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Characterization of a Variant of *vga*(A) Conferring Resistance to Streptogramin A and Related Compounds

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A variant of the *vga*(A) gene (1,575 bp), encoding an ATP-binding cassette protein conferring resistance to streptogramin A and related antibiotics, was cloned from the chromosome of a *Staphylococcus aureus* clinical isolate and sequenced. The sequence of the variant was similar to that of the *vga*(A) gene (83.2% identity). However, the G+C content of the variant (35.6%) was higher than that of *vga*(A) (29%) and there was no cross hybridization between *vga*(A) and the variant at high stringency (≥60°C), the highest temperature at which a signal was detected being 55°C. Unlike previous reports for *vga*(A) and *vga*(B), the variant of *vga*(A) may be present in multiple copies in the genome. These copies are chromosomal in some isolates and both chromosomal and plasmid-borne in others. Nucleotide sequences hybridizing at 65°C with the *vga*(A) variant were found in all the staphylococcal strains harboring plasmids carrying both *vga*(B) and *vat*(B), which also encode resistance to streptogramin A.

Streptogramins and related antibiotics are produced by streptomycetes and are classified as A and B compounds, according to their basic primary structures (13). These compounds bind different targets in the peptidyltransferase domain of the 50S ribosomal subunit and inhibit protein elongation at different steps. A compounds provoke a conformational modification of the bacterial ribosome at the B compound binding site and decrease the dissociation constant of B compounds (14). Thus, A and B compounds, which are bacteriostatic when used separately, act synergistically when combined and may become bactericidal, mainly against gram-positive bacteria. Natural mixtures, such as pristinamycin, synergistin, virginiamycin, and mikamycin, are used orally and topically. Quinupristin-dalfopristin (Synercid), consisting of an injectable semisynthetic mixture of derivatives of streptogramins B and A, respectively, is now available in hospitals for the treatment of infections due to gram-positive cocci resistant to other antibiotics.

Staphylococcal resistance to synergistic mixtures of A and B compounds (pristinamycin MICs of ≥2 mg · liter^{-1}) is always associated with resistance to A compounds (pristinamycin IIA MICs of ≥8 mg · liter^{-1}) but not necessarily with resistance to B compounds (1, 16). To date, seven genes encoding resistance to A compounds have been isolated from staphylococcal and enterococcal plasmids. Genes *vat* (8) [now named *vat*(A) (28)], *vat*(B) (2), *vat*(C) (5), *sat*(A) (27) [renamed *vat*(D) (28)], and *sat*(G) (20, 32) [renamed *vat*(E) (28)] encode related proteins (50.4 to 60.1% identical amino acids) conferring resistance to streptogramin A and similar compounds by acetylation of the drugs. The degenerate primers M and N can be used to detect any of these genes by PCR experiments (1). The staphylococcal *vga* gene (6) [renamed *vga*(A)] and *vga*(B) (3) encode related ATP-binding proteins (58.8% identical amino acids) probably involved in the active efflux of A compounds (16).

We report here the cloning, sequencing, and distribution of a variant of the staphylococcal gene *vga*(A), which encodes an ATP-binding protein conferring resistance to A compounds.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The relevant characteristics of the strains and plasmids used are reported in Table 1. Two collections of strains resistant to streptogramin A were screened for the presence of the variant of *vga*(A). One collection consisted of 52 staphylococci belonging to five species (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus cohnii* subsp. *urealyticum*, and *Staphylococcus simulans*) (1) and included the clinical isolates described in Table 1 and the *S. cohnii* strain harboring pIP1714, which carries *vat*(C) and *vgb*(B) (5). The other collection consisted of 51 *Enterococcus faecium* strains isolated from fecal samples from poultry, pigs, farmers, and suburban residents in The Netherlands (20).

Media. Staphylococci were grown in brain heart infusion (Difco Laboratories, Detroit, Mich.), and *Escherichia coli* cells were grown in Luria broth. Susceptibility to antibiotics was tested on Mueller-Hinton agar (Diagnostics Pasteur, Marnes-la-Coquette, France).

Susceptibility to antimicrobial drugs. Susceptibility to antibiotics was determined by a disk diffusion assay (12) with commercially available antibiotic disks (Diagnostics Pasteur) and disks prepared in our laboratory as described previously (19). MICs of pristinamycin IIA and pristinamycin (Rhône-Poulenc Rorer, Vitry, France) were determined with serial 1:2 dilutions of antibiotics in Mueller-Hinton agar (17).

