

# Tn5406, a New Staphylococcal Transposon Conferring Resistance to Streptogramin A and Related Compounds Including Dalfopristin

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Received 5 November 2001/Returned for modification 11 February 2002/Accepted 16 April 2002

**We characterized a new transposon, Tn5406 (5,467 bp), in a clinical isolate of *Staphylococcus aureus* (BM3327). It carries a variant of *vgaA*, which encodes a putative ABC protein conferring resistance to streptogramin A but not to mixtures of streptogramins A and B. It also carries three putative genes, the products of which exhibit significant similarities (61 to 73% amino acid identity) to the three transposases of the staphylococcal transposon Tn554. Like Tn554, Tn5406 failed to generate target repeats. In BM3327, the single copy of Tn5406 was inserted into the chromosomal *att554* site, which is the preferential insertion site of Tn554. In three other independent *S. aureus* clinical isolates, Tn5406 was either present as a single plasmid copy (BM3318), as two chromosomal copies (BM3252), or both in the chromosome and on a plasmid (BM3385). The Tn5406-carrying plasmids also contain two other genes, *vgaB* and *vatB*. The insertion sites of Tn5406 in BM3252 were studied: one copy was in *att554*, and one copy was in the additional *SCCmec* element. Amplification experiments revealed circular forms of Tn5406, indicating that this transposon might be active. To our knowledge, a transposon conferring resistance to streptogramin A and related compounds has not been previously described.**

Streptogramins (SGs) and related antibiotics are naturally produced by streptomycetes. They are classified as A and B compounds, according to their basic primary structures (9). The two classes of compounds bind different targets in the peptidyltransferase domain of the 50S ribosomal subunit and inhibit protein elongation at different steps (10). A and B compounds are bacteriostatic when used separately but act synergistically when combined, such that in some cases they are bactericidal, mainly against gram-positive bacteria. Natural mixtures, such as pristinamycin, synergistin, and mikamycin, are used in human medicine orally and topically. Quinupristin and dalfopristin (7), derivatives of streptogramin B (SGB) and A SGA, respectively, is an injectable semisynthetic mixture which has been available since 1999 for use in hospitals to treat infections due to gram-positive cocci that are resistant to other antibiotics. Virginiamycin, a related SG, was long used as a growth promoter in both Europe and the United States but was banned in Europe in 1999.

Staphylococcal resistance to synergistic mixtures of A and B compounds (pristinamycin MICs of  $>2$  mg liter<sup>-1</sup>) is always associated with resistance to A compounds (pristinamycin IIA MICs of  $\geq 8$  mg liter<sup>-1</sup>) but not necessarily with resistance to B compounds (1, 12). Seven genes and a variant encoding resistance to A compounds have been isolated from staphylococcal and enterococcal plasmids. The genes *vataA* (6, 31), *vatB* (3, 31), *vatC* (4, 31), *vatD* (30, 31), and *vatE* (13, 31, 35) encode related proteins (50.4 to 60.1% identical amino acids [aa]) conferring resistance to SGA and similar compounds by acetylation of the drugs. The staphylococcal genes *vgaA* (5, 31) and *vgaB* (2, 31) encode related putative ATP-binding proteins (58.8% identical aa) that are probably involved in the active

efflux of A compounds. The variant of *vgaA* (14), referred to here as “*vgaAv*,” was recently described in a clinical strain of *Staphylococcus aureus* (BM3327) and confers resistance to SGA but not to pristinamycin. Despite 83.2% identity with *vgaA*, *vgaAv* is distinguishable by its higher G+C content (35.6% instead of 29% for *vgaA*). In clinical isolates of *S. aureus* hybridizing with *vgaAv*, one to two copies of this gene are present on the chromosome and/or plasmids. Analysis of the sequences flanking *vgaAv* led to the identification of a new transposon, Tn5406 (5,467 bp) similar to Tn554 (11, 23, 24), also named Tn3853 (34). Tn554 (6,691 bp) contains three transposase genes (*tnpA*, *tnpB*, and *tnpC*), the gene *ermA* conferring inducible resistance to macrolide-lincosamide-SGB (MLS<sub>B</sub>) antibiotics, and the gene *spc* conferring spectinomycin resistance. Tn554 has no inverted terminal repeats: it fails to generate target repeats and transposes preferentially to a single chromosomal site, *att554*, with a frequency close to 100% (19, 23, 25, 27, 29). When *att554* is occupied, deleted, or naturally absent, Tn554 can insert at a lower frequency into secondary insertion sites most of which are in the chromosome (*att155* and *att137*) (11, 17, 23, 25, 32, 33). In some cases it inserts into penicillinase plasmids (pI524 and pI258) (23, 24). Five copies of Tn554 have been detected in the methicillin-resistant *S. aureus* strain N315, whose chromosome has been sequenced (21). One of the five copies is in the staphylococcal cassette chromosome *mec* (*SCCmec*) element (16, 21). Here we describe the sequence, distribution, and insertion sites of the transposon Tn5406 harboring *vgaAv*.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The relevant characteristics of the strains used are reported in Table 1. We used pUC18 as cloning vector. pIP1799 (14), pIP1692 (3), and pIP1705 (2) were used as probes to detect *vgaAv*, *vatB*, and *vgaB*, respectively.

