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Comparison of Tn*1546*-Like Elements in Vancomycin-Resistant *Staphylococcus aureus* Isolates from Michigan and Pennsylvania

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In 2002, the first two clinical isolates of vancomycin-resistant *Staphylococcus aureus* (VRSA) containing *vanA* were recovered in Michigan and Pennsylvania. Tn*1546*, a mobile genetic element that encodes high-level vancomycin resistance in enterococci, was present in both isolates. With PCR and DNA sequence analysis, we compared the Tn*1546* elements from each isolate to the prototype Tn*1546* element. The Michigan VRSA element was identical to the prototype Tn*1546* element. The Pennsylvania VRSA element showed three distinct modifications: a deletion of nucleotides 1 to 3098 at the 5′ end, which eliminated the *orf1* region; an 809-bp IS*1216V*-like element inserted before nucleotide 3099 of Tn*1546*; and an inverted 1,499-bp IS*1251*-like element inserted into the *vanSH* intergenic region. These differences in the Tn*1546*-like elements indicate that the first two VRSA isolates were the result of independent genetic events.

The first two clinical isolates of vancomycin-resistant *Staphylococcus aureus* (VRSA) were recovered from patients in Michigan and Pennsylvania in June and September of 2002, respectively (2, 3). The MIC of vancomycin was 1,028 μg/ml for the Michigan VRSA (MI-VRSA) isolate and 32 μg/ml for the Pennsylvania VRSA (PA-VRSA) isolate by broth microdilution (7). The *vanA* gene was detected in both isolates by PCR amplification and localized to a plasmid of either 58 (Michigan) or 127 (Pennsylvania) kb by Southern hybridization (2, 3, 11, 12). The MI-VRSA *vanA* plasmid was transferable to *S. aureus* COL by filter mating (12), but conjugal transfer of the PA-VRSA plasmid to other staphylococcal or enterococcal recipients has been unsuccessful. The SmaI pulsed-field gel electrophoresis patterns of both isolates fall within the USA100 lineage (New York/Japan clone), which is the most common staphylococcal pulsed-field type found in U.S. hospitals. However, the patterns are clearly distinguishable, indicating that the MI-VRSA and PA-VRSA isolates are not epidemiologically linked (11, 12).

The prototype *vanA* gene is present on transposon Tn*1546*, a mobile genetic element containing genes responsible for high-level glycopeptide resistance among enterococci and several other gram-positive organisms (1). Acquisition of vancomycin resistance usually involves horizontal transfer of a Tn*1546*-containing plasmid. Tn*1546* is composed of nine genes: *vanR*, *vanS*, *vanH*, *vanA*, and *vanX* are required for the expression of resistance; *orf1* and *orf2* encode transposase and resolvase enzymes, respectively; *vanY* encodes a carboxypeptidase; and *vanZ* has an unknown function, although expression of this gene is associated with teicoplanin resistance (1, 5). In 1992, Noble et al. reported in vitro transfer of glycopeptide resistance from *Enterococcus faecalis* to *S. aureus* (8), but natural transfer was not documented until 2002. This report describes the similarities and differences of the genetic elements encoding vancomycin resistance in the MI-VRSA and PA-VRSA isolates and compares them with the prototype Tn*1546* transposon from enterococci.

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PCR amplification of the individual genes comprising Tn*1546* was performed with an Applied Biosystems 9600 thermocycler (Applied Biosystems, Foster City, Calif.) in accordance with previously described protocols (4). PCR primers included P1 to P19, described by Arthur et al. (1), and additional primers selected from the sequence of Tn*1546* with Oligo 6 software (Molecular Biology Insights, Inc., Cascade, Colo.) (Table 1). The DNA sequences were determined by dRhodamine Dye Terminator Cycle Sequencing and an ABI 377 automated DNA sequencer (Applied Biosystems). DNA sequences were aligned and compared with DNAsis for Windows (version 2.5; MiraiBio, Inc., San Francisco, Calif.).

