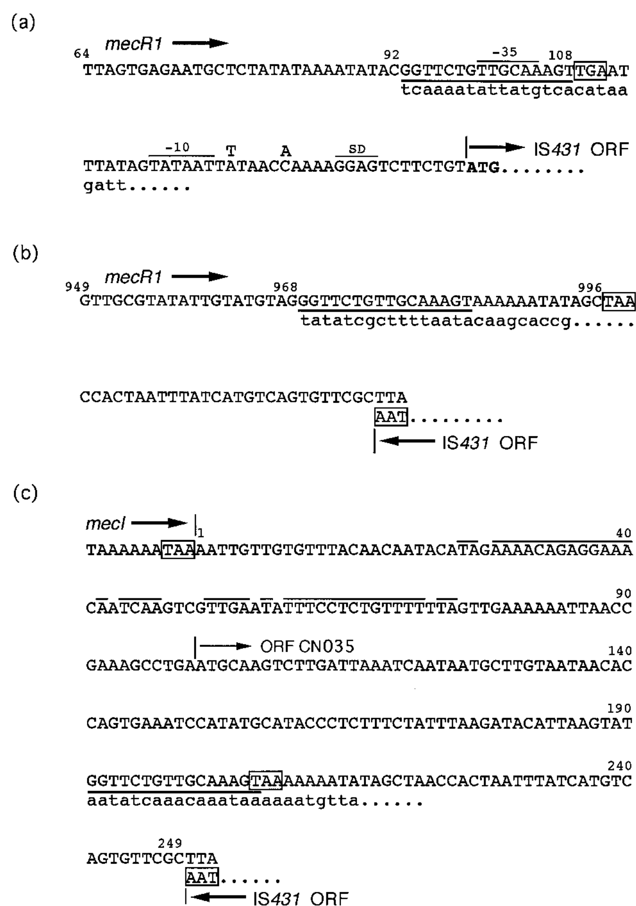

FIG. 2. (a and b) Nucleotide sequences of the 3′-end portions of Δ*mecR1*a (a) and Δ*mecR1*b (b), and partial sequences of IS*431* [referred to as IS*431*-F (a) and IS*431*-R (b) in the text] integrated into *mecR1*, determined for staphylococcal isolates with incomplete *mec* regulator genes. Nucleotide numbers from the initial base of the *mecR1* start codon are indicated on the sequences, and termination codons for the rearranged *mecR1* sequence or IS*431* are boxed. Underlining shows locations of the terminal inverted repeat (IR) of IS*431* [IR-L (a) and IR-R (b)] (3). Lowercase letters indicate the *mecR1* nucleotide sequence of a prototype MRSA strain, N315 (8). Nucleotides of IS*431*mec (4) which are different from those of IS*431*-F are indicated above the sequence. (c) Nucleotide sequence of a junction (numbered on the sequence from 1 to 249) between the 3′-end portion of *mecI* and IS*431*-I detected in *S. haemolyticus* isolates. Lowercase letters indicate the nucleotide sequence found in the prototype MRSA strain N315 (11) (GenBank accession no. D86934). Terminal codons of *mecI*, IS*431*, and the rearranged ORF of CN035 are boxed. IR-R of IS*431* is shown by an underline, and inverted repeats downstream of *mecI* are shown by lines above the sequence.

TABLE 1. Grouping of staphylococcal isolates based on genetic organization of IS*431* and the *mec* regulator region

Staphylococcal species	Isolate(s) (biological and/or genetic type)[a]	Position, orientation, and genotype of IS*431* located near the *mecA* and *mec* regulator genes[b]
S. aureus	SH15 (IV,C,7), SH73 (IV,C,7), SH273 (VII,B,7), SH313 (VII,B,7), SH363 (VII,B,7), SH423 (II,A,13), SH443 (II,A,10), SH457 (II,A,10)	IS*431*-A [A]; *mecA*; *mecR1*; *mecI*
S. epidermidis	SH350, SH214, SH366, SH390, SH418, SH520	
S. aureus	SH20041 (II,E,10)	IS*431*-A [A]; *mecA*; Δ*mecR1*a; IS*431*-F [B]
S. epidermidis	SH513	
S. haemolyticus	SH83 (i), SH84 (iii), SH335 (iv), SH426 (iii), SH514 (i), SH533 (i), SH534 (i), SH535 (i), SH536 (iii), SH538 (iii)	
S. haemolyticus	SH517 (ii), SH529 (ii)	IS*431*-A [A]; *mecA*; Δ*mecR1*b; IS*431*-R [A]
S. haemolyticus	SH339 (v), SH537 (vi)	IS*431*-A [A]; *mecA*; *mecR1*; *mecI*; IS*431*-I [C]