**Media.** Staphylococci were grown in brain heart infusion (Difco Laboratories, Detroit, Mich.), and *Escherichia coli* was grown in Luria broth (Difco). Suscep-

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TABLE 1. Relevant characteristics of the strains used in this study

Strain	Characteristics	Resistance <sup>a</sup>	MIC ( $\mu\text{g ml}^{-1}$ )		MLSB and SGA resistance genes <sup>b</sup>	Tn5406 copy no. (location) <sup>c</sup>	Insertion site of Tn5406	Detection of Tn5406 circular forms	Source or reference
			PIA	PRI					
XL2-Blue	<i>E. coli</i> recipient (Stratagene)	No drug resistance marker							
RN450	<i>S. aureus</i> NCTC 8325-4	No drug resistance marker	1	0.250	None				28
RN4220	<i>S. aureus</i> NCTC 8325-4 <sup>r</sup>	No drug resistance marker	2	0.06	None				18
BM3252	<i>S. aureus</i> clinical isolate: <i>mecA</i>	LIN SGA PEN MET TET MIN KAN NEO TOB GEN STR SUL CAD ARS MER ETB	64	1	<i>vgaA</i> <sub>v</sub>	2 (C)	One copy in <i>att554</i> site; one copy in <i>SCCmec</i> mobile element	No	14
BM3318	<i>S. aureus</i> clinical isolate; <i>mecA</i> ; Tn554	MLSB <sub>pc</sub> SGA PRI PEN MET TET MIN KAN NEO TOB GEN STR SPT SUL CAD ARS MER ETB ABR	128	16	<i>ermA ermB vatB vgaB</i> <i>vgaA</i> <sub>v</sub>	1 (P)	ND <sup>d</sup>	Yes	14
IPF110	BM3318 derivative, susceptible to SGA obtained after serial pas- sages on drug-free medium; <i>mecA</i> ; Tn554	MLSB <sub>pc</sub> PEN MET TET MIN KAN NEO TOB GEN STR SPT SUL CAD ARS MER ETB ABR	1	0.12	<i>ermA ermB</i>				This study
BM3327	<i>S. aureus</i> clinical isolate	MLSB <sub>pc</sub> SGA PRI PEN TET MIN KAN NEO TOB GEN STR SUL PEF	128	8	<i>ermC vgaA</i> <sub>v</sub>	1 (C)	<i>att554</i> site	Yes	14
BM3385	<i>S. aureus</i> clinical isolate; pIP1156	LIN SGA PRI PEN SUL TMP RIF CAD ARS ETB MER ABR	128	4	<i>vatB vgaB vgaA</i> <sub>v</sub>	2 (C + P)	One copy (C) in <i>att554</i> site; one copy (P): ND	No	14
BM12940	BM3385 derivative, susceptible to SGA obtained after serial pas- sages on drug-free medium	RIF CAD ARS MER	2	0.06	None				This study

<sup>a</sup> Abbreviations: ABR, acetyltrimethylammonium bromide; AMP, ampicillin; ARS, sodium arsenate; CAD, cadmium acetate; ETB, ethidium bromide; GEN, gentamicin; KAN, kanamycin; LIN, lincomycin; MER, mercuric nitrate; MET, methicillin; MLSB<sub>pc</sub>, constitutive resistance to macrolides-lincosamides-SGB; MIN, minocycline; NEO, neomycin; PEN, penicillinase; PEF, pefloxacin; PRI, pristinamycin IIA; RIF, rifampin; SGA, streptogramin A; SPT, spectinomycin; SUL, sulfonamide; TET, tetracycline; TOB, tobramycin; TMP, trimethoprim.

<sup>b</sup> The strains were screened by PCR experiments for the following genetic elements: Tn554, *ermA*, *ermB*, *ermC*, *mecA*, *vatB*, *vgaA*, *vgaB*, *vgaA*<sub>v</sub>, *vgaB*<sub>v</sub>, *vgaA* and *vgaB*.

<sup>c</sup> Location: C, chromosome; P, plasmid.

<sup>d</sup> ND, not done.