DNA isolation and analysis. Total cellular DNA was isolated from staphylococcal strains and was purified using the QIAamp tissue kit from Qiagen (Hilden, Germany). Plasmid DNA was extracted and purified from *E. coli* 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 less than 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 (15).

Cloning and DNA sequencing. Fragments amplified by PCR were cloned using the TOPO TA cloning kit (Invitrogen, Groningen, The Netherlands) following the manufacturer's instructions. DNA restriction fragments were inserted into *E. coli* vectors using the ligase of the Fast-Link ligation kit (Epicentre Technologies Corporation, Madison, Wis.), and the recombinant plasmids were introduced by transformation into competent *E. coli* XL2-Blue cells (Stratagene, La Jolla, Calif.) following the manufacturer's instructions.

An Applied Biosystems (Foster City, Calif.) automated 373A DNA sequencer and the protocol described by the manufacturer were used for sequencing.

Labeling of DNA probes, blotting, and hybridization. Plasmid DNA was labeled with [α-^{32}]dCTP (110 TBq mmol^{-1}) by random priming using the Megaprime DNA labeling system (Amersham).

Hybond N^{+} membranes (Amersham) were used for blotting as described previously (1). Prehybridization and hybridization were done at various temperatures (65, 60, 55, 50, 45, and 42°C) in a mixture containing 5× SSPE (1× SSPE is 0.15 M NaCl and 8 mM NaH_2PO_4), 5× Denhardt's solution, 0.5% (wt/vol) sodium dodecyl sulfate (SDS), and 100 μg of fish sperm DNA (DNA, molecular

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

Strain or plasmid	Characteristic(s)	Resistance[a]	Reference(s)
Strains			
BM225	*S. aureus* recipient; mutant of RN8325-4	Fa Nv Rf	18
BM3093	*S. aureus* transductant; BM225 + pIP680; *vga*(A) *vat*(A) *vgb*(A)	Fa Nv Rf SgB SgA (64) Pt (4)	4, 7
BM3249	*S. aureus* clinical isolate; *vga*(A) variant	L SgA (64) Pt (1) Pc Mc Tc Mn Km Nm Tm Gm Sm Su Pf Fm Cd As Hg Eb	1
BM3250	*S. aureus* clinical isolate; *vga*(A) variant	L SgA (64) Pt (1) Pc Mc Tc Mn Km Tm Gm Sm Su Cm Cd As Hg Eb	2, 3
BM3252	*S. aureus* clinical isolate; *vga*(A) variant	L SgA (64) Pt (1) Pc Mc Tc Mn Km Nm Tm Gm Sm Su Cd As Hg Eb	2, 3
BM3318	*S. aureus* clinical isolate; *vga*(A) variant, *vga*(B) *vat*(B)	MLSc SgA (128) Pt (16) Pc Mc Tc Mn Km Nm Tm Gm Sm Sp Su Cd As Hg Eb Ba	2, 3
BM3327	*S. aureus* clinical isolate; *vga*(A) variant	MLSc SgA (128) Pt (8) Pc Tc Mn Km Nm Tm Gm Sm Su Pf	1, 3
BM3385	*S. aureus* clinical isolate; *vga*(A) variant + pIP1156; *vga*(B) *vat*(B)	L SgA (128) Pt (4) Pc Su Tp Rf Cd As Eb	2, 3
ISP1127	*S. aureus* recipient	Nv	26
RN4220	*S. aureus* recipient	No drug resistance marker	22
BM12926	*S. aureus* transformant; RN4220 [pIP1810; *vga*(A) variant]	SgA (32) Km Nm Tm	This study
BM12927	*S. aureus* transformant; RN4220 (pRB374)	Km Nm Tm	This study
TOP10F′	*E. coli* recipient (Invitrogen)	No drug resistance marker	
XL2-Blue	*E. coli* recipient (Stratagene)	No drug resistance marker	
Plasmids			
pUC18		Ap	
pCR2.1-TOPO	*E. coli* cloning vector (Invitrogen)	Ap	
pRB374	Shuttle vector with *vegII* promoter	Ap Km Nm Tm in *E. coli*; Km Nm Tm in *S. aureus*	11
pIP1652	pUC18 + 619-bp insert from within *vat*(A); accession no. L07778; nt 269 to nt 887	Ap	8
pIP1653	pUC18 + 476-bp insert from within *vga*(A); accession no. M90056; nt 1199 to nt 1674	Ap	6
pIP1654	pUC18 + 920-bp insert from within *vgb*(A); accession no. JO3313; nt 624 to nt 1543	Ap	7
pIP1692	pUC18 + 603-bp insert from within *vat*(B); accession no. L38809; nt 79 to nt 681	Ap	2
pIP1705	pUC18 + 1,040-bp insert from within *vga*(B); accession no. U82085; nt 352 to nt 1391	Ap	3
pIP1740	pUC18 + 565-bp insert from within *vat*(C); accession no. AFO15628; nt 1310 to nt 1874	Ap	5
pIP1741	pUC18 + 714-bp insert from within *vgb*(B); accession no. AFO15628; nt 520 to nt 1233	Ap	5
pIP1795	pCR2.1-TOPO + 594-bp insert from within *vat*(D); accession no. L12033; nt 189 to nt 782	Ap	This study
pIP1799	pCR2.1-TOPO + 580-bp insert from within *vga*(A) variant; accession no. AF186237; nt 668 to nt 1247	Ap	This study
pIP1802	pUC18 + 528-bp insert from within *vat*(E); accession no. AF153312; nt 363 to nt 890	Ap	20
pIP1810	pRB374 + 1,811-bp insert including *vga*(A) variant from BM3327; accession no. AF186237; nt 50 to nt 1860	Ap Km Nm Tm in *E. coli*; SgA Km Nm Tm in *S. aureus*	This study