DNA sequence analysis revealed that the *vanA* sequences from the two strains were identical to the *vanA* sequence of Tn*1546* (GenBank accession no. M97297). However, PCR amplification of additional transposon elements from the MI-VRSA and PA-VRSA isolates suggested significant differences. By Long PCR (14), the entire 10.8-kb transposon was amplified from the MI-VRSA isolate and Tn*1546*-containing *E. faecalis* A256 (4, 9). The restriction patterns of the MI-VRSA and A256 Long PCR products obtained with EcoRI, EcoRV, HindIII, and XbaI were identical (data not shown) (14). With the same oligonucleotide primers, the complete transposon could not be amplified from the PA-VRSA isolate.

The PCR products obtained from the MI-VRSA isolate and *E. faecalis* A256 with all of the primer pairs reported by Arthur et al. from *orf1* through *vanZ* were of the expected sizes. The DNA sequences of the MI-VRSA products were identical to those of the prototype Tn*1546* element. Although amplifica-

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TABLE 1. Additional primers used for PCR and sequencing

Primer[e]	Sequence (5′→3′)	Reference
Tn1546R	GGAAAATGCGGATTTACAACGCTAAG	14
VanR4 (+)	(3907)[a] ATGCTTATAAATTCGGCCCTA	This study
VanS3 (+)	(4771)[a] CCGAGGGAAACTTGGGGATTG	This study
VanA3 (−)	(7002)[a] TATTGCAACTTTTATTCTATTCATG	This study
VanA4 (+)	(7984)[a] TTGATCGTATTAGCGTTAAAGGGG	This study
IS1251-1 (+)	(106)[b] AACCCAAAAGGAGGAATCAAG	This study
IS1251-2 (−)	(1057)[b] CTTTGAAGCCAGGTCGC	This study
IS1251-4 (+)	(1041)[b] GCGACCTGGCTTCAAAG	This study
6113R (−)	(6113)[a] TATCGTTGCCATAACGC	13
7875F (+)	(7875)[a] CCGCATTGTACTGAACG	13
8544F (+)	(8544)[a] GCATATAGCCTCGAATGG	13
9519F (+)	(9519)[a] ACCAGCAGGTTATAGTGAGC	13
10687F (+)	(10687)[a] CTCGCCCGTAGGTGTGAAGTG	This study
10778F (+)	(10778)[a] TTTAGTGCTGAGGAATTGG	13
IS1216V.A (+)	(90)[c] GGAAAGCAATTTCAGCAG	13
IS1216V.C (−)	(495)[c] CACTTGTAATAGAGGGGGC	13
IS1216V.E (+)	(749)[c] AGCTTAAATCATAGATACCGTAAGG	13
UP211 (+)	(211)[d] CCATCGATTATGAGGCTAGACA	This study
DN645 (−)	(663)[d] ATTCTTGCACCCAACGATA	This study

[a] The primer positions correspond to the sequence of Tn*1546* (GenBank accession no. M97297).
[b] The primer positions correspond to the sequence of IS*1251* (GenBank accession no. AF148130).
[c] The primer positions correspond to the sequence of IS*1216V* (GenBank accession no. AF093508).
[d] The primer positions correspond to the sequence of the Tn*1546*-like element reported in this study.
[e] +, forward primer; −, reverse primer (relative to the respective sequence).

tion products from the *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* regions of the PA-VRSA isolate were of the predicted sizes, no products were obtained for the *orf1* gene. However, PCR products that were larger than expected were generated from the *orf1-orf2* and *vanS-vanH* intergenic regions. These data suggested that insertion sequences were present. This was confirmed by DNA sequence analysis, which revealed truncation of the 5′ region of the Tn*1546*-like element resulting in the loss of nucleotides 1 to 3098, which eliminated the *orf1* region. Upstream from the truncated Tn*1546* element was a 419-bp sequence with homology to bases 58293 to 58711 of *E. faecalis* V583 pTEF1 (GenBank accession no. AE016833). This sequence, represented by the dotted line in Fig. 1, was followed by an 809-bp sequence designated an "IS*1216V*-like" element (GenBank accession no. AF093508) that is in the same 5′→3′ orientation as