TABLE 2. Primers used in this study

Primer	Sequence (5'-3')	Target	Accession no.	Positions (nt)	Source or reference
1	GTTTCGATTGTACATCCACG	<i>att554</i>	AF186237	1156–1174	25
2	ATCCACTACTATTTTTGCAC	Tn554 <i>tnpA</i> -like	AF186237	1410–1390	This study
3	GTGTAAAGGTTATTACATCGGC	Tn5406 3' end	AF186237	6665–6687	This study
4	CAATGAAAGTACTTCGTAGG	3' region from <i>att554</i>	AF186237	6920–6901	This study
5	GATGAACAATTTGAAGTAGTTGAACC	Tn554 <i>tnpA</i> -like	AF186237	1441–1466	This study
6	GATACTCATATTGAGTGGG	Tn554 <i>tnpB</i> -like	AF186237	3084–3066	This study
12	CCCGCTTCTACAAGACTGG	<i>att554</i>	AF186237	6842–6824	25
15	ATGAAAATATTTGTTAGAGG	<i>vgaAv</i>	AF186237	5065–5083	This study
16	AGACATGTCCTTAAAGTGATTC	Tn5406 3' region	AF186237	6659–6638	This study
<i>mecA</i> <sub>1</sub>	GGGATCATAGCGTCATTATTC	<i>mecA</i>	Y14051	3851–3871	36
<i>mecA</i> <sub>2</sub>	TATCGTCAACGATTGTGACACG	<i>mecA</i>	Y14051	4384–4363	36
<i>ermA</i> <sub>1</sub>	TATCTTATCGTTGAGAAGGGATT	<i>ermA</i>	K02987	4913–4891	22, 24
<i>ermA</i> <sub>2</sub>	CTACACTTGGCTTAGGATGAAA	<i>ermA</i>	K02987	4775–4796	22, 24
<i>spc</i> <sub>1</sub>	GTTGTCCCTTGGCAATATCCTC	<i>spc</i>	K02987	3928–3949	24
<i>ermB</i> <sub>1</sub>	CTATCTGATTGTTGAAGAAGGATT	<i>ermB</i>	U35228	366–389	22
<i>ermB</i> <sub>2</sub>	GTTTACTCTTGGTTTAGGATGAAA	<i>ermB</i>	U35228	484–507	22
<i>ermC</i> <sub>1</sub>	CTTGTTGATCACGATAATTTC	<i>ermC</i>	M17990	214–235	22
<i>ermC</i> <sub>2</sub>	ATCTTTTAGCAAACCCGTATTC	<i>ermC</i>	M17990	382–403	22

tibility to antibiotics was tested on Mueller-Hinton agar (Bio-Rad, Hercules, Calif.).

**Susceptibility to antimicrobial drugs.** Susceptibility to antibiotics was determined by a disk diffusion assay with commercially available antibiotic disks (Bio-Rad) performed according to the recommendations of the French Society of Microbiology and with disks prepared in our laboratory as described previously (14). The MICs of pristinamycin IIA and pristinamycin (Rhône-Poulenc Rorer, Vitry, France) were determined with serial 1:2 dilutions of antibiotics in Mueller-Hinton agar (1).

**DNA isolation and analysis.** Total cellular DNA was isolated from staphylococcal strains and purified by using the QIAamp tissue kit from Qiagen (Hilden, Germany). Plasmid DNA was extracted and purified from *E. coli* by using the QIAprep spin plasmid kit from Qiagen. Restriction endonucleases were obtained from Amersham-Pharmacia Biotech, Inc. (Piscataway, N.J.), and were used according to the manufacturer's instructions. DNA fragments of <500 bp were separated by electrophoresis in 4% NuSieve GTG agarose gels (FMC BioProducts, Rockland, Maine). *Sma*I digestion and pulsed-field gel electrophoresis were performed as described previously (14).

**Cloning and DNA sequencing.** DNA restriction fragments were inserted into *E. coli* vectors by using the ligase of the Fast-Link ligation kit (Epicenter Technologies Corp., Madison, Wis.), and recombinant plasmids were introduced into competent *E. coli* XL-2 Blue cells (Stratagene, La Jolla, Calif.) by transformation according to the manufacturer's instructions.

An Applied Biosystems (Foster City, Calif.) automated 373A DNA sequencer was used for sequencing according to the protocol provided by the manufacturer.

**Labeling of DNA probes, blotting, and hybridization.** Hybridization experiments were performed at 65°C as described previously (14)

**PCR.** DNA was amplified by PCR by using the Ready-To-Go kit (Amersham), according to the manufacturer's instructions, in a Crocodile III apparatus (Appligène, Illkirch, France). The primers used are described in Table 2. Primer pairs 1 and 2, 3 and 4, 2 and 3, 5 and 6, *mecA*<sub>1</sub> and *mecA*<sub>2</sub>, *ermB*<sub>1</sub> and *ermB*<sub>2</sub>, and *ermC*<sub>1</sub> and *ermC*<sub>2</sub> were used for amplification by PCR at high stringency (initial cycle of 5 min at 95°C and 2 min at 55°C, followed by 35 cycles of 1 min at 72°C, 30 s at 95°C, and 1 min at 55°C, with a final extension step of 5 min at 72°C). All other amplifications by PCR used the same conditions, replacing the annealing temperature of 55°C by the following temperatures depending on the primers: 53°C with primers *ermA*<sub>1</sub> and *spc*<sub>1</sub> (to amplify internal region of Tn554), 51°C with primers *ermA*<sub>2</sub> and 12 (to amplify the 3' region of Tn554 inserted in *att554*), 47°C with primers 1 and 12, and 42°C with primers 15 and 16 (to amplify *vgaAv*).