[a] Abbreviations: Ap, ampicillin; As, sodium arsenate; Ba, cetyltrimethylammonium bromide; Cd, cadmium acetate; Cm, chloramphenicol; Eb, ethidium bromide; Fa, fusicid acid; Fm, fosfomycin; Gm, gentamicin; Hg, mercuric nitrate; Km, kanamycin; L, lincosamide; Mc, methicillin; MLSc, constitutive resistance to macrolides-lincosamides-streptogramin B; Mn, minocycline; Nm, neomycin; Nv, novobiocin; Pc, β-lactam (penicillinase production); Pf, pefloxacin; Rf, rifampin; SgA, streptogramin A; SgB, streptogramin B; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim. Numbers in parentheses after SgA are MICs (milligrams per liter) of pristinamycin IIA; those after Pt are MICs (milligrams per liter) of pristinamycin.

biology grade; Roche Molecular Biochemicals, Mannheim, Germany) ml^{-1}. The membranes carrying DNA transferred from agarose gels were treated with 10 ng of radiolabeled DNA probe ml^{-1}. The washing began with two successive immersions in 2× SSPE–0.1% SDS at room temperature for 10 min, followed by one immersion in 1× SSPE–0.1% SDS at the hybridization temperature for 15 min, and finally by one immersion in 0.1× SSPE–0.1% SDS at the hybridization temperature for 10 min. Washed blots treated with the radiolabeled probe were placed against Hyperfilm (Amersham) at −80°C.

PCR. DNA was amplified by PCR using the Ready-To-Go kit (Amersham) according to the manufacturer's instructions in a Crocodile III apparatus (Appligène, Illkirch, France). The following primers were used: A, 5′-AAYTAYWCNAAYTAYRTNGARCARAARGA-3′ [nucleotide (nt) 1386 to nt 1414 in *vga*(A), accession no. M90056]; B, 5′-NACRTTYTCNARNATNGAYTT-3′ [nt 1967 to nt 1947 in *vga*(A), accession no. M90056]; C, 5′-CTTCAATTGGGATCCTCAGGATAGG-3′ [nt 40 to nt 64 in the *vga*(A) variant, accession no. AF186237; *Bam*HI site is underlined]; D, 5′-GTTATGGTACCTTCTTGTTAGG-3′ [nt 1866 to nt 1845 in *vga*(A) variant, accession no. AF186237; *Bam*HI site is underlined]; E, 5′-CTCTTTGTACGAGTATATGG-3′ [nt 612 to nt 631 in *vga*(A) variant, accession no. AF186237]; and F, 5′-GTTTCTTAGTAGCTCGTTGAGC-3′ [nt 809 to nt 788 in *vga*(A) variant, accession no. AF186237]. PCR experiments with primers A and B were carried out at low stringency (initial cycle of 5 min at 95°C followed by 35 cycles of 30 s at 40°C, 30 s at 72°C, and 30 s at 95°C with a final extension step of 4 min at 40°C and 10 min at 72°C), and those with primers C and D, and E and F, were carried out 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 and a final extension step of 5 min at 72°C).

