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Distribution of Genes Encoding Resistance to Streptogramin A and Related Compounds among Staphylococci Resistant to These Antibiotics

JEANINE ALLIGNET, SYLVIE AUBERT, ANNE MORVAN AND NÉVINE EL SOLH*

National Reference Center for Staphylococci, Unité des Staphylocoques, Institut Pasteur, 75724 Paris Cedex 15, France

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The levels of resistance to pristinamycin (Pt) and to its major constituents, pristinamycin IIA and IB (PIIA and PIB, respectively; classified as streptogramins A and B, respectively) were determined for 126 staphylococcal isolates. The results suggest tentative susceptibility breakpoints of ≤2, ≤8, and ≤0.5 μg/ml for PIIA, PIB, and Pt, respectively. Fifty-six isolates that were inhibited by ≥4 μg of PIIA per ml were investigated for the presence of staphylococcal genes encoding resistance to PIIA (*vga, vat*, and *vatB*) and PIB (*vgb*). None of these genes was found in the 4 isolates inhibited by 4 μg of PIIA per ml or in 4 of the other 52 isolates tested. The remaining 48 isolates harbored plasmids carrying *vatB* and *vga* or combinations of genes (*vga-vat-vgb* or *vga-vat*). The absence of any known PIIA resistance gene from the four *Staphylococcus aureus* isolates inhibited by ≥8 μg of PIIA per ml suggests that there is at least one PIIA resistance mechanism in staphylococci that has not yet been characterized.

Streptogramin A (SgA) and its related compounds (pristinamycin IIA [PIIA], virginiamycin M, mikamycin A, and synergistin A) and streptogramin B (SgB) and its related compounds (pristinamycin IB [PIB], virginiamycin S, mikamycin B, and synergistin B) are bacteriostatic when they are used separately. When they are used in association, they act synergistically and become bactericidal, mainly against gram-positive bacteria (15). Natural mixtures such as pristinamycin (Pt), synergistin, virginiamycin, or mikamycin have been available on the European market since 1955. They are used orally and topically for the treatment of cutaneous, bone, and respiratory infectious diseases, mostly those caused by staphylococci (15). A semisynthetic injectable streptogramin, RP59500, consisting of a mixture of derivatives of the A and B compounds (quinupristin and dalfopristin, respectively) is undergoing clinical trials (see the entire volume [volume 30, supplement A, 1992] of the *Journal of Antimicrobial Chemotherapy*) (7, 8, 24, 27, 28, 32, 34, 41).

In France, the level of resistance to mixtures of the A and B compounds in staphylococci, which was first encountered in 1975 (19, 23), is currently low (≤5%) in most hospitals (5, 22). There have been rare outbreaks in intensive care units due to the dissemination of epidemic *Staphylococcus epidermidis* strains resistant to the A compounds (31, 42). In staphylococci, resistance to the synergistic mixtures of the A and B compounds (Pt MICs, ≥2 μg/ml) is always associated with resistance to the A compounds (PIIA MICs, ≥8 μg/ml), but not necessarily with resistance to the B compounds (22, 23). Three genes encoding resistance to the A compounds by different mechanisms, *vga* (2), *vat* (4), and *vatB* (1), and one gene encoding a lactonase which inactivates the B compounds, *vgb* (3), have been detected on *Staphylococcus aureus* plasmids conferring resistance to mixtures of the A and B compounds. The gene *vga*, also detected on plasmids in *S. epidermidis* (31), encodes an ATP-binding protein probably involved in the active efflux of A compounds. The genes *vat* and *vatB* encode acetyltransferases which inactivate streptogramin A (and similar compounds) and which are related (50% amino acid identity). The two enzymes exhibit significant similarity (58 and 47% amino acid identity, respectively) to the *Enterococcus faecium* acetyltransferase that inactivates A compounds and that is encoded by the gene *satA* (35).

The MICs of PIIA, PIB, and Pt for 126 staphylococcal isolates were determined to choose tentative breakpoints for identifying susceptibility and resistance to these antibiotics. We also report the distributions and locations of the genes *vga, vat, vatB*, and *vgb* among 52 staphylococcal strains of various species that are resistant to SgA and related compounds, including PIIA.