**Nucleotide sequence accession number.** The nucleotide sequences of Tn5406 in the *att554* site of *S. aureus* BM3327 have been submitted to GenBank under accession no. AF186237. The 5'- and 3'-flanking regions of Tn5406 in the *SC-Cmec* type element of *S. aureus* BM3252 are registered under accession no. AF411128 and AF411129, respectively.

## RESULTS

**Characterization of Tn5406 isolated from the chromosome of BM3327.** We recently characterized *vgaAv* (accession no. AF186237, nucleotides [nt] 5065 to 6639) (14). The two overlapping restriction fragments carrying the 5' and 3' parts of the sequenced copy of *vgaAv*, i.e., a 1.3-kb *Hind*III fragment and a 7-kb *Eco*RI fragment, respectively, were separately ligated into pUC18 cleaved by the same enzymes. The resulting recombinant plasmids, pIP1805 and pIP1809, respectively, were used to sequence the chromosomal regions adjacent to *vgaAv* (accession no. AF186237, nt 1 to 7089, including *vgaAv*). The three putative genes upstream from *vgaAv* exhibited significant similarities with the genes involved in the transposition of Tn554: *tnpA*, *tnpB*, and *tnpC* (70, 71.5, and 65% identities, respectively) (23, 24). The amino acid sequences of the predicted translation products of Tn5406 transposases were 71, 73, and 61% identical to those of Tn554, respectively (Fig. 1). Significant similarities with the 5' and 3' ends of Tn554 (accession no. K02987) (66.7 and 69.7% identity, respectively) were also observed between nt 1251 and 1383 and between nt 6642 and 6717 (accession no. AF186237). In contrast, the similarity between the region from the end of the Tn554-*tnpC*-like gene to the start of *vgaAv* (318 nt), as well as that of Tn554 between the end of *tnpC* and the start of *spc* (152 nt), is restricted to a region of 27 nt only (66.7% identity).

The region extending from nt 1251 to 6717 (accession no. AF186237) and including *vgaAv* and the three transposase genes is flanked by sequences corresponding to *att554* (accession no. K02985) (23, 29), the preferential insertion site of Tn554 in the chromosome of *S. aureus* strain RN450. Indeed, from nt 1135 to 1250 (accession no. AF186237), the sequence is identical to that of RN450 *att554* from nt 1 to 116 (accession no. K02985): both end with GATGTA, which is the 6-bp central core of *att554*. Moreover, from nt 6717 to 6851 (accession no. AF186237), the sequence is almost identical (one additional nucleotide in position 6843) to that of the RN450 *att554* site from nt 117 to 249. These data suggested that *vgaAv* of BM3327 was within a Tn554-like transposon extending from nt

