

## Clonal Diversity among Streptogramin A-Resistant *Staphylococcus aureus* Isolates Collected in French Hospitals

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We analyzed 62 clinical isolates of streptogramin A-resistant (SGA<sup>r</sup>) *Staphylococcus aureus* collected between 1981 and 2001 in 14 hospitals located in seven French cities. These isolates, including five with decreased susceptibility to glycopeptides, were distributed into 45 antibiotypes and 38 *Sma*I genotypes. Each of these genotypes included between 1 and 11 isolates, the *Sma*I patterns of which differed by no more than three bands. Although numerous clones were identified, we observed the spread of monoclonal isolates either within the same hospital or within hospitals in distinct cities and at large time intervals. Hybridization with probes directed against 10 SGA<sup>r</sup> genes (*vatA*, *vatB*, *vatC*, *vatD*, *vatE*, *vgaA*, *vgaB*, *vgaAv*, *vgbA*, and *vgbB*) revealed six patterns: *vgaAv* (21 isolates), *vatA-vgbA* (24 isolates), *vgaAv-vatB-vgaB* (14 isolates), *vgaAv-vatA-vgbA* (1 isolate), *vgaAv-vatA-vgbA-vatB-vgaB* (1 isolate), and *vgaA* (1 isolate). We detected at least one SGA<sup>r</sup> determinant in all of the tested isolates. *vgaAv*, which is part of the recently characterized transposon Tn5406, was found in 59.7% of the tested isolates. Of the 16 streptogramin B-susceptible isolates, 14 carried *vgaAv* alone and were susceptible to the mixtures of streptogramins, whereas the 2 isolates carrying *vgaAv-vatB-vgaB* were resistant to these mixtures. *vatA-vgbA* was found on plasmids of the same apparent size in 26 (42%) of the tested clinical isolates from 18 unrelated *Sma*I genotypes. The possible dissemination of some of the multiple clones characterized in the present study with an expected increased selective pressure of streptogramins following the recent licensing of Synercid (quinupristin-dalfopristin) must be carefully monitored.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen worldwide. Glycopeptides have been the reference drugs for the treatment of MRSA infections (16, 21, 30). After the emergence of clones with decreased susceptibility to these antibiotics, first reported as sporadic cases in several countries and more recently also as outbreaks, alternative treatments such as quinupristin-dalfopristin (Synercid) have been promoted (17, 21, 33). Quinupristin and dalfopristin are derivatives of pristinamycins IA and IIA, respectively (10). Quinupristin-dalfopristin is an injectable, semisynthetic, mixture with a synergistic activity against most gram-positive pathogens. Since 1999, quinupristin-dalfopristin has been available for use in hospitals for the treatment of infections caused by gram-positive cocci that are resistant to other antibiotics.

Quinupristin-dalfopristin and the natural antibiotics produced by streptomycetes, such as streptogramin, pristinamycin, synergistin, mikamycin, and virginiamycin, are mixtures of two classes of compounds, A and B, with distinct primary structures (10, 14). The A compounds are polyunsaturated cyclic macrolactones, and the B compounds are cyclic hexadepsipeptides. Both types of compounds bind different targets in the peptidyltransferase domain of the 23S ribosomal subunit and inhibit protein elongation at different steps. Compounds A and B are bacteriostatic when used separately but act synergistically when combined, such that in some cases they are bactericidal, mainly against gram-positive bacteria.

In countries such as France, where natural mixtures (pristinamycin and synergistin) have been used orally and topically since 1960, the prevalence of clinical isolates of staphylococci resistant to mixtures of compounds A and B (pristinamycin MICs of >2 mg liter<sup>-1</sup>) varies from 0 to 44% in hospitals (22). Virginiamycin was long used in animal feed as a growth promoter in both Europe and the United States but was banned in Europe in 1999. The first staphylococcal clinical isolates resistant to the mixtures were reported in France in 1975 (14). Staphylococcal resistance to synergistic mixtures is always associated with resistance to A compounds (pristinamycin IIA MICs of ≥8 mg liter<sup>-1</sup>) but is not necessarily associated with resistance to B compounds (1).

In staphylococci, resistance to B compounds is mediated by (i) the methylation of 23S rRNA, which confers resistance to macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) when the *erm* genes are expressed constitutively (32); (ii) the hydrolysis of the drug by lyases (24) encoded by the *vgbA* (7) and *vgbB* (5) genes, which are always located on plasmids conferring resistance to A compounds; (iii) probable efflux ABC proteins encoded by the *msr* genes, which confers resistance to streptogramin B (SGB) and C<sub>14</sub>-C<sub>15</sub> macrolides (26, 27); or (iv) a mutation in the L22 ribosomal protein of *S. aureus*, which is responsible for cross-resistance to SGB and erythromycin (23). Resistance to A compounds may be conferred by two categories of genes: (i) *vatABCDE* (4, 5, 8, 18, 25, 35), encoding related acetyltransferases that inactivate the drug and are found on staphylococcal and/or *Enterococcus faecium* plasmids, and (ii) *vgaAB* (2, 6) and *lsa* (28), encoding related ATP-binding proteins, the resistance mechanism of which has not been elucidated. *vga*-type genes are found in staphylococcal plasmids but not in *E. faecium* strains, whereas *lsa*

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conferring resistance to SGA and to clindamycin is present in the chromosome of all tested *E. faecalis* strains. A variant of *vgaA* (*vgaAv*) (19) carried by a transposon found in staphylococci, Tn5406 (20), which is located on plasmids and/or the chromosome, was recently characterized.