MATERIALS AND METHODS

Bacteria and plasmids. The 126 staphylococcal isolates studied included 82 *S. aureus*, 38 *S. epidermidis*, 4 *Staphylococcus haemolyticus*, 1 *Staphylococcus simulans*, and 1 *Staphylococcus cohnii* subsp. *urealyticum* strains. They all belonged to different phage types and/or genotypes. Fifty-one of the 126 isolates were from our collection of staphylococci resistant to the A compounds (PIIA MICs, ≥16 μg/ml). Nine of the PIIA^r isolates were collected in an Algerian hospital in 1989 and 1990, and the remaining isolates were from hospitals in various French cities (collected from 1975 to 1993). In both countries, pristinamycin is used in therapy. The *S. aureus* and *S. epidermidis* species were identified by phenotypic characterization (17), including the use of the ID32 Staph system (11). The hybridization patterns of *Hin*dIII-digested cellular DNA with a pBA_2 probe containing a *Bacillus subtilis* 16S rRNA gene were used to assign the remaining isolates to species other than *S. aureus* or *S. epidermidis* (16, 17). The isolates were classified as distinct if their *Sma*I profiles differed by more than five fragments, if the percentages of similarity between the hybridization patterns of *Eco*RI-digested cellular DNA with an IS*256* probe (31, 33, 42) were ≤75, and/or if the phage patterns of the *S. aureus* isolates differed by more than three strong lytic reactions with phages used at 100 routine test dilutions (9).

S. aureus BM224, resistant to rifampin and fusidic acid, was used as a recipient in mating experiments (21).

pUC18 derivatives carrying part of the *vat* (4), *vga* (2), *vgb* (3), or *vatB* (1) gene were used as hybridization probes.

Media. Brain heart infusion (BHI) and BHI agar (Difco Laboratories, Detroit, Mich.) were used for staphylococcal growth. Susceptibility was tested on Mueller-Hinton agar (MHA) or Mueller-Hinton broth (MHB) (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France).

Susceptibility to antibiotics. Susceptibility to antibiotics was determined by a disk diffusion assay (12) with commercially available antibiotic disks (Sanofi Diagnostics Pasteur). Additional disks prepared in our laboratory contained PIIA (20 μg) or PIB (40 μg). All antibiotic resistance markers found among staphylococci to date were investigated (33).

The MICs of PIB, PIIA, and Pt were determined by serial twofold dilutions of

* Corresponding author. Phone: (33-1) 01 45 68 83 63. Fax: (33-1) 01 40 61 31 63.