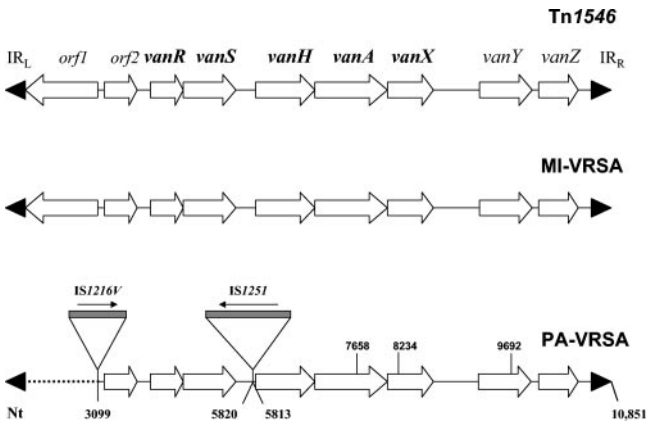

FIG. 1. Comparison of the Tn*1546*-like elements in the MI-VRSA and PA-VRSA isolates to the prototype Tn*1546* element. Numbers correspond to the nucleotide (Nt) positions in the prototype Tn*1546* element. Deletions are indicated by dotted lines. IR, inverted repeat.

the transposon (6). For confirmation of this result, we generated a 1,417-bp product with a primer (UP211) within the pTEF1 sequence that preceded the IS*1216V*-like element and the primer P6 (1), located in the *orf2* region.

The Tn*1546*-like element sequence from the PA-VRSA isolate began at nucleotide 3099 of the prototype Tn*1546* sequence (located between *orf1* and *orf2*) and continued through nucleotide 5820 (in the *vanS-vanH* intergenic region). The elimination of the *orf1* (transposase) region of the element may affect the mobility of the truncated transposon (13). The expression of vancomycin resistance may also be affected by the presence of the transposon on such a large plasmid in *S. aureus* (11). DNA sequence analysis also revealed a 1,499-bp sequence designated "IS*1251*-like" (GenBank accession no. AF148130) (10). This element was inserted downstream from position 5820 in the opposite orientation relative to the transposon but was in the same position and orientation as an insertion described by Handwerger et al. (5) in a Tn*1546*-like element from an *E. faecium* isolate. IS*1251* and IS*1251*-like elements have been reported almost exclusively in U.S. isolates. The exceptions are two isolates from Ireland and one from Norway (5, 10). The IS*1251*-like element in the PA-VRSA isolate was also flanked by an 8-bp duplication of the target sequence (ATAATTTT) corresponding to bases 5813 to 5820 of Tn*1546* (5, 13). The remainder of the Tn*1546*-like element (5,049 bp) had 99% nucleotide homology with the reported prototype sequence. Nucleotide substitutions identified at positions 7658 (T→C), 8234 (G →T), and 9692 (C →T) (Fig. 1) were the same as those described by Willems et al. (13) for Tn*1546* type F2. The PA-VRSA isolate differs in that the IS*1216V*-like element is not in the reverse orientation and there is a larger deletion at the 5′ end of the PA-VRSA transposon (3,098 versus 889 bp). Tn*1546* type F2 isolates have only been found in hospitalized patients in the United States (13).

Ligation of PA-VRSA EcoRI plasmid fragments into plas-

mid vector pUC19 enabled us to determine the sequence of the 3′ inverted repeat. The ligation mixture was used as the template for PCR amplification with the M13 reverse sequencing primer (GGAAACAGCTATGACCATG) [Strategies **6**(1)**:**15, 1993; Stratagene, La Jolla, Calif.] and a Tn*1546*-specific primer (10687F or 10778F). The PA-VRSA inverted repeat was identical to that reported in Tn*1546*.

In summary, vancomycin resistance in the MI-VRSA and PA-VRSA isolates is due to acquisition of *vanA* on plasmid-borne Tn*1546*-like elements. The MI-VRSA isolate acquired a Tn*1546* element on 58-kb plasmid pLW1043 (12). The Tn*1546*-like element on the 127-kb plasmid (11) in the PA-VRSA isolate is quite different, lacking *orf1* because of a truncation at the 5′ end and containing two insertion sequences; an IS*1216V*-like insertion sequence preceded the truncated Tn*1546* sequence, and an IS*1251*-like insertion sequence was located in the *vanS-vanH* intergenic region. These differences in the Tn*1546*-like elements indicate that the first two VRSA isolates arose from independent genetic events.

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