We have become aware of the *vatA-vgbA* combination (33; data not shown), which was not found in the 52 unrelated staphylococci tested previously (1). Indeed, *vatA-vgbA* was always associated with *vgaA*. Related plasmids (15 to 45 kb) carrying this combination have been detected in strains belonging to various staphylococcal species and isolated prior to 1997 (3).

The aim of the present study was to type and analyze the distribution of SGA resistance ( $SGA^r$ ) genes, because of their uncommon phenotype, in a collection of 62  $SGA^r$  clinical isolates of *S. aureus* sent to us from 1981 to 2001 by French hospital microbiologists.

#### MATERIALS AND METHODS

**Bacterial strains and plasmids.** The relevant characteristics of the 62 *S. aureus*  $SGA^r$  clinical isolates used are reported in Table 1. These isolates were collected from different patients between 1981 and 2001 in 14 hospitals located in seven French cities. Thirty-five patients were infected (56.5%), whereas seventeen (27.4%) were colonized. No information was available for the other 10 patients. *S. aureus* strains BM3093 (3), BM3318 (19), and BM10692 (3), *S. cohnii* subsp. *cohnii* BM10711 (5), and *E. faecium* strain K14 (18) were used as positive controls for the detection of streptogramin resistance genes. *S. aureus* strain NCTC 8325, the *SmaI* pattern of which served as a size standard in pulsed-field gel electrophoresis, was also used. The following recombinant plasmids were used as probes in hybridization experiments: pIP1652-*vatA* (8), pIP1692-*vatB* (4), pIP1740-*vatC* (5), pIP1795-*vatD* (25), pIP1802-*vatE* (18), pIP1653-*vgaA* (6), pIP1799-*vgaAv* (19), pIP1705-*vgaB* (2), pIP1654-*vgbA* (7), pIP1741-*vgbB* (5), and pIP1644-*IS257mp* (12).

**Media.** Staphylococci were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.). Susceptibility to antibiotics was tested on Mueller-Hinton agar (Bio-Rad, Hercules, Calif.).

**Susceptibility to antimicrobial drugs.** All clinical isolates were stored at  $-80^\circ\text{C}$  and were isolated on Mueller-Hinton agar containing  $10\ \mu\text{g}$  of pristinamycin IIA  $\text{ml}^{-1}$ . Susceptibility to antibiotics was determined by a disk diffusion assay with commercially available antibiotic disks (Bio-Rad), according to the recommendations of the French Society of Microbiology and with disks prepared in our laboratory as described previously (1). The MICs of vancomycin and teicoplanin were determined by the E-test according to the manufacturer's recommendations (AES, Combourg, France). A population analysis on brain heart infusion broth containing 4 mg of vancomycin  $\text{liter}^{-1}$  was carried out as previously described to screen for heterogeneous vancomycin intermediate *S. aureus* (hetero-VISA) strains (11).

**DNA isolation and analysis.** Total cellular DNA was isolated from staphylococcal strains and purified by using the QIAamps tissue kit from Qiagen (Hilden, Germany). *S. aureus* plasmid DNA was extracted and purified by using the QIAprep spin plasmid kit from Qiagen according to the manufacturer's instructions but with an additional step consisting of 1 h at  $37^\circ\text{C}$  in the presence of  $10\ \mu\text{l}$  of lysostaphin ( $1\ \text{mg}\ \text{ml}^{-1}$ ). Restriction endonucleases were obtained from Amersham-Pharmacia Biotech, Inc. (Piscataway, N.J.) and used according to the manufacturer's instructions. Instagene matrix DNA preparations were used for PCR experiments according to the manufacturer's instructions (Bio-Rad). The DNA was digested with *SmaI* and subjected to pulsed-field gel electrophoresis as described previously (Fig. 1) (13). Concatameric bacteriophage lambda DNA molecules (48.5 kb; Bio-Rad) and the *SmaI* fragments of the cellular DNA from *S. aureus* NCTC 8325 were used as size standards. Macrorestriction fingerprints were compared visually and were scanned by using GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). A similarity matrix was created by use of the band-based Dice similarity coefficient (tolerance of 1% and optimization of 2%). The unweighted pair-group method with average linkages was used to cluster the strains on the basis of the *SmaI* patterns. If the dendrograms revealed clusters that included strains with similarity of at least 80%, the patterns of the strains in the same cluster were compared visually on the same gel. The strains were clustered according to the following criteria proposed by Tenover et al. (31): (i) strains were grouped in the same major genotype if their patterns