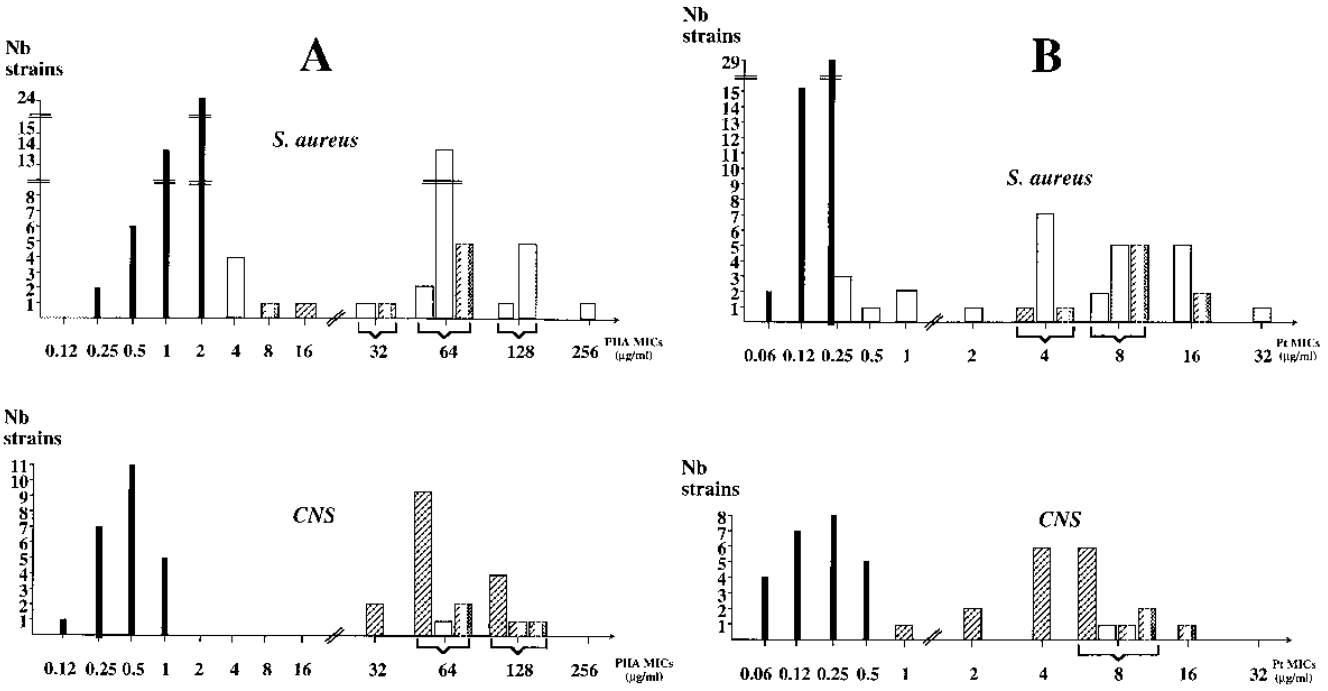

FIG. 1. Distribution of 126 epidemiologically unrelated staphylococci (82 *S. aureus* isolates and 44 coagulase-negative staphylococci [CNS]) according to their levels of resistance to PIIA (A) and Pt (mixture of PIB and PIIA) (B) and to the presence of *vga, vat, vatB*, and *vgb* sequences in the 56 staphylococci for which the PIIA MICs ≥4 μg/ml. ■, strains susceptible to PIIA (MICs ≤2 μg/ml); □, strains in which none of the tested genes was present; ▨, strains in which *vga* was present; ▩, strains in which *vatB* was present; [·], strains in which *vat-vga* was present; [·], strains in which *vga-vat-vgb* is present. Nb, number.

antibiotics in MHA (25). The staphylococcal isolates tested were kept at −80°C. After thawing, the strains were isolated on MHA and on MHA supplemented with 10 μg of PIIA per ml to select PIIA^r staphylococci known to be PIIA^r strains (PIIA MICs, ≥16 μg/ml) (1, 2, 4, 23, 31). Isolated colonies from each bacterial culture was picked either from the medium containing PIIA or from MHA for the strains which did not grow on MHA containing PIIA. The latter colonies were cultured for 18 h at 37°C in MHB, whereas the former colonies were cultured in MHB supplemented with 10 μg of PIIA per ml. The cultures were diluted 20-fold in MHB, incubated with shaking for 6 h at 37°C, diluted 20-fold in MHB, and incubated by shaking for 18 h at 37°C. These cultures were deposited in the wells of a multipoint inoculator, which was used to apply the organisms to the plates at approximately 10^4 CFU per spot.

Mating procedure. The mating procedure was performed as described previously (21).

DNA isolation and analysis. Total cellular DNA was isolated from staphylococcal strains and plasmids were isolated from staphylococci and *Escherichia coli* and purified as described previously (14, 18, 37). For the PCR experiments, cellular DNA was prepared according to the instructions of the manufacturer of the InstaGene DNA purification matrix (Bio-Rad, Hercules, Calif.).

Total cellular DNA and plasmids were analyzed by 0.7% agarose gel electrophoresis in Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA) as described by Sambrook et al. (37). *Sma*I digestion and pulsed-field gel electrophoresis were performed as described previously (40).

The DNA fragments amplified by PCR were separated by electrophoresis in Nusieve agarose (FMC Product, Rockland, Maine) gels (4% [wt/vol]) prepared and run in Tris-acetate buffer.

PCR. PCR experiments were carried out at high or low stringency, depending on the primers used. High-stringency PCR involved a precycle of 3 min at 95°C and 2 min at 60°C and then 30 cycles of 20 s at 72°C, 20 s at 95°C, and 20 s at 60°C; this was followed by a cycle of 1 min at 72°C. Low-stringency PCR involved a precycle of 5 min at 95°C and then 35 cycles of 2 min at 40°C, 1 min and 30 s at 72°C, and 30 s at 95°C; this was followed by a cycle of 4 min at 40°C and 12 min at 72°C. Other conditions were as reported previously (1).

