Analysis of Pristinamycin-Resistant *Staphylococcus epidermidis* Isolates Responsible for an Outbreak in a Parisian Hospital

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In 1990, over a 6-month period, an increase from 1 to 10% in the incidence of pristinamycin resistance among coagulase-negative staphylococci was observed in four intensive care units of a Parisian hospital. Twenty-three such isolates, as well as 25 pristinamycin-susceptible *Staphylococcus epidermidis* isolates, were collected and typed by analyzing various bacterial constituents. Two structurally related plasmids of 7.3 and 14.3 kb, carrying the gene *vga* encoding resistance to pristinamycin, were detected in the 23 pristinamycin-resistant coagulase-negative staphylococci which were identified as *S. epidermidis*. Although related by numerous common characteristics, 20 of these 23 isolates could be divided into two types, A (17 isolates) and B (three isolates). These types were characterized on the basis of their plasmid contents and hybridization patterns obtained when the *EcoRI*-digested DNA was probed with plasmid pIP1551 containing an internal fragment of the insertion sequence IS256. These findings suggest that the dissemination of type A epidemic strains was, in large part, responsible for the outbreak.

The antibiotics of the virginiamycin family, which are produced by streptomycetes, are mixtures of two chemically unrelated molecules (A and B compounds) which act synergistically in vivo and in vitro (7). Compounds of group A, including streptogramin A, pristinamycin IIA, and virginiamycin M, are polyunsaturated cyclic peptolides. Compounds of group B, which include streptogramin B, pristinamycin IB, and virginiamycin S, are cyclic hexadepsipeptides. The target of these antibiotics, as well as of the synergistic effect, is the ribosome. The combination of the naturally occurring A and B compounds, which has a synergistic association, such as pristinamycin and staphylomycin, has been used in Europe as an oral antistaphylococcal agent. A novel semi-synthetic injectable streptogramin (RP59500) made of a mixture of A and B compounds (RP54476 and RP57669, respectively) is presently being studied in clinical trials. Supplement A to volume 30 of the *Journal of Antimicrobial Chemotherapy* (1992) is entirely devoted to the properties of RP59500.

In France, resistance to pristinamycin was first encountered among staphylococci in 1975 (4, 12, 17, 18). To date, the prevalence of such strains remains low ($\leq 2\%$) (16, 23). Resistance to the synergistic mixtures of A and B compounds (MIC of pristinamycin, ≥ 2 mg/liter) is always associated with resistance to A compounds (MIC of pristinamycin IIA, ≥ 8 mg/liter) but not necessarily with resistance to B compounds, whereas resistance to B compounds is not always associated with resistance to pristinamycin. In *Staphylococcus aureus* clinical isolates, the pristinamycin resistance determinants have been shown to be located on structurally related nonconjugative plasmids of 26 to 32 kb that encode resistance to numerous antibiotics, including A and B compounds (17, 18), and on conjugative determinants (14, 23) that confer resistance to various antibiotics, including A compounds but not B compounds. The latter determi-

nants are occasionally associated with the presence of plasmids in transconjugants.

Various mechanisms of resistance to the A and B compounds have been observed among staphylococci. An acetyltransferase that inactivates the A compounds was detected in the lysates of two pristinamycin-resistant staphylococcal isolates (10, 22). The gene encoding this enzyme, *vat*, was recently isolated from a staphylococcal plasmid and sequenced (3). A mechanism involving the active transport of A compounds was suspected because of the presence, on a staphylococcal plasmid (15), of a gene, *vga*, that encoded a protein with two ATP-binding domains and that conferred resistance to both A compounds and pristinamycin (1). Three mechanisms of resistance to B compounds have been described: (i) target site modification mediated by inducible or constitutive enzymes which methylate 23S rRNA in 50S ribosomal subunits, giving rise to coresistance to erythromycin, lincosamides, and B compounds (28); (ii) modification of the transport of the antibiotic probably mediated by an ATP-binding protein encoded by the gene *msrA*, which confers coresistance to macrolides and B compounds (24); and (iii) inactivation of B compounds by a hydrolase that splits the lactone ring (2, 21), resulting in resistance to B compounds but not to pristinamycin.