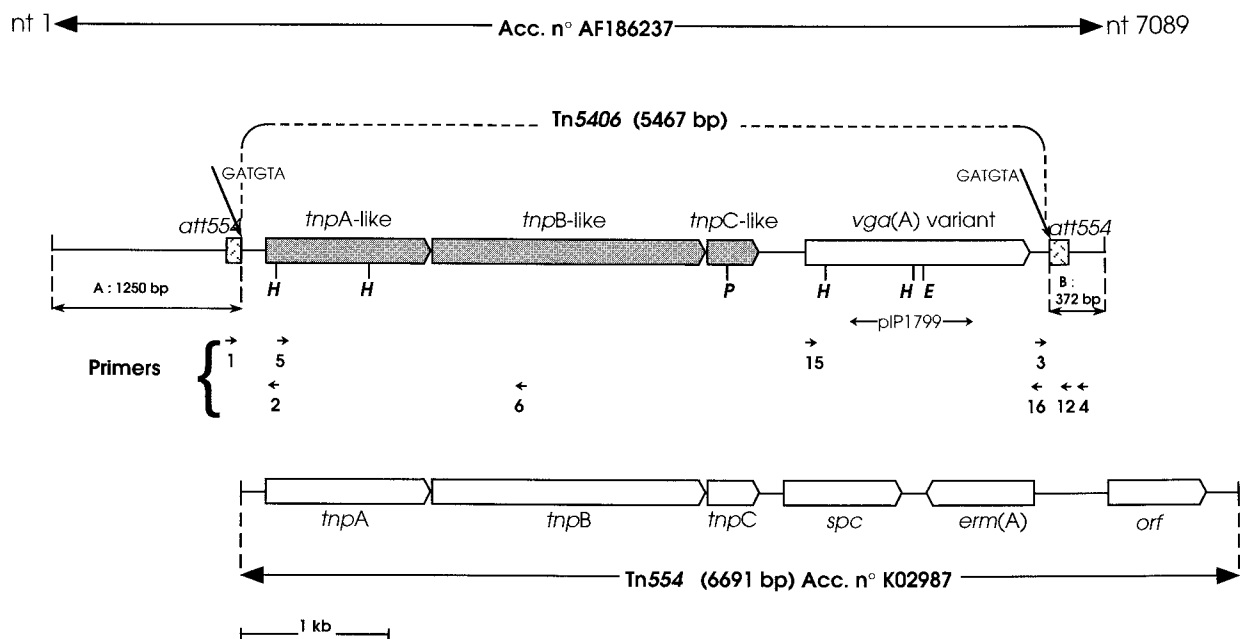


FIG. 1. Schematic representation of Tn554 and the sequenced copy of Tn5406 from the chromosome of *S. aureus* BM3327. The position and orientation on Tn5406 of primers described in Table 2 are indicated by arrows. The *vgaAv* intragenic fragment inserted in pIP1799 used as a probe is indicated by an arrow. Abbreviations: *H*, *Hind*III; *P*, *Pst*I; *E*, *Eco*RI. Regions (indicated by double-headed arrows): A:1,250 bp, region adjacent to the 5' part of Tn5406 and exhibiting 99.9% identity with chromosomal DNA of *S. aureus* N315 (accession no. AP003134, nt 192697 to 191446); B:372 bp, region adjacent to the 3' part of Tn5406 and exhibiting 99.2% identity with chromosomal DNA of *S. aureus* N315 (accession no. AP003134, nt 184733 to 184362).

1251 to 6717. This 5,467-bp putative transposon was named Tn5406 (Fig. 1).

#### Analysis of the regions flanking Tn5406 in strain BM3327.

We sequenced 1,250 nt 5' to Tn5406 and 372 nt 3' to Tn5406 in BM3327 (Fig. 1). The 1,250 nt 5' to Tn5406 (nt 1 to 1250) exhibited 99.9% identity with the *S. aureus* N315 genome from nt 192697 to 191446 (accession no. AP003134) (21). From nt 32 to 712 there is a putative gene whose translation product is a hydrophobic protein of 227 aa. A gene encoding a putative and closely related protein (98.7% aa identity) similar to type IV prepilin peptidase was detected at the same position relative to the 5' end of Tn554 in the chromosome of N315 (accession no. AP003134, nt 192689 to 191982). The sequenced region of 372 nt 3' to Tn5406 (nt 6718 to 7089) (Fig. 1) exhibited 99.2% identity with the 3' part of the copy of Tn554 in the *att554* site of the *S. aureus* N315 genome extending from nt 184733 to 184362 (accession no. AP003134) (21).

In the chromosome of BM3327, as well as in N315 (21) and NCTC8325 (<http://www.genome.ou.edu/staph.html>), the *att554* sequence is within a putative 669-bp gene (Fig. 2). The translation product of this gene is similar (53.1% aa identity) to the *Bacillus subtilis* protein Ysxa (accession no. Z99118, nt 65971 to 66666) (20) believed to be involved in DNA repair. In the chromosomes of BM3327 and N315, this putative gene is interrupted at the same site by either Tn5406 or Tn554, respectively. A frameshift mutation is present in the gene of BM3327 interrupted by Tn5406.

**Testing for circular forms of Tn5406 in the cellular DNA of clinical isolates.** Murphy (23) proposed a transposition model, including excision and circularization steps, preceding integration in the new target. To check whether this model can be

proposed for Tn5406, we looked for the presence of circular forms with primers 2 and 3 (Table 2). Amplification of the cellular DNA of strains BM3318 and BM3327 (Table 1) by PCR with these primers gave a 213-bp amplicon. This demonstrates the presence of circular forms of Tn5406, suggesting that it might be functional according to the model proposed for Tn554. We were not able to detect these forms in strains BM3252 and BM3385.

**Investigation for presence of Tn5406 in clinical isolates carrying *vgaAv*.** We have already shown (14) that a *vgaAv*-probe (pIP1799) hybridizes with (i) a single chromosomal *Sma*I fragment in BM3327; (ii) two chromosomal *Sma*I fragments in BM3252; (iii) a single plasmid *Sma*I fragment containing two other SGA resistance genes, *vatB* and *vgaB*, in BM3318; and (iv) both a chromosomal *Sma*I fragment and a large *vatB-vgaB* plasmid not cleaved by *Sma*I in BM3385. We used PCR, hybridization, and sequencing to determine whether these various copies of *vgaAv* are carried by Tn5406.

Each of the four wild-type strains tested (Table 1) gave two to four *Eco*RI fragments and two or three *Hind*III fragments hybridizing with the *vgaAv* probe, including the 0.57-kb *Hind*III intragenic fragment (results not shown). Since the intragenic probe used (from pIP1799) contained a *Hind*III site (14), these data suggested that each strain contained no more than one or two copies of *vgaAv*.