The oligonucleotides used as primers were as follows: oligonucleotide I, 5′-A TTATGAATGGAG$^{C}_{T}$AAACC$^{A}_{T}$TAG$^{A}_{C}$A$^{T}_{G}$G-3′; oligonucleotide J, 5′-$^{A}_{T}$CC$^{A}_{T}$ATCCA$^{C}_{T}$ACATC ATT$^{T}_{A}$CC-3′; oligonucleotide M, 5′-AT$^{A}_{T}$ ATGAA$^{T}_{C}$GG**I**GC**I**AA$^{C}_{T}$CA$^{TA}_{CC}$G**I**ATG-3′; and oligonucleotide N, 5′-**I**CC$^{G}_{A}$ ATCCA**I**AC $^{A}_{G}$TC$^{A}_{G}$TT**I**CC-3′ (**I** indicates inosine).

Oligonucleotides I (1) and M (this study) encode the conserved motif III from Vat, VatB, and SatA. Oligonucleotides J (1) and N (this study) encode the conserved motif IV of these proteins (1). The oligonucleotides encoding the same motifs differed by their degrees of degeneracy (16-fold for oligonucleotides I and J, 3,072-fold for oligonucleotide M, and 768-fold for oligonucleotide N). Primer pairs I-J and M-N were designed to amplify a 147-bp DNA fragment from the genes *vat* (4), *vatB* (1), and *satA* (35).

Blotting and hybridization. DNA was transferred from agarose gels to Hybond-N$^+$ membranes (Amersham International) by the capillary blotting method of Southern (39). Prehybridization and hybridization were performed under stringent conditions as described previously (13). The plasmids used as probes were labeled with [α^{-32}P]dCTP (110 TBq · mmol^{-1}) by random priming with the Megaprime DNA labeling system (Amersham International). The blots were exposed to Hyperfilm (Amersham International) at −80°C.

RESULTS

Staphylococcal resistance to PIIA, PIB, and Pt. Of the 126 staphylococcal isolates (82 *S. aureus* and 44 coagulase-negative staphylococci) included in the study, 51 grew and 75 did not grow on MHA containing 10 μg of PIIA per ml. The levels of resistance of the 126 isolates to PIIA and Pt are reported in Fig. 1.

The 51 isolates resistant to 10 μg of PIIA per ml belonged to the following taxa: *S. aureus* (31 isolates), *S. epidermidis* (14 isolates), *S. haemolyticus* (4 isolates), *S. simulans* (1 isolate), and *S. cohnii* subsp. *urealyticum* (1 isolate). For these isolates, the MICs of PIIA and Pt ranged from 16 to 256 μg/ml and from 1 to 32 μg/ml, respectively. In the absence of selection with PIIA, the MICs decreased by 1 or 2 dilutions (data not shown).

Of the 75 isolates susceptible to 10 μg of PIIA per ml, 51 were *S. aureus* and 24 were *S. epidermidis*. For 70 of these isolates (including the 24 *S. epidermidis* isolates), the MICs of PIIA and Pt did not exceed 2 and 0.5 μg/ml, respectively (Fig. 1). The five remaining *S. aureus* isolates were inhibited by either 4 or 8 μg of PIIA per ml (Fig. 1), and the MIC of Pt was from 0.25 to 0.50 μg/ml for the four *S. aureus* isolates inhibited

TABLE 1. Relevant characteristics of the 56 staphylococci carrying either resistance to SgA (PIIA MIC, ≥8 μg/ml) or intermediate resistance to SgA (PIIA MIC, 4 μg/ml)

Species (no. of isolates)	Resistance phenotype[a]	No. of isolates	Detection of[b]				Distribution of isolates according to the following Pt MICs (μg/ml) (no. of isolates):							
			vga	*vat*	*vatB*	*vgb*	0.25	0.50	1	2	4	8	16	32
S. aureus (36)	Mc M L SgB SgA Pt	11	−	−	+	−						5	5	1
	Mc M L SgB SgA Pt	3	+	+	−	+						2	1	
	Mc M L SgB SgA Pt	2	−	−	−	−						2		
	Mc M L SgB SgA Pt	1	+	−	−	−					1			
	Mc L SgB SgA Pt		+	+	−	+							1	
	L SgB SgA Pt	1	+	+	−	+						1		
	Mc SgB SgA Pt	1	+	+	−	+						1		
	SgB SgA Pt	1	+	+	−	+						1		
	M L SgA Pt	1	−	−	+	−					1			
	Mc L SgA Pt	1	−	−	+	−					1			
	L SgA Pt	7	−	−	+	−				1	6			
	Mc L SgA Pti	2	−	−	−	−			2					
	Mc L SgAi	1	−	−	−	−	1							
	L SgAi	1	−	−	−	−	1							
	SgAi	1	−	−	−	−	1							
	M SgAi	1	−	−	−	−		1						
S. epidermidis (14)	Mc M L SgB SgA Pt	12	+	−	−	−				1	5	6		
	Mc M L SgB SgA Pt	1	−	−	+	−						1		
	Mc M L SgB SgA Pt	1	+	+	−	−						1		
S. haemolyticus (4)	Mc M L SgB SgA Pt	1	+	+	−	+						1		
		1	+	−	−	−					1			
	Mc M L SgA Pt	1	+	−	−	−				1				
	Mc L SgA Pti	1	+	−	−	−			1					
S. cohnii subsp. *urealyticum* (1)	Mc SgB SgA Pt	1	+	+	−	+						1		
S. simulans (1)	Mc SgB SgA Pt	1	+	+	−	+							1	