Nucleotide sequence accession number. The nucleotide sequences of the *vga*(A) variant gene and the flanking regions (19 nt upstream and 305 nt downstream) have been submitted to GenBank under accession no. AF186237.

RESULTS AND DISCUSSION

Filter mating experiments. Each of the four *S. aureus* isolates (BM3249, BM3250, BM3252, and BM3327 [Table 1]) resistant to pristinamycin IIA (MICs of 64 to 128 mg · $liter^{-1}$)

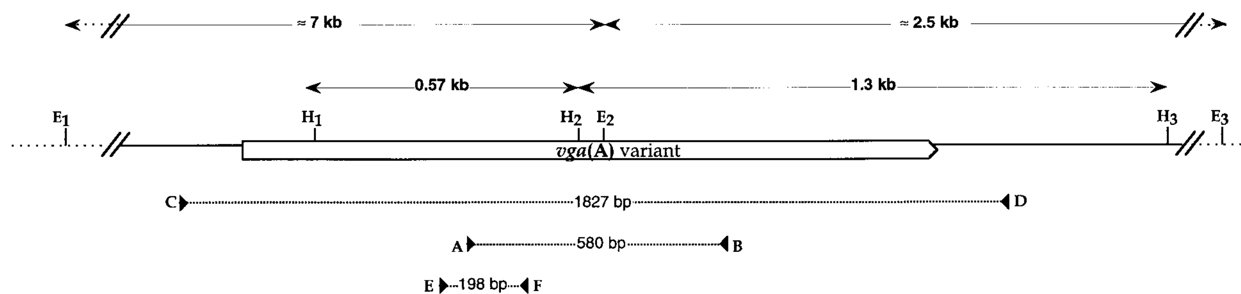

FIG. 1. Restriction map of the chromosomal region of BM3327 carrying the *vga*(A) variant (accession no. AF186237). The primers A, B, C, D, E, and F, indicated by arrows, are described in Materials and Methods, and the sizes of the amplicons are indicated below the map.

and to related antibiotics was crossed on a membrane filter with the *S. aureus* recipient strain ISP1127 (26). No transcipients were obtained by selection on pristinamycin IIA (frequency $< 10^{-10}$ transcipients/donor CFU).

Hybridization experiments with SgA^r gene probes at various temperatures. The total cellular DNA of the four *S. aureus* isolates cited above does not hybridize at high stringency (65°C) with the *vat*(A), *vat*(B), *vat*(C), *vat*(D), *vat*(E), *vga*(A), or *vga*(B) probes (1, 3). We repeated this experiment at 42°C, the lowest temperature at which no nonspecific signals were observed with the chromosomal DNA of the *S. aureus* strain RN4220 (22), susceptible to all antibiotics. At 42°C, a single 0.57-kb *Hin*dIII fragment hybridizing with the *vga*(A) probe was detected in the DNA of each of the four *S. aureus* clinical isolates (results not shown). A hybridizing *Hin*dIII fragment of the same size was detected in the DNA of the *S. aureus* clinical isolate, BM3385, harboring pIP1156 (≈60 kb), which carries *vga*(B) and *vat*(B), which are contiguous and are located in a 7-kb *Hin*dIII fragment. In the *S. aureus* transductant, BM3093, harboring pIP680 (4), the *vga*(A) probe hybridized at 42°C only with a *Hin*dIII fragment of 5.6 kb carrying *vga*(A), confirming the absence of nonspecific hybridization at 42°C.

To determine the highest temperature at which the *vga*(A) probe detected the 0.57-kb *Hin*dIII fragment in the four *S. aureus* clinical isolates, hybridization experiments were carried out with the *vga*(A) probe at various temperatures. The *vga*(A) probe hybridized with the 0.57-kb *Hin*dIII fragment at 45, 50, and 55°C but not at 60 and 65°C. This strongly suggested that these isolates carried a gene related to, but divergent from, *vga*(A).