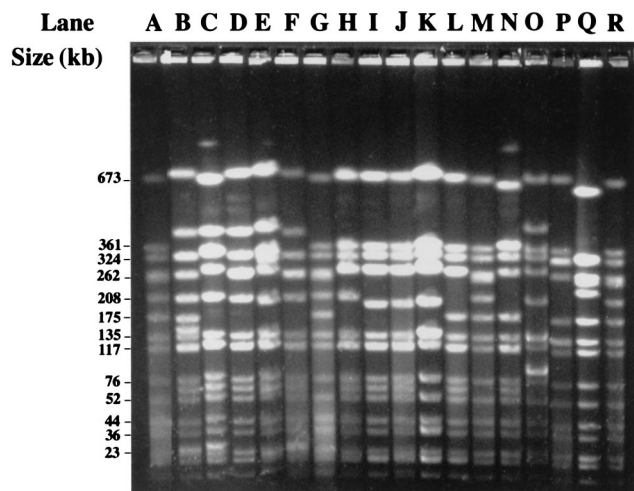


FIG. 1. Pulsed-field gel electrophoresis of *SmaI*-digested total DNA from  $SGA^r$  *S. aureus* clinical isolates. Lanes A, G, M, and R, NCTC 8325 used as standard; lane B, genotype 17a strain; lane C, genotype 15a strain; lane D, genotype 17c strain; lane E, genotype 16b strain; lane F, genotype 17b strain; lane H, genotype 24h strain (Paris-A, 1997); lane I, genotype 24a strain; lane J, genotype 24a strain (Toulouse, 1998); lane K, genotype 24c strain (Paris-A, 1999); lane L, genotype 30a strain; lane N, genotype 30b strain; lane O, BM3318; lane P, BM3093; lane Q, BM10692.

differed by no more than three bands (these strains were considered to be closely related and monoclonal); (ii) if patterns differed by between four and six bands, the strains were scored as being possibly related but were, nevertheless, classified into distinct genotypes to discriminate them from the closely related strains; and (iii) if patterns differed by seven or more bands, strains were considered to be different. Major genotypes are designated by Arabic numerals. Strains with undistinguishable patterns were classified within the same subtype. Subtypes are designated by Arabic numerals with letter suffixes.

**Labeling of DNA probes, blotting, and hybridization.** Hybridization experiments were performed at  $65^\circ\text{C}$  as described previously (19).

**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 to detect antibiotic resistance genes were those described previously: *vatA* (8), *vatB* (4), *vgaB* (2), and *vgbA* (7). A DNA fragment from within *vgaAv* (19) was amplified with primer A ( $5'$ -CTCC GTGTTGAAGATGTTTCG-3'; nucleotides [nt] 5881 to 5901; accession no. AF186237) and primer B ( $5'$ -GGATCAAACGCCTCTATAGCC-3'; nt 6339 to 6318; accession no. AF186237). The pIP1680 DNA fragment extending from pAM $\beta$ 1 *repE*-like gene to *vatA* was amplified with the primers RepE ( $5'$ -ATTG CTGAAGGTAAGG-3'; nt 4343 to 4362; accession no. AF007787) and VatA2 ( $5'$ -CAATGACCATGGACCTGATC-3'; nt 269 to 288; accession no. L07778). PCR experiments were carried out at high stringency (initial cycle of 5 min at  $95^\circ\text{C}$  and 2 min at  $55^\circ\text{C}$ , followed by 35 cycles of 1 min at  $72^\circ\text{C}$ , 30 s at  $95^\circ\text{C}$ , and 1 min at  $55^\circ\text{C}$ , with a final extension step of 5 min at  $72^\circ\text{C}$ ).

#### RESULTS

**Analysis of antibiotypes.** As shown in Table 1, 48 of the 62  $SGA^r$  isolates tested (77.4%) were also resistant to the mixtures of A and B compounds, including pristinamycin. Of these 48 isolates, 46 were resistant to B compounds. These 46  $SGA^r$  isolates were distributed into three  $MLS_B$  phenotypes:  $MLS_{Bc}$  (31 isolates),  $SGB$  (11 isolates), and  $MLS_{Bf}/SGB$  (4 isolates). Of the 16  $SGA^r$  isolates susceptible to B compounds, 14 were also resistant to lincomycin, and 2 of these 14 isolates were also resistant to clindamycin.

A total of 46 of the 62  $SGA^r$  *S. aureus* isolates studied were MRSA (74.2%) (Table 1). These MRSA isolates could be di-

TABLE 1. Relevant characteristics of 62 *S. aureus* clinical isolates resistant to SGA