PCR with primer pairs 1-2 and 3-4 (Table 2) amplified fragments of 255 and 256 bp, respectively, from BM3327, BM3252, and BM3385. Thus, each of these strains contained a copy of Tn5406 inserted in *att554*. These results were confirmed by hybridization experiments with an "*att554* probe" consisting of a 220-bp fragment amplified from within the *att554* of RN4220



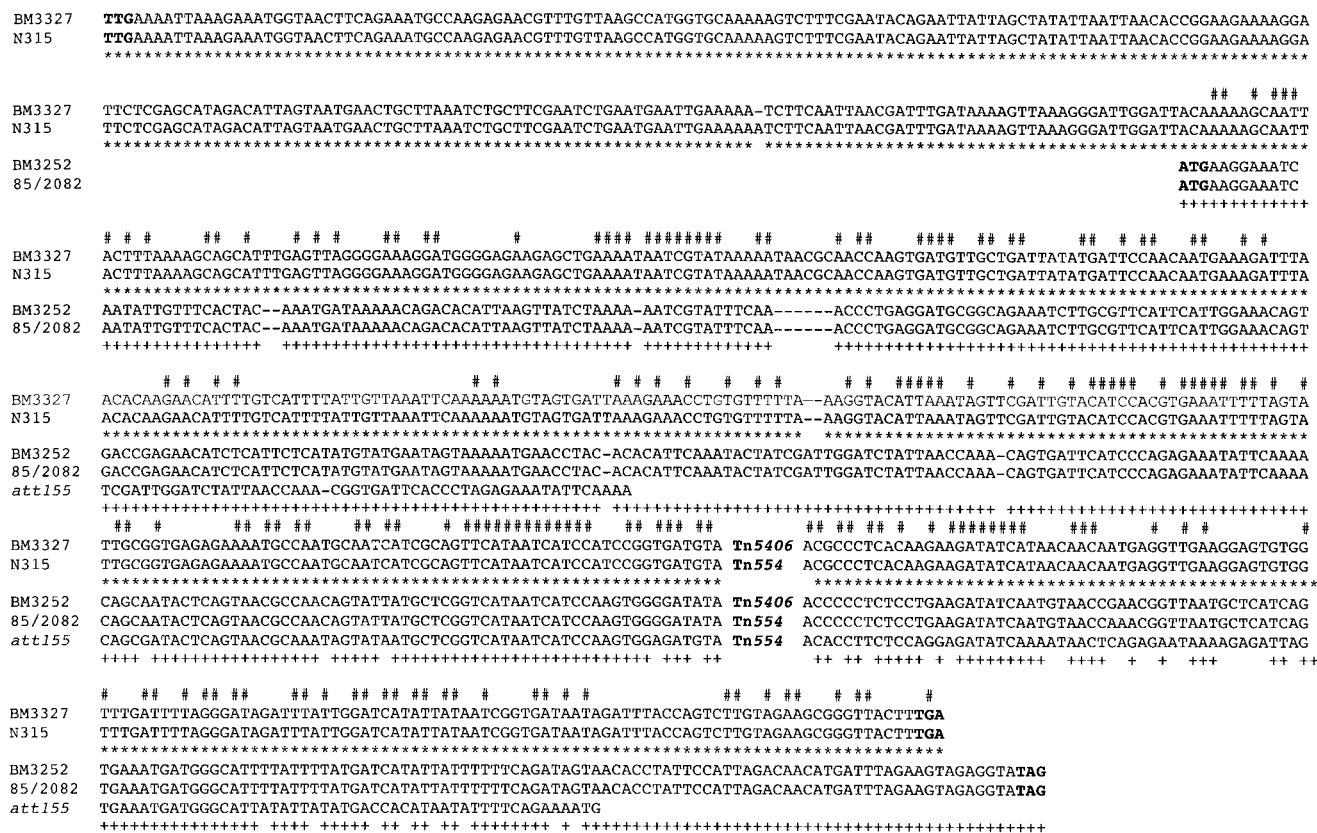


FIG. 2. Alignments of the nucleotide sequences of *att155* (33) and those flanking *Tn546* and *Tn554* in the chromosomes of the following *S. aureus* strains: BM3327 (accession no. AF186237, nt 713 to 1250, and nt 6718 to 6851), N315 (accession no. AP003134, nt 191453 to 191985 and nt 184362 to 184503), BM3252 (accession no. AF411128, nt 844 to 1146, and accession no. AF411129, nt 124 to 265), and 85/2082 (accession no. AB037671, nt 57652 to 58803 and nt 49780 to 50963). The sequences from these latter strains are delimited by the start and stop codons (reported in boldface) of the genes interrupted by the transposons. Symbols: #, nucleotides common to all the aligned sequences; \*, nucleotides common to BM3327 and N315; +, nucleotides common to BM3252, 85/2082, and *att155*.

with the primer pair 1-12 (Table 2): each of the three strains contained two *att554*-hybridizing *Hind*III fragments (a 1.3-kb fragment cohybridizing with *vgaAv* and a 3.2-kb fragment) (results not shown).

The finding that BM3318 appears to contain a circular form of *Tn5406* suggests that the single plasmid copy of *vgaAv* is carried by this transposon. Amplification with the primer pairs *spc*<sub>1</sub>-*ermA*<sub>1</sub> and *ermA*<sub>2</sub>-12 (Table 2) and the detection of two *Hind*III fragments hybridizing with the *att554* probe but not with *vgaAv* probe indicated that the single chromosomal *att554* site in BM3318 was occupied by a copy of *Tn554*.