[a] The phenotypes of resistance to the following antibiotics are reported: methicillin (Mc), macrolides (M), lincosamides (L), SgB, SgA, and Pt. The isolates with intermediate resistance to SgA (PIIA MIC, 4 μg/ml) or Pt (MIC, 1 μg/ml) are designated SgAi or Pti, respectively.

[b] Hybridization with probes for the genes *vga* (2), *vat* (4), *vatB* (1), and *vgb* (3).

by 4 μg of PIIA per ml, and the MIC of Pt was 4 μg/ml for the single *S. aureus* strain inhibited by 8 μg of PIIA per ml.

These results suggest a tentative breakpoint of susceptibility for PIIA-susceptible staphylococci to be 2 μg of PIIA per ml, and thus, the strains inhibited by ≥8 μg of PIIA per ml may be considered resistant. Similar to previous reports (38), the staphylococcal strains inhibited by ≤0.5 μg of Pt per ml may be considered susceptible and those inhibited by Pt at ≥2 μg/ml may be considered resistant. Strains inhibited by 4 μg of PIIA per ml or 1 μg of Pt per ml may be considered to have an intermediate level of resistance. Thus, the levels of resistance to PIIA for the isolates tested were distributed as follows: 52 isolates were resistant to PIIA (MICs, ≥8 μg/ml), four isolates had intermediate PIIA resistance (MICs, 4 μg/ml), and 70 isolates were susceptible to PIIA (MICs, ≤2 μg/ml).

The phenotypes of resistance of the 56 staphylococci inhibited by at least 4 μg of PIIA per ml to macrolides, lincosamides, SgB, SgA, and Pt are reported in Table 1. The isolates resistant to SgB and related antibiotics, including PIB, were inhibited by 32 to more than 256 μg of PIB per ml, whereas SgB-susceptible isolates were inhibited by 4 to 8 μg of PIB per ml. Pt, which consists of a natural mixture of PIIA and PIB, acted synergistically against 117 of the 126 staphylococci tested, as assessed by the fractional inhibitory concentration index (≤0.5) (20). Of the nine *S. aureus* isolates against which no synergistic activity was observed, six isolates were resistant to PIIA but susceptible to PIB and three were resistant to both PIIA and PIB.

Distribution of the staphylococcal genes *vga, vat, vatB*, and *vgb* among the 56 staphylococci inhibited by at least 4 μg of PIIA per ml. The cellular DNA of the isolates was screened by hybridization for the presence of the *vga, vat, vatB*, and *vgb* genes. The 56 staphylococci inhibited by ≥4 μg of PIIA per ml and 10 of the staphylococci inhibited by ≤2 μg of PIIA per ml were tested. None of the four genes was detected in the PIIA-susceptible controls or in the four *S. aureus* isolates with intermediate resistance to PIIA (MIC, 4 μg/ml) (Fig. 1).

Of the 52 PIIA^r isolates (PIIA MICs, ≥8 μg/ml), 48 carried either a single *vga* or *vatB* gene or a combination of two or three genes: *vat-vga* or *vat-vga-vgb* (Table 1). The gene *vatB* alone was mostly detected in *S. aureus* (20 of the 36 isolates analyzed), whereas only 1 of the 21 coagulase-negative staphylococci carried this gene. The *vga* gene alone was more widespread among the coagulase-negative staphylococci (15 of the 21 coagulase-negative staphylococci) and was detected alone in only one *S. aureus* isolate. The combination *vga-vat-vgb* was found in isolates belonging to four taxa (*S. aureus, S. haemolyticus, S. cohnii* subsp. *urealyticum*, and *S. simulans*) from both France and Algeria.