PCR experiments with degenerate primers, A and B, which encode conserved motifs in *vga*(A) and *vga*(B). Conserved motifs in the peptide sequences deduced from *vga*(A) and *vga*(B) were chosen outside the regions containing the Walker motifs A and B, as they are widespread in ATP-binding cassette proteins. Primers A (coding strand) and B (complementary strand) (Materials and Methods) were expected to amplify 582- and 579-bp DNA fragments from within the *vga*(A) and *vga*(B) genes, respectively.

The cellular DNA of BM3093 containing *vga*(A) was primed with oligonucleotides A and B in PCR experiments at low stringency (40°C). A DNA fragment of the expected size (≈580 bp) was amplified from the cellular DNA of BM3093 and the five *S. aureus* clinical isolates, BM3249, BM3250, BM3252, BM3327, and BM3385. The sequences of the 580-bp fragments amplified from BM3250 and BM3327 were identical and exhibited 80 and 61% nucleotide identity with those of *vga*(A) and *vga*(B), respectively. The G+C contents of the amplicons (36.1%) were higher than those of *vga*(A) (29%) or *vga*(B) (27.2%).

The 580-bp amplicon from BM3250 was inserted into linearized pCR2.1-TOPO, and the resulting recombinant plasmid was named pIP1799.

Hybridization experiments using pIP1799 as a probe. Cellular DNA extracted from BM3249, BM3250, BM3252, BM3327, BM3093, and BM3385 was cleaved with *Hin*dIII and probed with pIP1799 at high stringency (65°C). Nucleotide sequences hybridizing with the probe were detected in all the strains, except BM3093 (results not shown). Each hybridization pattern contained two or four *Hin*dIII fragments, two of which (0.57 and 1.3 kb) were common to all the patterns; additional hybridizing fragments were detected in the patterns of BM3249 and BM3250 (3 kb), BM3252 (1.1 and 3 kb), and BM3385 (1.1 kb). These results suggest that the 580-bp insert of pIP1799, which hybridized with neither *vga*(A) nor *vga*(B) at high stringency, did not originate from either of these genes.

Cloning and sequencing of the putative new gene carried by the cellular DNA of BM3327. BM3327 was chosen because it carried only the two *Hin*dIII fragments of 0.57 and 1.3 kb hybridizing with pIP1799 and common to the hybridization patterns of all five *S. aureus* clinical isolates tested. Each of the two fragments was inserted separately into the *Hin*dIII site of pUC18 and sequenced. Each *Hin*dIII insert contained part of the amplicon from pIP1799; thus, they were contiguous. An open reading frame of 1,418 nt including the 0.57-kb *Hin*dIII insert (575 nt) and 843 nt of the 1.3-kb *Hin*dIII insert was identified. The region of the genome upstream from the 0.57-kb *Hin*dIII fragment was sequenced to obtain the part of the putative gene encoding the N terminus of the putative protein.

Two *Eco*RI fragments of 2.5 and 7 kb were found in the hybridization pattern of BM3327 (results not shown). To identify the *Eco*RI fragment carrying the start of the gene, a 198-bp DNA fragment was amplified from BM3327 with oligonucleotides E and F (Fig. 1). The 198-bp amplicon used as a probe hybridized with the 7-kb *Eco*RI fragment in the cellular DNA of BM3327, but not with the 2.5-kb *Eco*RI fragment. Thus, the 7-kb *Eco*RI fragment was inserted into the *Eco*RI site of pUC18 and sequenced with a primer corresponding to a region within the 0.57-kb *Hin*dIII fragment. The first start codon (ATG) upstream from the *Hin*dIII site H_1 is 8 nt downstream from a 6-nt putative ribosome binding site. The ΔG of interaction of the most stable structure between this putative ribosome binding site and the 3′ end of the 16S rRNA (25), calculated according to the method of Tinoco et al. (29), is −64.4 kJ · mol^{-1}. Thus, the sequence registered in the GenBank-EMBL Data Library under accession no. AF186237 contains a 1,575-bp gene delimited by the ATG codon at nt 191 to 193 and the TGA stop codon at nt 1763 to 1765. This gene encodes a 524-amino-acid putative protein of 58,216 Da and displays 83.2% nucleotide identity to *vga*(A) and 57.4% nucleotide