Smal geno- type <sup>a</sup>	Yr	Source (city or city-hospital)	Antibiotic resistance markers <sup>b</sup>	Presence or absence of streptogramin resistance gene <sup>c</sup> :								
				<i>vgaAv</i>	<i>vgaA</i>	<i>vatA</i>	<i>vatB</i>	<i>vgaB</i>	<i>vgbA</i>			
1 <sup>A</sup>	1999	Paris-A	CHL PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	+	(9.5)	
2 <sup>A</sup>	1997	Grenoble	PEN STR KAN TOB GEN MLS <sub>Bi</sub> PRI PIIA LIN CLI SUL TMP	+	(0.6 + 1.1 + 1.3)	–	–	+	(5.5)	+	(5.5)	
3	2001	Cahors	PRI PIB PIIA	–	–	+	(9.5)	–	–	–	+	(9.5)
4	1999	Villiers-St Denis	PEN MET PRI PIB PIIA TEC <sub>1</sub>	–	–	+	(8.0)	–	–	–	+	(8.0)
5	1999	Paris-A	PEN TET MIN PRI PIB PIIA SUL FUC	–	–	+	(9.5)	–	–	–	+	(9.5)
6	1999	Paris-A	PEN MET STR SPT KAN NEO TOB GEN TET MLS <sub>Bc</sub> PRI PIIA SUL PEF RIF	–	–	+	(9.5)	–	–	–	+	(9.5)
7	2000	Remiremont	PEN STR SPT KAN TOB GEN TET MLS <sub>Bc</sub> PRI PIIA SUL PEF RIF TEC <sub>1</sub>	+	(0.6 + 1.1)	–	–	–	+	(5.0)	+	(5.0)
8a	1998	Paris-A	PEN MET KAN NEO TOB PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
8b	1998	Paris-A	PEN PRI PIB PIIA	–	–	+	(9.5)	–	–	–	+	(9.5)
9	2000	Blois	PEN MET SPT MLS <sub>Bc</sub> PRI PIIA TMP PEF RIF FUC FOF VAN <sub>1</sub> TEC	–	+	(3.2)	–	–	–	–	–	
10	2000	Paris-C	MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
11	2000	Paris-C	PEN KAN TOB GEN MLS <sub>Bc</sub> PRI PIIA FUC	+	(0.6 + 1.1 + 1.3)	–	–	–	+	(5.5)	+	(5.5)
12a	1999	Villiers-St Denis	PEN MET SPT KAN NEO TOB MLS <sub>Bi</sub> PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
12a	2001	Paris-G	PEN MET SPT KAN NEO TOB MLS <sub>Bi</sub> PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
12b	2000	Orsay	PEN MET KAN NEO TOB MLS <sub>Bi</sub> PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
13	1981	Paris-A	PEN MET STR SPT KAN NEO TOB GEN TET MIN MLS <sub>Bc</sub> PRI PIIA	+	(0.6 + 1.1)	–	–	–	+	(7.5)	+	(7.5)
14a	1998	Toulouse	PEN MET KAN NEO TOB GEN PRI PIIA LIN PEF TMP	+	(0.6 + 1.1 + 1.3)	–	–	–	+	(5.5)	+	(5.5)
14b	1999	Toulouse	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF TEC <sub>1</sub>	+	(0.6 + 1.3)	–	–	–	–	–	–	
15a	1991	Paris-A	PEN MET STR SPT KAN NEO TOB GEN TET MIN MLS <sub>Bc</sub> PRI PIIA PEF RIF hVANI TEC <sub>1</sub>	–	–	+	(9.5)	–	–	–	+	(9.5)
15b	1999	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
15c	1993	Paris-A	PEN MET STR SPT KAN NEO TOB GEN TET MIN MLS <sub>Bc</sub> PRI PIIA SUL TMP PEF RIF FOF hVANI TEC	+	(0.6 + 1.1)	–	–	–	+	(5.0)	+	(5.0)
16a <sup>B</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA TMP PEF	–	–	+	(8.0)	–	–	–	+	(8.0)
16b <sup>B</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA PEF RIF	–	–	+	(9.5)	–	–	–	+	(9.5)
17a <sup>B</sup>	1998	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	–	–	+	(8.0)	–	–	–	+	(8.0)
17b <sup>B</sup>	1996	Grenoble	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA TMP PEF FOF	+	(0.6 + 1.1)	–	–	–	+	(4.5)	+	(4.5)
18 <sup>C</sup>	1990	Paris-A	PEN STR KAN NEO MLS <sub>Bi</sub> PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
19 <sup>C</sup>	1999	Paris-A	PEN MET STR KAN NEO MLS <sub>Bc</sub> PRI PIIA SUL TMP FUC	–	–	+	(9.5)	–	–	–	+	(9.5)
20 <sup>D</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
21 <sup>D</sup>	1998	Paris-A	PEN MET KAN NEO TOB PRI PIB PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)