In BM3385, the 3' part of the plasmid-borne *vgaAv* is carried by a 1.1-kb *Hind*III fragment (results not shown). An indistinguishable fragment is present in BM3318 (results not shown). The 5' part of this *vgaAv* copy in BM3385 is on an *Eco*RI fragment of ~20 kb giving no hybridization signal with the *att554* probe. However, this *Eco*RI fragment hybridized with a "Tn5406 transposase A-B probe" (a 1,640-bp amplicon obtained with primers 5 and 6 [Table 2]) (results not shown). Thus, the plasmid copy of *vgaAv* in BM3385 is, like the chromosomal copy, carried by *Tn5406*.

*Hind*III and *Eco*RI digests of BM3252 and BM3327 DNAs were hybridized with the *vgaAv* probe (results not shown).

BM3252 gave three *Hind*III fragments hybridizing with *vgaAv* probe, two of which were also present in BM3327: *Hind*III fragments of 0.57 kb (internal to *vgaAv*) and of 1.3 kb. The third *Hind*III fragment (3 kb), unique to BM3252, was suspected to contain a *vgaAv* not on the copy of *Tn5406* inserted in *att554*. It was inserted into pUC18. The ends of this fragment were sequenced: it indeed carried part of a *vgaAv* gene, including 123 nt identical to the 3' end of BM3327 *Tn5406* (accession no. AF186237, nt 6594 to 6717). Two of the four *Eco*RI fragments (7 and 7.5 kb) of BM3252 hybridized with both the *vgaAv* probe and the probe obtained with primers 1 and 2 (Table 2). The 7.5-kb *Eco*RI fragment of BM3252 was not found in BM3327. Therefore, it was suspected to carry the 5' part of the copy of *vgaAv*, which is not in the *Tn5406* copy at the *att554* site. This fragment was inserted into pUC18, and its ends were sequenced: it was mapped to the 5' part of the transposon. It contained 134 nt identical to the 5' end of BM3327 *Tn5406* (accession no. AF186237, nt 1251 to 1385). These data suggested that both copies of *vgaAv* in BM3252 were carried by *Tn5406*-related transposons.

**Sequence analysis of the regions flanking the chromosomal copy of *Tn5406* in BM3252 not inserted in the *att554***

**site.** We sequenced 1,146 nt 5' and 1,185 nt 3' to the chromosomal copy of Tn5406 in BM3252 that was not inserted into *att554*. These flanking regions were more similar to the 240-nt *att155* insertion site of Tn554 in methicillin-resistant *S. aureus* and *S. epidermidis* clinical strains isolated in 1988 (33) (81.7% identity on a 229-nt overlap) than to *att554* (58.1%). Nevertheless, this Tn5406 insertion site in BM3252 was clearly neither *att554* nor *att155*: the central hexanucleotide core sequence GATATA, differed by a single nucleotide from those (GATGTA) of *att155* and *att554*. The sequence of the region 5' to Tn5406 in BM3252 from nt 1 to 1146 (accession no. AF411128) is at least 87.6% identical to the 5'-flanking regions of Tn554 inserted in the type III *SCCmec* of *S. aureus* strains 85/3907 (accession no. AB047089, nt 68 to 1213) (16) and 85/2082 (accession no. AB037671, nt 57652 to 58803) (16). The sequence of the region 3' to Tn5406 (accession no. AF411129, nt 124 to 1308) is highly similar (97.5% nt identity) to the 3'-flanking region of Tn554 in the type III *SCCmec* of strain 85/2082 (accession no. AB037671, nt 49780 to 50963) (16). Moreover, the 128-nt 3' to BM3252 Tn5406 are also identical to the right arm of the secondary Tn554 attachment site (accession no. M32312, nt 1 to 128) in the methicillin-resistant *S. aureus* isolate, ANS46. This strain is typical of endemic and epidemic methicillin-resistant *S. aureus* strains found in Australia and England (8).

The BM3252 Tn5406 chromosomal copy which is not in *att554* appears to be inserted into a gene encoding a 148-aa putative protein. This gene is closely related to those of strains 85/3907 and 85/2082 (16), which are interrupted by Tn554 at the same relative position (Fig. 2). These genes are assumed to be involved in DNA repair because of the significant similarity of their products with the *Bacillus* sp. strain NEB-606 RadC (15).

**Analysis of SGA-susceptible derivatives of *S. aureus* clinical isolates, BM3318 and BM3385.** BM3318 and BM3385 derivatives susceptible to SGA, IPF110 and BM12940, respectively (Table 1), were obtained after 20 passages of each strain on drug-free medium. The BM3318 derivative, IPF110, had lost resistance to SGA, *vatB*, *vgaB*, Tn5406, and also the single plasmid *SmaI* band (results not shown), suggesting the loss of the large SGA resistance plasmid. This loss was not associated with the loss of LIN resistance (*ermB* conferring constitutive MLS<sub>B</sub> resistance) or with that of penicillinase (BM3318 carries two penicillinase plasmids). The SGA-susceptible variant of BM3385, BM12940, whose *SmaI* pattern was identical to that of BM3385, had lost the antibiotic resistances conferred by pIP1156 and Tn5406 (resistance to SGA, lincomycin, trimethoprim, and penicillinase). With *SmaI*-digested DNA from strain BM12940, no hybridization was detected with *vgaB*, *vatB*, and *vgaAv* probes (results not shown). Conversely, with the same probes and *SmaI*-digested BM3385 DNA, hybridization was detected at the level of the wells and ~670 kb (14). The *att554* probe hybridized with a single *HindIII* fragment of 3.6 kb in the cellular DNA of BM12940 as in RN4220 (results not shown). Primer pair 1-12 (Table 2) amplified a 220-bp fragment from BM12940. Its sequence was identical to part of *att554*. This indicates that the chromosomal copy of Tn5406 had been precisely excised from BM3385.