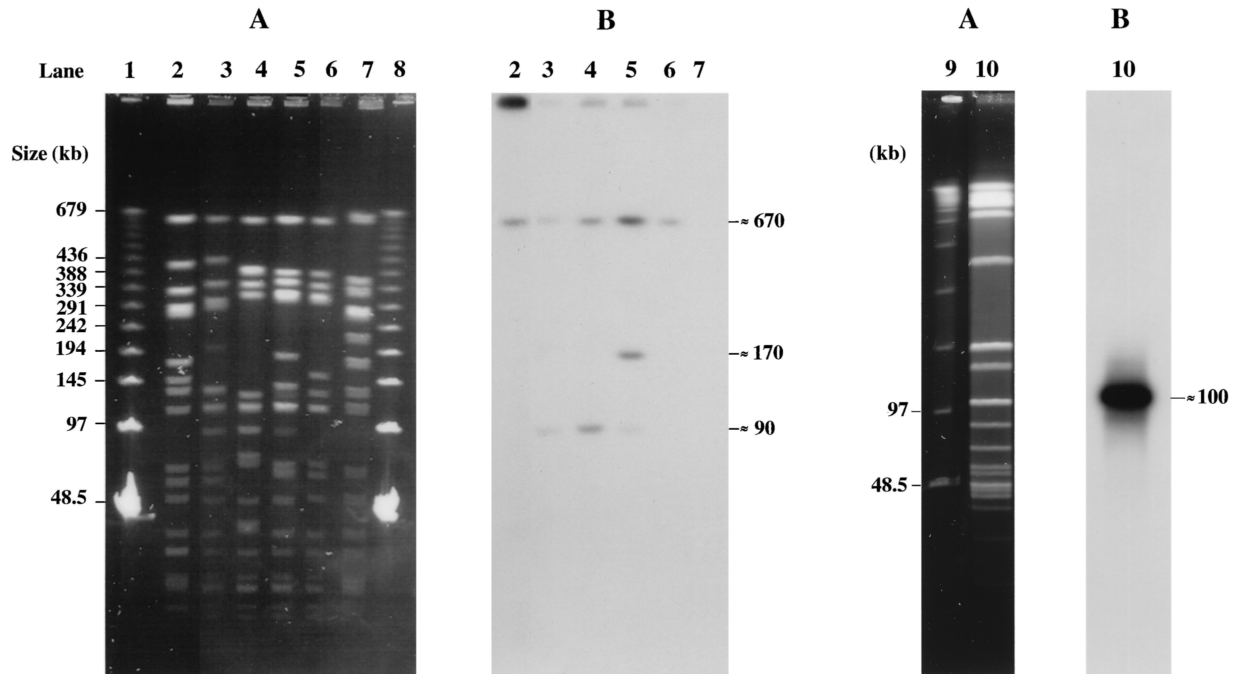

FIG. 2. Pulsed-field gel electrophoresis of *Sma*I-digested total DNA from clinical *S. aureus* strains resistant to streptogramin A. (A) *Sma*I macrorestriction patterns; (B) hybridization patterns with the *vga*(A) probe (pIP1799) at high stringency (65°C, 5× SSPE buffer). Lanes 1, 8, and 9, bacteriophage lambda DNA concatemers (Bio-Rad); lanes 2, BM3385; lanes 3, BM3250; lanes 4, BM3252; lanes 5, BM3249; lanes 6, BM3327; lanes 7, NCTC8325 used as standard; lanes 10, BM3318.

identity to the *vga*(B) gene. The G+C content of the sequenced gene is 35.6%. This value is higher than those of *vga*(A) (29%) and *vga*(B) (27.2%) but similar to those of the staphylococcal genome (32 to 36%).

The predicted translation product of the sequenced gene has a calculated pI of 7.27. According to the algorithm of Kyte and Doolittle (23), the protein is hydrophilic. It contains no sequence similar to known signal sequences of secreted proteins (30). The amino acid sequence was compared to the sequences available in databases (GenBank, release 114; EMBL, release 60). Significant similarities to the ATP-binding domains of numerous ATP-binding cassette proteins were found. The proteins giving the best matches were Vga(A) (81.2% identical amino acids) and Vga(B) (57.4% identical amino acids). Each of these three proteins contains two ATP-binding domains, each including the two ATP-binding motifs described by Walker et al. (31) and a highly conserved SGG signature sequence found between the two ATP-binding motifs of all investigated ATP-binding proteins (10, 21).

Despite the lack of hybridization with *vga*(A) at high stringency (≥60°C), the gene characterized in this study should be considered to be a variant of *vga*(A) according to the nomenclature recently proposed by Roberts et al. (28).

