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# Distribution of Insertion Sequence-Like Element IS*1272* and Its Position Relative to Methicillin Resistance Genes in Clinically Important Staphylococci

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**The distribution of insertion sequence-like element IS*1272* was analyzed for clinical isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus haemolyticus*. In each of the staphylococcal species, IS*1272* was detected in both methicillin-resistant (MR) and methicillin-susceptible strains of different genetic types. In MR isolates, IS*1272* was generally located downstream of the truncated *mecR1* gene (Δ*mecR1*), with an identical junction sequence occurring between Δ*mecR1* and IS*1272*, although insertion of an additional gene sequence in the junction sequence was detected in one *S. epidermidis* isolate. These findings suggest that the *mec* element with the rearranged form of *mecR1* (Δ*mecR1*-IS*1272*) has been transmitted to multiple clones of staphylococci.**

Methicillin resistance of staphylococci is defined by production of penicillin-binding protein 2a (5, 15), encoded by the *mecA* gene, which is located on the bacterial chromosome (13). Expression of *mecA* is primarily controlled by regulator elements MecR1 and MecI, encoded by *mecR1* and *mecI*, which are located upstream of *mecA* (6). Although isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with intact *mec* regulatory genes show inducible resistance to beta-lactams, most recent isolates of MRSA are constitutively resistant to methicillin and have changes in the *mec* regulatory region (7, 8). Indeed, point mutations in the *mecI* or *mecA* operator region which are believed to have inactivated MecI were detected (7, 11, 14).

Another genomic variation, the deletion of *mecI* accompanied by a partial deletion of *mecR1*, has been reported (1, 10, 14). Archer and coworkers analyzed the MRSA strain COL, which lacks *mecI* and the 3′ half of *mecR1*, and described the presence of the insertion sequence-like element IS*1272* adjacent to the incomplete *mecR1* gene (1). IS*1272* contains two open reading frames (ORFs) and an inverted repeat (IR) comprising 16 bases at each terminus (IRR and IRL) (2). The truncated *mecR1* gene (Δ*mecR1*) adjacent to IS*1272* has also been observed in *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*; furthermore, this *mecR1* deletion junction sequence was the same in *S. aureus* and *S. epidermidis* strains, as well as in an *S. haemolyticus* strain, Y176 (1, 2). These observations suggested that an IS*1272*-mediated *mecR1* deletion event may have occurred in coagulase-negative staphylococci, with subsequent horizontal transfer of the rearranged region to *S. aureus*.

However, it is not clear whether there are other rearranged forms of Δ*mecR1* and IS*1272* different from the one detected in strain COL or whether the COL-type *mec* DNA was transmitted from coagulase-negative staphylococci into a single clone or multiple clones of *S. aureus*. To investigate these points, a total of 176 clinical isolates of staphylococci from different patients admitted to the Sapporo Medical University Hospital, Sapporo, Japan, during the period from 1993 to 1998 (99 *S. aureus* isolates, 59 *S. epidermidis* isolates, and 18 *S. haemolyticus* isolates) were analyzed in this study. *S. aureus* isolates of various coagulase types were selected for the present study. In addition, we analyzed three Japanese MRSA strains, MR108, MR6, and JO18, possessing truncated *mecR1* genes (14), which were provided by the Culture Collection Laboratory of the Institute of Medical Science at the University of Tokyo, Tokyo, Japan, and five type strains of *S. haemolyticus* (ATCC 15796, ATCC 29968, ATCC 29969, ATCC 29970, and ATCC 43253).

The presence of *mecA*, *mecI*, and the 5′ and 3′ halves of *mecR1* was examined by PCR as described previously (9, 10). Based on the results, staphylococcal strains were classified into four groups: type 1 methicillin-resistant (MR) strains, which have both *mecR1* and *mecI* (type 1 *mec* region); type 2 MR strains, which lack *mecI* and the 3′ half of *mecR1* (type 2 *mec* region); type 3 MR strains, lacking both *mec* regulator genes (type 3 *mec* region); and methicillin-susceptible (MS) strains possessing neither the *mecA* nor *mec* regulator gene.

Detection of IS*1272* was performed by PCR with two primer pairs that amplify different portions of IS*1272* ORFs. IS*1272* was detected in a total of 52 MR and MS isolates of the three staphylococcal species (Table 1). In *S. aureus*, IS*1272* was found in all 10 type 2 MRSA and four MS *S. aureus* (MSSA) isolates, while it was not detected in isolates of other *mec* region types. Similarly, in *S. epidermidis*, all of the type 2 and some of the MS isolates possessed IS*1272*. However, some isolates of type 1 and type 3 MR *S. epidermidis* were also IS*1272* positive. IS*1272* was detected in all of the MR and MS *S. haemolyticus* isolates, regardless of the *mec* region type, except for a type 1 isolate (SH339) and an MS strain (ATCC 29968).