[a] Coagulase types, coagulase gene types (13), and protein A types (15) (in this order) are shown for *S. aureus*, and genetic groups (i to vi) discriminated by AP-PCR fingerprinting are shown for *S. haemolyticus*.

[b] Arrows indicate direction of transcription. IS*431*-A, IS*431*-F, IS*431*-R, and IS*431*-I were designated according to their positions relative to methicillin resistance genes. Genotypes (A, B, and C) of IS*431* are given in brackets.

modifying functions of *mec* regulator proteins. Considering the function of IS*431* associated with gene transfer, it may be also possible that recombination between the IS*431* copies occurs, leading to deletion of *mecA* and its transfer to other bacterial genomes, although no analysis of this was done in the present study.

In the present study, *mec*DNA with a rearranged form, Δ*mecR1*a-IS*431*-F, was detected in the three staphylococcal species, suggesting the transmission of this type of *mec*DNA among different staphylococcal species. However, other IS*431*-mediated genomic variations, Δ*mecR1*b-IS*431*-R and *mecR1*-*mecI*-IS*431*-I, were found only in *S. haemolyticus*. Taken together with our observation of a higher prevalence of IS*431* in methicillin-susceptible CNS than in methicillin-susceptible *S. aureus* (unpublished data), it was assumed that IS*431* had originally been prevalent in CNS, resulting in the occurrence of various forms of *mec*DNA with inserted IS*431* in CNS, and that subsequently some of the *mec*DNAs having the rearranged *mec* regulator region, including those with Δ*mecR1*a-IS*431*-F, had been transmitted to *S. aureus*, as suggested for IS*1272*-integrated *mec*DNA (1). It is also noteworthy that the IS*431* located 190 bases downstream of *mecI*, which was found in two *S. haemolyticus* isolates in the present study, had been observed in *Staphylococcus sciuri* subsp. *rodentius* strain K8 isolated from a rodent (23) (GenBank accession no. Y13096), suggesting the transmission of this type of *mec*DNA between these two staphylococcal species.

Since genomic diversity in the *mec* regulator region seems to

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             1                                                                                       G                          100
Genotype A   ATGAACTATT TCAGATATAA ACAATTTAAC AAGGATGTTA TCACTGTAGC CGTTGGCTAC TATCTAAGAT ATACATTGAG TTATCGTGAT ATATCTGAAA
Genotype B   .......... .......... .......... .......... .......... .......... .......... ..G....... .......... ..G.......
Genotype C   .......... .......... ........-- ---------- -----..... .......... .GCT...... ..G....... ......[...]. ..........

                                                                                  A`      A    G                        200
Genotype A   TATTAAGGGA ACGTGGTGTA AACGTTCATC ATTCAACGGT CTACCGTTGG GTTCAAGAAT ATGCCCCAAT TTTGTATCAA ATTTGGAAGA AAAAGCATAA
Genotype B   .......... .......... .......... ...T...... .[..G].....A .......... .......G.. ...A...... .......... ..........
Genotype C   .......... ..T....... .......... .......... .......... .......... .......... ...A...... .......... ..........
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FIG. 3. Alignment of partial nucleotide sequences of the three IS*431* genotypes, A, B, and C, identified in this study. Sequences corresponding to ORF of IS*431*mec (4), numbered from the first nucleotide of the start codon, are shown. The sequence of genotype A is shown as the IS*431*mec sequence, and only substituted nucleotides found in some isolates are indicated above the sequence. In genotype B and C sequences, dots represent nucleotides identical to those of genotype A, while dashes denote gaps. Putative termination codons of genotypes B and C are boxed.

be a good marker to discriminate *mec*DNA, further extensive studies to search for other rearranged forms of *mec* regulator genes from various staphylococcal species may be significant in understanding diverse routes of *mec*DNA dissemination among staphylococci.

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