Analysis of the drug resistance pattern conferred by the *vga*(A) variant gene. An 1,827-bp DNA fragment including the *vga*(A) variant was amplified with primers C and D (Fig. 1) from the cellular DNA of BM3327 and inserted into the shuttle vector pRB374 (11). The resulting plasmid, pIP1810, was introduced by electroporation into the *S. aureus* recipient RN4220; it conferred resistance to pristinamycin IIA (MICs of 32 mg · liter^{-1}), whereas RN4220 harboring the vector pRB374 was inhibited by 2 mg of pristinamycin IIA liter^{-1}. Pristinamycin MICs were the same (0.06 mg · liter^{-1}) for the recipient strain RN4220 and for transformants harboring pIP1810 or pRB374. The four *S. aureus* clinical isolates carrying the *vga*(A) variant only, which were inhibited by 1 mg of pristinamycin liter^{-1} (Table 1), may be considered intermediately resistant to pristinamycin, because, for pristinamycin-resistant *S. aureus* strains, the MICs are ≥2 mg · liter^{-1} (1). None of the antibiotic resistance markers carried by these wild-type clinical isolates was conferred by pIP1810.

Distribution and location of the *vga*(A) variant gene among two collections of isolates resistant to A compounds. The isolates were screened for the presence of the *vga*(A) variant by hybridization at 65°C with pIP1799. Sequences hybridizing with pIP1799 were found in the 20 *S. aureus* isolates carrying *vga*(B) and *vat*(B) (1) including BM3318 and BM3385 (Table 1); in one *S. epidermidis* isolate carrying *vga*(A) only (1); and in the four *S. aureus* isolates BM3249, BM3250, BM3252, and BM3327 (Table 1). The hybridizing sequences comigrated with the chromosomal DNA fragment in all isolates, and in 15 isolates an additional signal was detected in extrachromosomal DNA bands (≥40 kb) (results not shown).

The cellular DNA of six *S. aureus* clinical isolates hybridizing with pIP1799 was digested with *Sma*I and probed with this plasmid at 65°C (Fig. 2). A ≈670-kb *Sma*I fragment was detected in BM3385, BM3250, BM3252, BM3249, and BM3327 (Fig. 2B, lanes 2 to 6). An additional *Sma*I fragment of ≈90 kb was detected in BM3250 and BM3252 (Fig. 2, lanes 3 and 4), and two additional *Sma*I fragments of ≈90 and ≈170 kb were present in the pattern of BM3249 (lanes 5).

In BM3318, the hybridizing *Sma*I fragment was ≈100 kb (Fig. 2B, lane 10). The same band also hybridized with the *vat*(B) and *vga*(B) probes (results not shown), suggesting that this ≈100-kb fragment originated from a plasmid carrying three SgA^r genes. In the BM3385 lane (Fig. 2B, lane 2), pIP1799 hybridized with DNA that did not migrate out of the well, and a similar signal was observed in hybridizations with *vat*(B) and *vga*(B) probes (results not shown). BM3385 contains pIP1156, which has no *Sma*I site and carries func-

tional *vat*(B) and *vga*(B) genes. Presumably, this plasmid also contains nucleotide sequences hybridizing with pIP1799.

To determine whether BM3249, BM3250, BM3252, BM3318, BM3327, and BM3385, which hybridize with pIP1799, carry a complete copy of *vga*(A) variant, PCR experiments were carried out using primers C and D (Fig. 1). For each strain tested, the size of the amplified fragment was the same as that of the BM3327 amplicon, suggesting that each strain carries at least one copy of *vga*(A) variant and adjacent regions [152 nt upstream and 102 nt downstream from the BM3327 *vga*(A) variant].

All attempts to detect a transposon carrying *vga*(A) in seven staphylococcal plasmids (24 to 45 kb) carrying *vga*(A), *vat*(A), and *vgb*(A) (4) and in two *S. epidermidis* plasmids (7.5 to 14.4 kb) carrying *vga*(A) failed (9, 24). In contrast to *vga*(A), which is disseminated via plasmids, the *vga*(A) variant may have been disseminated by a transposon, as in some strains it is carried by a *vat*(B)-*vga*(B) plasmid (BM3318), in others it is carried by a *vat*(B)-*vga*(B) plasmid and by the chromosome (BM3385), and in a third group it is on the chromosome in one or multiple copies. It is currently unclear whether the *vga*(A) variant is part of a transposon, and this is currently under investigation.

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