*S. aureus* isolates were genetically classified by protein A typing, which measures the number of 24-bp repeating units in the *Xr* region of the protein A gene, as described previously (4, 12). As shown in Table 2, IS*1272* was found in coagulase type III, IV, and VII MRSA with different protein A types (repeat no. 5, 8, 9, 10, 11, and 12). The three MRSA strains not from the Sapporo Medical University Hospital—MR108, MR6, and JO18—possessed IS*1272* and showed identical coagulase (IV)

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TABLE 1. Detection of IS*1272* in staphylococci with different *mecA* and *mec* regulator gene statuses

| Bacterial species (no. of strains) | *mecA* | *mec* regulator gene | | Classification of the *mec* region (*mec* region type) | Total no. of isolates | No. of isolates with: | |
|---|---|---|---|---|---|---|---|
| | | *mecR1* (*mecRA*[a]) | *mecI* | | | IS*1272* | Δ*mecR1*-IS*1272* junction sequence |
| *S. aureus* (102) | + | + | + | 1 | 38 | 0 | |
| | + | (+) | − | 2 | 10 | 10 | 10 |
| | + | − | − | 3 | 2 | 0 | |
| | − | − | − | | 52 | 4 | |
| *S. epidermidis* (59) | + | + | + | 1 | 40 | 1 | |
| | + | (+) | − | 2 | 12 | 12 | 12[b] |
| | + | − | − | 3 | 1 | 1 | |
| | − | − | − | | 6 | 3 | |
| *S. haemolyticus* (23) | + | + | + | 1 | 2 | 1 | |
| | + | (+) | − | 2 | 3 | 3 | 1 |
| | + | − | − | 3 | 10 | 10 | |
| | − | − | − | | 8 | 7 | |

[a] (mecRA) denotes the 5′ portion of *mecR1* (10).
[b] In one *S. epidermidis* isolate (SH194), a junction sequence longer than those of other isolates was detected (Fig. 1).

and protein A (repeat no. 9) types. IS*1272* was detected in coagulase type IV, V, and VII MSSA isolates with different protein A types. In contrast, no coagulase type II MRSA and MSSA possessed this genetic element.

*S. epidermidis* and *S. haemolyticus* were typed by arbitrarily primed PCR fingerprinting with ERIC2 primer (16) and M13 reverse primer (3) as described previously (16). As a result, *S. epidermidis* and *S. haemolyticus* carrying IS*1272* were differentiated into seven and two genetic types, respectively (data not shown).

The presence of the junction sequence between Δ*mecR1* and IS*1272* was examined for all of the type 2 isolates by PCR with primer pair mecRA1 (10) and mDA2 (5′-GATGTCTGTCGA GGACTC-3′), which are complementary to the sequences in *mecR1* and IS*1272*, respectively. A PCR product of 1,287 bp, which was expected from the sequence of *S. aureus* COL (GenBank accession no. L14017), was obtained for 22 isolates, although no PCR products were generated for the two MR *S. haemolyticus* isolates with a type 2 *mec* region. However, a PCR product which was approximately 1,400 bp longer than those from other isolates was generated for one *S. epidermidis* strain (SH194).

The nucleotide sequence between the 3′ end of Δ*mecR1* and the IRL of IS*1272* was determined directly from these PCR products by the dideoxynucleotide chain termination method (12). The 3′ ends of the *mecR1* genes of the 23 type 2 isolates were identical to those of *S. aureus* COL and *S. haemolyticus* Y176 (1, 2) (GenBank accession no. L14017 and U35635, respectively), containing a 9-base divergent sequence not found in *mecR1* (Fig. 1a). The truncated *mecR1* had an ORF of 984 bp and was suggested to encode an incomplete MecR1 product comprising 328 amino acids. The Δ*mecR1*-IS*1272* junction sequences of all isolates except for SH194 consisted of 248 bases, a length almost identical to those of strains COL and Y176. However, compared with published sequences of these strains, two additional nucleotides, T and A, were identified in all staphylococcal genomes examined (Fig. 1a). Furthermore, a single nucleotide was substituted in the junction sequence of *S. aureus* SH475 (Fig. 1a).

In *S. epidermidis* SH194, the truncated portion of *mecR1*, the Δ*mecR1*-side 247-base junction sequence (including two additional bases), and the IRL of IS*1272* were identical to those of most other isolates. However, an insertion of additional DNA was found in the junction sequence at the site close to the IRL. Partial sequences of both sides of the additional DNA are shown in Fig. 1.