## DISCUSSION

The sequencing of regions adjacent to *vgaAv* in BM3327 allowed us to characterize a new transposon, Tn5406, that is similar to Tn554. We detected circular forms of Tn5406 in some clinical isolates, and it was able to excise precisely from its insertion site. There is thus strong evidence of its ability to transpose. Four wild-type *S. aureus* clinical strains were studied, and in all cases *vgaAv* was on Tn5406 (one or two copies per isolate). Thus, *vgaAv* may always be carried by Tn5406. This transposon is therefore presumably not uncommon among SGA-resistant staphylococcal strains, since *vgaAv* was detected in 25 of the 56 independent staphylococci belonging to five species investigated (14). All plasmid copies of Tn5406 were on large self transferable plasmids, carrying two other SGA resistance genes, *vatB* and *vgaB*, which are cotranscribed and functional in BM3385. These plasmids are unstable in the absence of selection with SGA. The advantage of Tn5406 being carried by these plasmids may be the maintenance of SGA resistance through its transposition to the multiple available chromosomal insertion sites. However, although Tn5406 is active in BM3318, it did not transpose to the chromosome of the SGA-susceptible derivative IPF110. This may have been because IPF110 was derived in the absence of selective pressure with SGA. In BM3252, which is susceptible to erythromycin and SGB, the two chromosomal copies of Tn5406 conferred resistance to SGA (pristinamycin IIA MIC of 64  $\mu\text{g ml}^{-1}$ ) but not to pristinamycin (MIC of 1  $\mu\text{g ml}^{-1}$ ). In contrast, BM3385, which carries *vgaB* and *vatB* in addition to two copies of Tn5406, is resistant not only to SGA (pristinamycin IIA MIC of 128  $\mu\text{g ml}^{-1}$ ) but also to pristinamycin (MIC of 4  $\mu\text{g ml}^{-1}$ ). It is not known which of the SGA resistance genes and Tn5406 copies is functional in any strain. The multiplicity of Tn554 copies in clinical isolates has been explained by mutational drift toward inactivation in the *spc* gene (32). Similarly, the presence of more than one copy of Tn5406 could possibly be a consequence of mutations inactivating some copies of *vgaAv*. Different copies of Tn5406 carried by individual strains should be cloned to test their expression.

The G+C content of the transposons Tn554 (32.6%) and Tn5406 (34.9%), including the antibiotic resistance genes, is similar to that of the staphylococcal genome (32 to 36%) but slightly higher than that of the two putative ATP-binding proteins conferring resistance to SGA, i.e., *vgaA* (29%) and *vgaB* (27.2%). The evolutionary ancestor of *vgaA* and *vgaB* probably belongs to a genus other than staphylococcus. If *vgaAv* has the same ancestor, it was acquired by staphylococci much earlier. Tn554 and Tn5406 have very similar transposases, and both were found in *S. aureus* chromosomal insertion sites that were either identical (*att554*) or very similar (*att155* in the *SCCmec* region). In both sites, the transposition of Tn554 or Tn5406 interrupts genes encoding putative DNA repair proteins (Ysxa-like or RadC-like) with 31.7% identical amino acids. This relatedness is significantly higher in the regions flanking Tn5406 and Tn554: 64.3% for the 28 aa upstream and 48.5% for the 33 aa downstream. The nucleotide sequences encoding these highly related flanking regions (84 nt upstream and 99 nt downstream), may thus serve as insertion sites for these two transposons.

All attempts to detect repeats in the regions flanking the

transposase genes in Tn554 and Tn5406 failed. Detection of such repeats, which may be subject to site-specific breakage and joining, would make the transposase region available as a cassette to pick up different antibiotic resistance genes and thus to trigger their transfer.

#### ACKNOWLEDGMENTS

We thank C. Tran for secretarial assistance. We are grateful to the *Staphylococcus aureus* Genome Sequencing Project and to B. A. Roe, Y. R. Tian, H. Jia, S. Li, S. Lin, S. Kenton, H. Lai, J. D. White, A. Dorman, F. Z. Najar, S. Clifton, V. Worrell, and J. Iandolo, with funding from the NIH and the Merck Genome Research Institute, for the use of NCTC 8325 DNA sequence data prior to publication.

J.H. received grants from the Fonds d'Etudes et de Recherche du Corps Médical des Hôpitaux de Paris, Institut Lilly (Bourse d'Etude en Infectiologie SPILF-Lilly), and from the Institut SmithKline Beecham.

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