22a <sup>E</sup>	1998	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	–	–	+	(8.0)	–	–	–	+	(8.0)
22a <sup>E</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.1)	–	–	–	+	(5.5)	+	(5.5)
22a <sup>E</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.1)	–	–	–	+	(5.5)	+	(5.5)
22a <sup>E</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.1)	–	–	–	+	(5.5)	+	(5.5)
23 <sup>E</sup>	1988	Paris-A	PEN MET STR SPT KAN NEO TOB GEN TET MIN MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.1)	–	–	–	+	(7.5)	+	(7.5)
24a <sup>F</sup>	1997	Toulouse	PEN MET PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24a <sup>F</sup>	1998	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24a <sup>F</sup>	1999	Toulouse	PEN MET KAN NEO TOB CHL PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24b <sup>F</sup>	2000	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24c <sup>F</sup>	1998	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24c <sup>F</sup>	1999	Paris-A	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24d <sup>F</sup>	1999	Paris-A	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24e <sup>F</sup>	1998	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24f <sup>F</sup>	1999	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
24g <sup>F</sup>	1999	Toulouse	PEN MET KAN NEO TOB PIIA LIN PEF TEC <sub>1</sub>	+	(0.6 + 1.3)	–	–	–	–	–	–	
24h <sup>F</sup>	1997	Paris-A	PEN MET KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA TMP PEF + 8)	+	(0.6 + 1.3 + 4.2	–	–	–	–	–	–	
25 <sup>F</sup>	2000	Toulouse	PEN MET MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.3 + 3)	–	–	–	–	–	–	
26 <sup>F</sup>	1993	Paris-D	PEN MET KAN NEO TOB PRI PIB PIIA PEF RIF	–	–	+	(9.5)	–	–	–	+	(9.5)
27	2000	Toulouse	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF TEC <sub>1</sub>	+	(0.6 + 5)	–	–	–	–	–	–	
28 <sup>G</sup>	1997	Paris-A	PEN PIIA LIN	+	(0.6 + 1.3)	–	–	–	–	–	–	
29 <sup>G</sup>	1993	Paris-A	PEN MET STR KAN NEO TOB GEN PIIA LIN RIF	+	(0.6 + 1.3)	–	–	–	–	–	–	
30a <sup>H</sup>	1999	Toulouse	PEN PIIA LIN PEF	+	(0.6 + 1.3)	–	–	–	–	–	–	
30b <sup>H</sup>	1988	Paris-A	PEN STR SPT KAN NEO TOB GEN PRI PIB PIIA LIN	+	(0.6 + 1.3)	–	+	(9.5)	–	–	+	(9.5)
31 <sup>H</sup>	1999	Paris-E	PEN KAN TOB GEN TET MLS <sub>Bc</sub> PRI PIIA FUC TEC <sub>1</sub>	+	(0.6 + 1.3)	–	–	–	–	–	–	
32	1999	Paris-A	PEN TET ERY PRI PIB PIIA PEF	–	–	+	(8.0)	–	–	–	+	(8.0)
33	1997	Paris-A	PEN KAN TOB GEN PIIA LIN CLI TMP FUC	+	(0.6 + 1.3)	–	–	–	–	–	–	
34	1999	Paris-A	PEN MET STR KAN TOB GEN MLS <sub>Bc</sub> PRI PIIA SUL PEF RIF FUC hVANI TEC	+	(0.6 + 1.1)	–	–	–	+	(7.5)	+	(7.5)
35	2001	Villiers-St Denis	PEN MET STR KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA SUL TMP PEF RIF FUC FOF VAN <sub>1</sub> TEC	+	(0.6 + 1.1 + 2.5)	–	–	–	+	(9.5)	+	(9.5)
36a	1996	Paris-B	PEN MET STR KAN NEO PRI PIB PIIA	–	–	+	(9.5)	–	–	–	+	(9.5)
36a	1996	Paris-B	PEN MET STR KAN NEO PRI PIB PIIA	–	–	+	(9.5)	–	–	–	+	(9.5)
37	1995	Paris-F	PEN STR KAN TOB GEN CHL MLS <sub>Bc</sub> PRI PIIA SUL TMP PEF RIF FOF	+	(0.6 + 1.1)	–	–	–	+	(5.5)	+	(5.5)
38a	2001	Villiers-St Denis	PEN MET SPT KAN NEO TOB GEN MLS <sub>Bc</sub> PRI PIIA PEF	+	(0.6 + 1.1)	–	+	(9.5)	+	(5.0)	+	(5.0)
38b	2001	Villiers-St Denis	PEN MET SPT KAN NEO TOB MLS <sub>Bc</sub> PRI PIIA PEF	–	–	+	(9.5)	–	–	–	+	(9.5)