In the present study, IS*1272* was found in *S. aureus*, *S. epidermidis*, and *S. haemolyticus* strains with the type 2 *mec* region, together with almost identical Δ*mecR1*-IS*1272* junction sequences. This finding supports the hypothesis that a *mec* element with Δ*mecR1* and IS*1272* has been transmitted horizontally among staphylococci. However, IS*1272* was also detected in type 1 and type 3 staphylococci and in MSSA in the present study. Hence, IS*1272* may have been disseminated among staphylococci irrespective of the presence of *mec* DNA,

TABLE 2. Distribution of IS*1272* in *S. aureus* strains of various coagulase and protein A types

| *S. aureus* strains | Coagulase type | No. of isolates examined | No. of isolates with IS*1272* | Protein A type[a] (no. of isolates) |
|---|---|---|---|---|
| MRSA | II | 32 | 0 | 7 (1), 9 (2), 10 (26), 11 (2), 13 (1) |
| | III | 2 | 2 | 10* (1), 11* (1) |
| | IV | 9 | 7 | 7 (2), 8* (2), 9* (4), 12* (1) |
| | VII | 7 | 1 | 5* (1), 7 (6) |
| MSSA | I | 1 | 0 | 8 (1) |
| | II | 9 | 0 | 4 (1), 8 (1), 9 (1), 10 (5), 11 (1) |
| | III | 6 | 0 | 6 (1), 10 (1), 11 (2), 12 (2) |
| | IV | 4 | 1 | 8 (1), 9 (1), 10* (2) |
| | V | 5 | 1 | 6 (3), 9 (1), UT* (1) |
| | VII | 25 | 2 | 4 (3), 5 (1), 6 (2), 7* (4), 8 (4), 9 (4), 10* (4), 11 (2), UT (1) |
| | VIII | 2 | 0 | 8 (2) |

[a] Protein A type is expressed as the number of 24-base repeating units in the *Xr* region of the protein A gene. *, protein A type of *S. aureus* in which IS*1272* was detected. UT, untypeable.

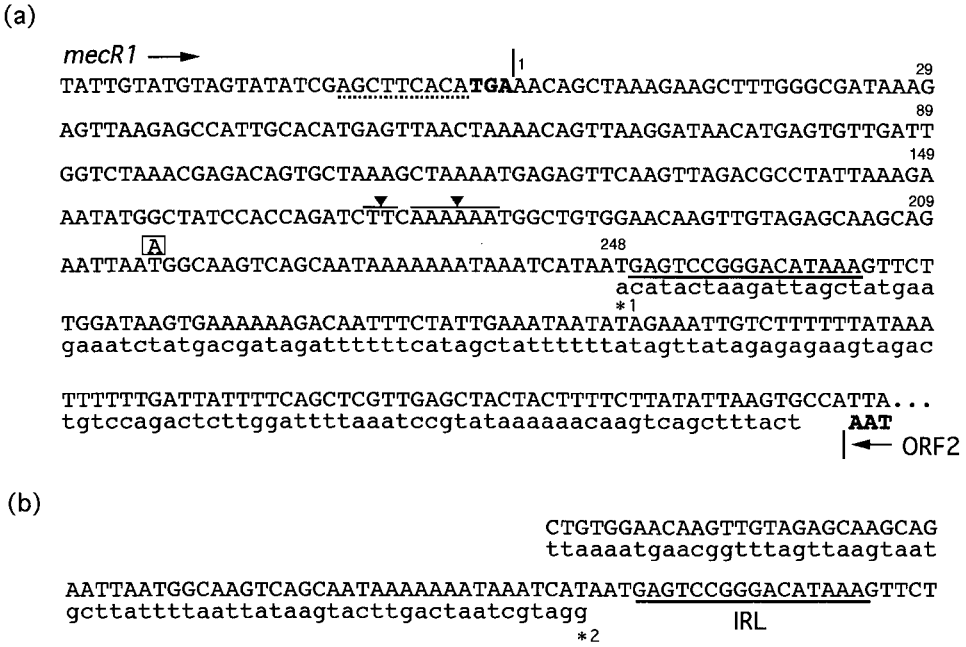


FIG. 1. (a) Nucleotide sequences of the 3′-end portion of Δ*mecR1*, the Δ*mecR1*-IS*1272* junction sequence (numbered on the sequence from 1 to 248), and the IRL side of IS*1272* determined for staphylococci in the present study. Termination codons for Δ*mecR1* and ORF2 of IS*1272* are shown in boldface type and are followed by vertical lines. Dotted underlining indicates divergent terminal sequence of Δ*mecR1*, while solid underlining shows the IRLs. Lines above the nucleotide sequences TT and AAAAAA, under solid inverted triangles, denote the regions where an additional T and A were identified, respectively, compared with those of strains COL and Y176 (1, 2). A single nucleotide in the junction sequence which was substituted in *S. aureus* SH475 is boxed. (b) In *S. epidermidis* SH194, an additional gene sequence (approximately 1,400 bp) was found in the IRL-side terminal portion of the junction sequence. Lowercase letters indicate partial nucleotide sequences at both ends of the inserted sequence, which initiates with adenine (*1) after the 247th nucleotide of the junction sequence (a) and terminates at the guanine (*2) close to the IRL of IS*1272* (b).

although this genetic element appears to have been originally resident in *S. haemolyticus* (2).