None of the four genes investigated was found in four PIIA^r

S. aureus isolates (PIIA MICs, 64 to 256 μg/ml). These strains were tested by PCR at high and low stringencies with degenerate primers I and J (1) to amplify a 147-bp DNA fragment from staphylococcal and enterococcal genes, *vat* (4), *vatB* (1), and *satA* (35). No amplified products were observed (data not shown). Another pair of primers, M and N, which anneal to all putative sequences encoding motifs III and IV, were designed. The probability of amplifying a DNA fragment from within any *vat*-related genes is higher with primers M and N than with primers I and J. At a low annealing temperature (40°C), a single DNA fragment of the expected size (147 nucleotides) was amplified by using these primers from the cellular DNA of the *S. aureus* strains carrying *vat* or *vatB* used as positive controls. No amplicon of 147 bp was detected with the cellular DNA of the four *S. aureus* isolates which, furthermore, did not hybridize at high stringency with the *vat, vatB, vga*, or *vgb* probe (data not shown).

Locations of the nucleotide sequences hybridizing with the four genes investigated. In the 48 PIIA^r staphylococcal isolates hybridizing with the probes, the corresponding nucleotide sequences were located in extrachromosomal DNA (data not shown). In each of the isolates carrying more than one of the tested genes, all hybridizing sequences were located on the same plasmid.

In all the tested coagulase-negative staphylococci hybridizing with *vga* (15 strains) or with *vga* and *vat* (1 strain) (Table 1), the homologous sequences were located on plasmids of 6 to 15 kb. In the 10 staphylococcal isolates hybridizing with *vga, vat*, and *vgb* (Table 1) and in the single *S. aureus* isolate hybridizing with *vga* only (Table 1), the plasmids carrying the homologous sequences were from 26 to ≈45 kb. The nucleotide sequences hybridizing with *vatB* (21 strains) were, in each strain, on plasmids of at least 50 kb, as estimated by agarose gel electrophoresis. *Sma*I digests of DNA from 10 *S. aureus* isolates carrying *vatB* were separated by pulsed-field gel electrophoresis and were tested with the *vatB* probe. The fragment from one of these strains that hybridized with *vatB* was 95 kb. The "*vatB* plasmid" in this isolate is therefore at least 95 kb (data not shown). For all other isolates, the hybridizing DNA sequences were located in the wells (data not shown), suggesting that the *vatB* plasmids were not cleaved by *Sma*I.

Filter mating experiments. Four *S. aureus* isolates and one *S. simulans* isolate harboring *vga-vat-vgb* plasmids and eight *S. aureus* isolates and one *S. epidermidis* isolate harboring *vatB* plasmids were mated with *S. aureus* BM224. Resistance to PIIA was transferred from only eight donors containing *vatB* plasmids (seven *S. aureus* isolates and one *S. epidermidis* isolate). The frequencies were from 3×10^{-7} to 6×10^{-8} PIIA^r transconjugants per donor. Resistance to lincosamides and penicillins was always cotransferred with resistance to PIIA and Pt. Additional markers, such as resistance to trimethoprim or to aminoglycosides (kanamycin, tobramycin, gentamicin, and neomycin), were also cotransferred from some donors. The hybridization patterns obtained with the *vatB* probe of total DNA from the transconjugants were similar to those for the corresponding donors (data not shown).

DISCUSSION

The results reported here reveal that there is considerable diversity among the plasmids and genes conferring resistance to PIIA. These genes may have been selected by the inclusion of mixtures of SgA and SgB in animal feed, a practice existing since 1957 (15). Because the Sg resistance genes are located on plasmids which may confer resistance to several other antibiotics, selection pressure may have been exerted by other antibiotics as well. We did not find any relationship between the presence of a given SgA resistance gene or a given combination of genes and the levels of resistance to PIIA or to Pt.