<sup>a</sup> Genotypes which include strains that are possibly related (less than seven band differences) are marked with the same superscript letter.

<sup>b</sup> Abbreviations: CHL, chloramphenicol; CLI, clindamycin; FUC, fucidic acid; FOF, fosfomicin; hVANI, heterogeneous vancomycin intermediate; KAN, kanamycin; LIN, lincomycin; MLS<sub>Bc</sub>, macrolide-lincosamide-SGB constitutive resistance; MLS<sub>Bi</sub>, macrolide-lincosamide-SGB inducible resistance; MET, methicillin; MIN, minocycline; NEO, neomycin; PEF, pefloxacin; PEN, penicillinase; PIB, pristinamycin IB (SGB); TEC, teicoplanin; TEC<sub>1</sub>, TEC intermediate; TOB, tobramycin; TMP, trimethoprim; VAN<sub>1</sub>, vancomycin intermediate.

<sup>c</sup> The following genes were investigated by hybridization at high stringency with the intragenic probes: *vatA*, *vatB*, *vatC*, *vatD*, *vatE*, *vgaA*, *vgaB*, *vgaAv*, *vgbA*, and *vgbB*. The genes reported were those found in at least one strain. The presence or absence of the cited genes are indicated by + or –, respectively. “+” is followed by the size(s) of the hybridizing *Hind*III fragment(s) in kilobases.

vide into gentamicin-sensitive (GEN<sup>S</sup>) and GEN<sup>R</sup> phenotypes (30 and 16 strains, respectively), whereas 9 of the 16 methicillin-susceptible *S. aureus* (MSSA) isolates were GEN<sup>S</sup>. Eighteen of the MRSA isolates (39.1%) and none of the MSSA isolates were spectinomycin resistant. Pefloxacin-resistant and rifampin-resistant strains were more prevalent among MRSA than among MSSA. Indeed, 40 of the 46 (87%) MRSA isolates were pefloxacin-resistant compared to just 7 of the 16 MSSA isolates (43.8%), and 9 of the MRSA isolates (19.6%) were rifampin resistant compared to one of the MSSA isolates (6.3%).

Of the 62 SGA<sup>R</sup> strains, 5 had reduced susceptibility to teicoplanin and vancomycin; three of these strains were hetero-VISA, and two were glycopeptide-intermediate *S. aureus*. These strains were isolated in three different hospitals between 1991 and 2001. All of these strains were MRSA and were either resistant or susceptible to GEN and related aminoglycosides. We also detected six teicoplanin-intermediate strains, four of which were MRSA.

**Analysis of the *Sma*I patterns.** As shown in Table 1, the 62 SGA<sup>R</sup> isolates were distributed into 38 distinct genotypes based on the comparison of *Sma*I patterns, and each genotype included between 1 and 11 isolates. Although grouped into distinct genotypes, the strains in the following genotypes may have been related because their *Sma*I patterns differed by six bands or less: genotypes 1 and 2; 16 and 17; 18 and 19; 20 and 21; 22 and 23; 24, 25, and 26; 28 and 29; and 30 and 31. It is noteworthy that major clonal diversity (20 genotypes) was observed among the 30 isolates from one hospital (Paris-A). Some of the isolates belonging to the same genotype were isolated in distinct hospitals located in different cities (12a and 12b, 17a and 17b, 24a to 24h, and 30a and 30b) and/or had different antibiotypes (14a and 14b; 15a to 15c; 24a, 24c, 24g, and 24h; 30a and 30b). Some strains grouped in the same genotype were also disseminated over fairly large time scales (15a in 1991 and 15b in 1999; 30b in 1988 and 30a in 1999).

**Distribution of the streptogramin resistance genes tested.** In addition to the eight SGA<sup>R</sup> genes tested, we looked for the presence of *vgbA* and *vgbB*, which are frequently localized on SGA<sup>R</sup> plasmids. We detected *vgaAv* in 37 of the 62 SGA<sup>R</sup> strains analyzed (59.7%). These strains were isolated between 1981 and 2001. *vgaAv* was found alone in 21 strains, in association with *vgaB-vatB* in 14 strains, in association with *vata-vgbA* in 1 strain, and in association with *vgaB-vatB* and *vata-vgbA* in 1 strain. In this latter strain, we confirmed the presence of each of these resistance genes by using PCR to amplify the cellular DNA of a single colony. Of the 25 isolates that did not carry *vgaAv*, 24 contained *vata-vgbA* and 1 contained *vgaA* alone. Thus, we detected at least one SGA<sup>R</sup> determinant in each of the 62 SGA<sup>R</sup> clinical isolates investigated. None of the isolates carried *vatC*, *vatD*, *vatE*, or *vgbB*.

We measured the sizes of the *Hind*III fragments that hybridized with the intragenic streptogramin resistance probes. Clinical isolates with the same hybridization pattern did not necessarily belong to the same genotype. In the 21 strains carrying only *vgaAv*, this gene was carried by *Hind*III fragments of 0.6 + 1.3 kb (18 isolates), 0.6 + 1.3 + 3 kb (1 isolate), 0.6 + 1.3 + 4.2 + 8 kb (1 isolate), and 0.6 + 5 kb (1 isolate). Two of these profiles were identical to those of *S. aureus* strains BM3327 (0.6 + 1.3 kb) and BM3252 (0.6 + 1.3 + 3 kb) (19), which carry one and two chromosomal copies of Tn5406, respectively (20).

In the 14 strains carrying *vgaAv* and *vatB-vgaB*, we found three *Hind*III patterns with *vgaAv*: 0.6 + 1.1 kb (10 strains), 0.6 + 1.1 + 1.3 kb (3 strains), and 0.6 + 1.1 + 2.5 (1 strain). Two of these profiles were not distinguishable from those of *S. aureus* strains BM3318 (0.6 + 1.1 kb) and BM3385 (0.6 + 1.1 + 1.3 kb) (19), which carry either one plasmid copy or one chromosomal copy and one plasmid copy of Tn5406, respectively (20). In each of the 14 strains containing *vgaAv-vatB-vgaB*, a single *Hind*III fragment hybridized with both *vatB* and *vgaB*. The size of this fragment varied (4.5, 5, 5.5, 7.5, and 9.5 kb). In *S. aureus* BM3385, in which *vatB* and *vgaB* were shown to be cotranscribed (2), the two genes were located on a 7-kb *Hind*III fragment (results not shown).