Molecular epidemiologic typing of *S. aureus* indicated that truncated *mecR1* and IS*1272* are distributed in *S. aureus* strains of different coagulase and protein A types and also in *S. epidermidis* and *S. haemolyticus* strains with some distinct genetic types. These findings suggested that the *mec* element containing Δ*mecR1* and IS*1272* might have been transmitted to multiple clones of staphylococci.

It is notable that an additional large DNA sequence is inserted in the IRL-side terminal portion of the Δ*mecR1*-IS*1272* junction sequence in *S. epidermidis* SH194, which exhibited a novel rearrangement of Δ*mecR1* with IS*1272*. Furthermore, the Δ*mecR1*-IS*1272* junction sequence was not detected in two isolates of type 2 MR *S. haemolyticus*, suggesting the presence of an unknown form of deletion junction sequence in *mec* regulator genes. Thus, *mec* regulator genes may contain various genomic changes, which seem to have occurred multiple times in coagulase-negative staphylococci.

## REFERENCES


1. **Archer, G. L., D. M. Niemeyer, J. A. Thanassi, and M. J. Pucci.** 1994. Dissemination among staphylococci of DNA sequences associated with methicillin resistance. Antimicrob. Agents Chemother. **38:**447–454.
2. **Archer, G. L., J. A. Thanassi, D. M. Niemeyer, and M. J. Pucci.** 1996. Characterization of IS*1272*, an insertion sequence-like element from *Staphylococcus haemolyticus*. Antimicrob. Agents Chemother. **40:**924–929.
3. **Fang, F. C., M. McClelland, D. G. Guiney, M. M. Jackson, A. I. Hartstein, V. H. Morthland, C. E. Davis, D. C. McPherson, and J. Welsh.** 1993. Value of molecular epidemiologic analysis in a nosocomial methicillin-resistant *Staphylococcus aureus* outbreak. JAMA **270:**1323–1328.
4. **Frénay, H. M. E., J. P. G. Theelen, L. M. Schouls, C. M. J. E. Vandenbroucke-Grauls, J. Verhoef, W. J. van Leeuwen, and F. R. Mooi.** 1994. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. J. Clin. Microbiol. **32:**846–847.
5. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:**513–516.
6. **Hiramatsu, K., K. Asada, E. Suzuki, K. Okonogi, and T. Yokota.** 1992. Molecular cloning and nucleotide sequence determination of the regulator region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). FEBS Lett. **298:**133–136.
7. **Hiramatsu, K.** 1995. Molecular evolution of MRSA. Microbiol. Immunol. **39:**531–543.
8. **Hiramatsu, K., N. Kondo, and T. Ito.** 1996. Genetic basis for molecular epidemiology of MRSA. J. Infect. Chemother. **2:**117–129.
9. **Kobayashi, N., H. Wu, K. Kojima, K. Taniguchi, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, and I. Kurokawa.** 1994. Detection of *mecA*, *femA*, and *femB* genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiol. Infect. **113:**259–266.
10. **Kobayashi, N., K. Taniguchi, K. Kojima, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, I. Kurokawa, and N. Watanabe.** 1996. Genomic diversity of *mec* regulator genes in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Epidemiol. Infect. **117:**289–295.
11. **Kobayashi, N., K. Taniguchi, and S. Urasawa.** 1998. Analysis of diversity of mutations in the *mecI* gene and *mecA* promoter/operator region of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **42:**717–720.
12. **Kobayashi, N., S. Urasawa, N. Uehara, and N. Watanabe.** 1999. Analysis of genomic diversity within the *Xr*-region of the protein A gene in clinical isolates of *Staphylococcus aureus*. Epidemiol. Infect. **122:**241–249.
13. **Song, M. D., M. Wachi, M. Doi, F. Ishino, and M. Matsuhashi.** 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. **221:**167–171.
14. **Suzuki, E., K. Kuwahara-Arai, J. F. Richardson, and K. Hiramatsu.** 1993. Distribution of *mec* regulator genes in methicillin-resistant *Staphylococcus* clinical strains. Antimicrob. Agents Chemother. **37:**1219–1226.
15. **Utsui, Y., and T. Yokota.** 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **28:**397–403.
16. **van Belkum, A., R. Bax, P. Peerbooms, W. H. F. Goessens, N. Van Leeuwen, and W. G. V. Quint.** 1993. Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. **31:**798–803.