The plasmids hybridizing with the PIIA^r genes are distinguishable by their sizes, which range from 6 to at least 95 kb, by their restriction profiles, by whether they transfer by conjugation, and by the presence of various combinations of genes including PIIA^r genes. The large plasmids (≥50 kb) that were transferred by conjugation and that carry *vatB* always confer resistance to lincosamides and penicillins by the production of penicillinase; therefore, they may have a common region where these genes are carried. A common fragment of 5.3 kb carrying the contiguous genes *vat* and *vgb* and flanked by two copies of the insertion sequence IS*257* (6, 36) was found in the three tested plasmids carrying *vat, vgb*, and *vga* (30). It is not known whether this fragment is present in all plasmids containing the three genes encoding resistance to PIIA and PIB or whether it is a composite transposon. The genesis in vivo of the plasmids which carry, in addition to *vat* and *vgb*, several genes encoding antibiotic resistance may be the result of a sequence of events. These events may include the translocation of the element carrying *vat-vgb* by transposition, the homologous recombination between the multiple copies of IS*257* found in staphylococcal plasmids, and/or the integration of *vga* plasmids similar to those found in coagulase-negative staphylococci.

Resistance to PIIA and Pt declined in the absence of selection on PIIA, with most strains becoming susceptible to Pt, although they remained resistant to PIIA. When tested by disc diffusion (20 μg of PIIA per disc), these cultures showed a large inhibition diameter containing isolated colonies close to the disc. Thus, these cultures presumably contain two distinct populations: one, the majority, is susceptible to PIIA and the other, the minority, is resistant to PIIA. Plasmid-free variants and variants containing plasmids deleted for the PIIA resistance genes have been isolated from such cultures (data not shown). The structural instability of the plasmids may be due in part to the presence of multiple copies of IS*257*. In hospital laboratories, most L SgA^r *S. aureus* strains (strains resistant to lincosamides and streptogramin A but susceptible to streptogramin B and macrolides) carrying *vatB* and coagulase-negative staphylococci carrying *vga* (Table 1) scored susceptible in vitro to Pt (MICs, ≤0.5 μg/ml). The therapeutic use of synergistic mixtures of the A and B compounds may contribute to the selection of the population containing the SgA^r plasmid and which may therefore be resistant to the treatment. Consequently, if the efficacy of treatment with the mixture of the A and B compounds cannot be evaluated in vivo, we do not recommend the use of such mixtures when the SgA^r genes are present and/or when the strains are resistant to the A compounds, even if the strains appear to be susceptible to Pt in vitro. Screening on MHA containing 6 μg of PIIA per ml should be considered, because we encountered one PIIA^r isolate harboring *vga-vat* and *vgb* which was inhibited by 8 μg of PIIA^r per ml but which was not detected by using our screening concentration of 10 μg/ml.

Synergistic mixtures are active in vitro against staphylococci susceptible to SgA but resistant to SgB because of either the constitutive production of methylases modifying the target (5, 10, 26, 29, 34) or the production of a lactonase inactivating SgB and related compounds (3). However, Fantin et al. (26) have recently shown that for half of the tested *S. aureus* strains carrying constitutive resistance to macrolide-lincosamide-SgB due to methylase production, there was a decrease in the bactericidal activity of the semisynthetic Sg. RP59500 both in vitro and in vivo against isolates in an experimental endocarditis model. Among our isolates susceptible to PIIA (MICs, ≤2

μg/ml), there were no detectable differences in the levels of Pt resistance between the SgB-resistant and the SgB-susceptible strains. In contrast, most of the 52 PIIA^r staphylococci resistant to SgB were more resistant to Pt than those susceptible to SgB (Table 1), despite the maintenance of the synergistic activity of Pt.

None of the three staphylococcal genes encoding resistance to PIIA, i.e., *vga, vat*, and *vatB*, or the enterococcal *satA* gene (35) was detected in four *S. aureus* isolates resistant to PIIA (MICs, 64, 128, and 256 μg/ml); therefore, we expect other resistance genes to be found in staphylococci, unless these four isolates were mutants. Mutants with similar levels of resistance to PIIA and related compounds have been selected with frequencies ranging from 2×10^{-9} to 3×10^{-10} (22), but they have not been characterized. Nevertheless, the hypothesis of the existence of a mutation may be tested by looking for reverse mutants susceptible to SgA. The putative SgA resistance gene(s) could be isolated from the four *S. aureus* strains by genetic transfer (transduction or filter mating) or cloning, or both. It would be worthwhile to sequence such putative genes to investigate all the staphylococcal genes encoding resistance to SgA and related compounds in isolates exhibiting low levels of resistance to SgA and to synergistic mixtures of the A and B compounds.

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