In each of the 26 strains containing *vata* and *vgbA*, associated or not with other genes (*vgaAv* in one isolate and *vgaAv-vatB-vgaB* in one isolate), *vata* and *vgbA* were found on a single *Hind*III fragment of 8 or 9.5 kb, whereas in the seven native *vgaA-vata-vgbA* plasmids tested previously (3), the cotranscribed genes, *vata-vgbA*, were on a 4.8-kb *Hind*III fragment.

**Plasmid content of the SGA<sup>R</sup> strains carrying *vata-vgbA*.** Plasmids were extracted from the 26 strains carrying *vata-vgbA* and probed with *vgbA* (pIP1654) and IS257*tmp* (pIP1644). The extrachromosomal bands (Fig. 2A) that hybridized with both probes had the same apparent sizes and were located just below the chromosomal DNA fragments of the uncleaved total cellular DNA (Fig. 2B). The hybridization patterns with *vgbA* of these plasmids cleaved by *Hind*III (results not shown) were identical to those observed with the cellular DNA cleaved by the same enzyme (8 and 9.5 kb). In 5 isolates collected in two hospitals in 1998 and 1999, the cohybridizing band was 8 kb, whereas in 21 isolates collected in seven hospitals between 1988 and 2001 the cohybridizing band was 9.5 kb. The plasmids generating 8-kb *Hind*III fragments hybridizing with *vgbA* seem to have emerged later.

PCR experiments were carried out with primers pairing with pAMβ1 *repE*-like gene and *vata* from pIP680 harbored by *S. aureus* strain BM3093. The cellular DNA of BM3093 and of five isolates of the present study carrying *vata-vgbA* plasmids (16b, 17a, 30b, 32, and 22a isolated in 1998; Table 1) were used as substrates. Fragments of the same size (2.6 kb [data not shown]) were amplified from the DNA of BM3093 and the two isolates (16b and 30b) carrying plasmids generating 9.5-kb *Hind*III fragments hybridizing with *vgbA*. In contrast, none of the three other isolates that carried plasmids generating 8-kb *Hind*III fragments hybridizing with *vgbA* generated amplicons. Thus, the plasmids generating 9.5-kb *Hind*III fragments hybridizing with *vgbA* seem to be related to pIP680, whereas such a conclusion cannot be drawn for the other plasmid type.

## DISCUSSION

The analysis of 62 SGA<sup>R</sup> *S. aureus* clinical isolates revealed numerous clones: 38 different genotypes were observed, each including closely related isolates whose *Sma*I patterns differed by no more than three fragments. Twenty-nine unrelated genotypes included possibly related isolates whose *Sma*I patterns differed by no more than six fragments. This multiplicity of unrelated clones is not surprising since these isolates were not necessarily collected during outbreaks, unlike those analyzed

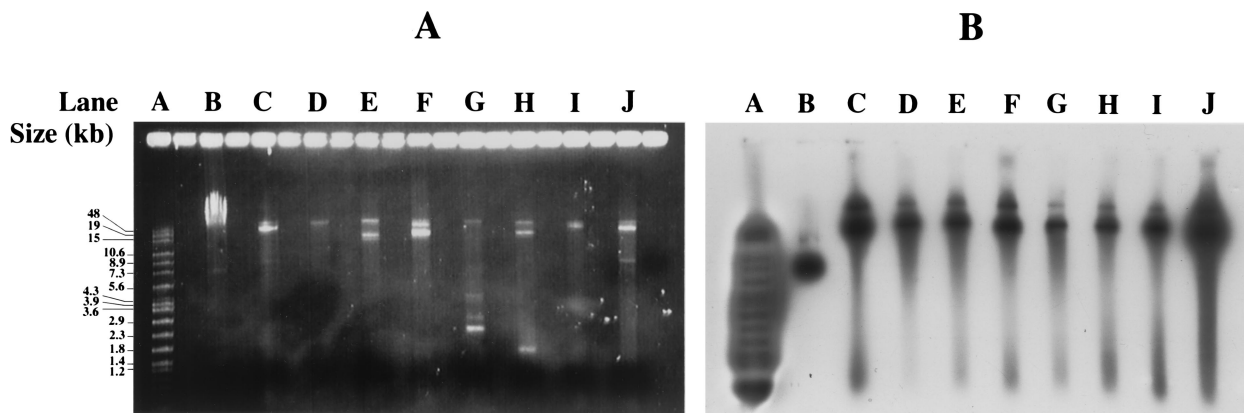


FIG. 2. Agarose gel electrophoresis patterns of plasmid DNA from SGA<sup>r</sup> *S. aureus* clinical isolates carrying *vata-vgbA* (lanes C to J). (A) Plasmid migration patterns; (B) hybridization patterns with the *vgbA* probe (pIP1654). Lanes A, the Raoul marker (Appligene) used as a standard; lanes B, total DNA from BM3093; lanes C, plasmid DNA from genotype 22a strain (Paris-A, 1998); lanes D, plasmid DNA from genotype 36a strain; lanes E, plasmid DNA from genotype 8a strain; lanes F, plasmid DNA from genotype 32 strain; lanes G, plasmid DNA from genotype 3 strain; lanes H, plasmid DNA from genotype 19 strain; lanes I, plasmid DNA from genotype 20 strain; lanes J, plasmid DNA from genotype 16a strain.

previously (15). Indeed, the clinical isolates included in the present study were mainly sent to us because of their uncommon antibiotic resistance patterns. Nevertheless, we observed the spread of monoclonal isolates both within the same hospital (Paris-A, Paris-B, Toulouse, or Villiers-St Denis) or in distinct hospitals, occasionally located in distinct cities (Villiers-St Denis, Paris-G, and Orsay; Paris-A and Toulouse; Paris-A and Grenoble), or at time intervals of up to 11 years, such as the genotype 30 strains collected in 1988 and 1999 (Table 1).

All of the staphylococcal isolates tested contained at least one SGA<sup>r</sup> gene. This finding is consistent with our previous findings concerning another collection of 52 SGA<sup>r</sup> independent staphylococci belonging to five species (1, 19). Since some of the clinical isolates in both collections carried several SGA<sup>r</sup> genes, we cannot rule out the possibility that staphylococci contain unknown genes, whereas among *E. faecium* strains new genes have to be characterized. In the study by Werner et al. (34), 7 of the 148 *E. faecium* isolates resistant to the mixtures did not contain any of the SGA<sup>r</sup> genes described to date and in the study by Soltani et al. (29), 8 of the 28 *E. faecium* mixture-resistant isolates did not contain any of these genes. Despite the multiplicity of unrelated clones detected in the present study, only six distinct SG<sup>r</sup> patterns were found. This may be due to the dissemination of structurally related plasmids in independent clones, as reported previously (3, 9). The *vata* and *vgbA* combination was not detected in our previous study (1), in which *vgaA* was always associated with *vata-vgbA*. A comparative analysis of seven native *vata-vgbA-vgaA* plasmids (15 to 45 kb) revealed the presence of a common 12.1-kb fragment carrying the three streptogramin resistance genes (3). This probably resulted from integration of a pAMβ1-like plasmid harboring *vata-vgbA* in which the replication gene is inactivated by IS257 insertion and a functional *vgaA* plasmid. A deletion between IS257 flanking *vgaA* may have resulted in the formation of *vata-vgbA* plasmids (Fig. 2A). The fact that these plasmids have the same size and are found in 42% of the isolates studied here is striking.

Zarrouk et al. (36) reported that the staphylococcal strains

resistant to A compounds but susceptible to B compounds remain susceptible to mixtures in vitro and in vivo. According to our previous (19) and present results, this is true when the strains carry *vgaAv* alone but not when they carry three SGA<sup>r</sup> genes. Indeed, the 14 SGB<sup>r</sup> isolates harboring *vgaAv* alone and originating from two hospitals (Paris-A and Toulouse) had the lincosamide and streptogramin A resistance phenotype. Like BM3385, the two lincosamide and streptogramin A-resistant isolates (genotypes 2 and 14a) (Table 1) that were susceptible to SGB and carried *vgaAv*, *vatB*, and *vgaB* were resistant to the mixtures (Table 1). Four of the seven *Hind*III hybridization patterns detected with *vgaAv* were identical to those of previously analyzed *S. aureus* clinical isolates in which *vgaAv* was shown to be carried by Tn5406 (19, 20). These results reflect a multiplicity of Tn5406 insertion sites in the collection of isolates tested.

We have found 45 antibiotypes among the SGA<sup>r</sup> clinical isolates tested. Some of them, such as genotypes 3 and 28 (Table 1), were susceptible to almost all other antibiotics, whereas genotype 15a, 15c, and 35 isolates were multiply resistant to various drugs, including glycopeptides (Table 1). The *Sma*I pattern of the genotype 15a isolate was identical to that of the major clone with reduced glycopeptide susceptibility found in European cities (17, data not shown). Coreistance to quinupristin-dalfopristin and glycopeptides (33), as in 5 of the 62 clinical isolates tested (8%), is of great concern since it may lead to therapeutic failure.

Since 1975, monoclonal staphylococcal isolates resistant to mixtures of the A and B compounds have occasionally been detected in the same hospitals or in hospitals located in geographically distinct French cities (15). However, in most French hospitals the prevalence of such isolates does not exceed 5% (14). The moderate use of natural mixtures in intensive care units, in which MRSA clinical isolates are easily spread, and the instability of SGA<sup>r</sup> plasmids, which are frequently lost or rearranged in the absence of selective pressure (1, 3), may explain why the numerous distinct isolates found in French hospitals have not disseminated often. The possible

dissemination of such clones with the increased selective pressure of streptogramins after the recent licensing of quinupristin-dalfopristin has to be prevented by improving hygiene and patient isolation measures.

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