In 1990, over a 6-month period, an increase from 1 to 10% in the incidence of pristinamycin resistance among coagulase-negative staphylococci was observed in four intensive care units of a Parisian hospital. During the same period, pristinamycin consumption continued without significant modification. We report here the typing of the pristinamycin-resistant coagulase-negative staphylococci involved in the outbreak. Typing was done by molecular methods including analysis of the genetic determinants conferring resistance to pristinamycin. By these methods, we characterized two types of epidemic strains harboring structurally related plasmids which carried the gene *vga* and found that the outbreak was mostly due to the dissemination of one type of epidemic

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strain, whereas the dissemination of the same plasmid in unrelated isolates had a minor contribution.

MATERIALS AND METHODS

Bacterial strains and plasmids. The 48 coagulase-negative staphylococci included in the present study were isolated from cultures of blood from 48 patients hospitalized in four intensive care units of the same Parisian hospital. They consisted of 23 pristinamycin-resistant coagulase-negative staphylococci and 25 pristinamycin-susceptible *Staphylococcus epidermidis* strains. Three of the 23 pristinamycin-resistant coagulase-negative staphylococci were isolated in March 1988 (see Table 1), whereas the other isolates were collected between January 1990 and March 1991. The *S. aureus* transducant, strain BM3093 harboring plasmid pIP680 (15), was also used in the study.

The *Escherichia coli* strains used were SF8 (20) and BM8132 (13); these strains harbored plasmids pBA2 and pIP1551, respectively.

Plasmid pBA2 consists of a 2.3-kb *Bacillus subtilis* DNA fragment encoding 16S rRNA inserted in the *Hind*III site of pBR322. Plasmid pIP1551 was constructed by cloning into pUC18 a 468-bp sequence from within the open reading frame, ORF390, of the staphylococcal insertion sequence IS256. Plasmid pIP680 (11.4 kb) was isolated by transduction from the *S. aureus* isolate BM3041 (17, 18); it carries the genes *vga* (1) and *vgb* (2), which encode resistance to A and B compounds of the virginiamycin antibiotics, respectively.

Media. Brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) and BHI agar (Difco) were used to grow staphylococci. Pristinamycin-resistant staphylococci were selected on medium containing 15 mg of pristinamycin IIA (Rhône-Poulenc Rorer) per liter. *E. coli* strains were grown on Luria broth (LB) containing 10 g of tryptone per liter, 5 g of yeast extract per liter, and 10 g of sodium chloride per liter or on LB containing 15 g of agar per liter supplemented with 100 mg of ampicillin (Panpharma, Fougères, France) per liter. Mueller-Hinton agar (Diagnostics Pasteur, Marne la Coquette, France) was used for disk susceptibility tests and MIC determinations.

Susceptibility to antimicrobial agents. The pattern of resistance to antimicrobial agents was determined by the disk diffusion method as described previously (16). The markers tested were those which enabled us to detect the drug resistance phenotypes described to date among staphylococci. Commercially available disks loaded with the following antibiotics were used: penicillin G, oxacillin, spectinomycin, streptomycin, kanamycin, neomycin, gentamicin, tobramycin, chloramphenicol, erythromycin, lincomycin, trimethoprim, sulfonamide, tetracycline, minocycline, pefloxacin, rifampin, fusidic acid, fosfomycin, and vancomycin (Diagnostics Pasteur). Additional disks prepared in our laboratory contained 20 µg of pristinamycin IIA, 40 µg of pristinamycin IB, 0.2 µmol of cadmium acetate, 0.2 µmol of sodium arsenate, 0.2 µmol of mercuric nitrate, 200 µg of ethidium bromide, 200 µg of acriflavine, 200 µg of propamidine isethionate, or 10 µg of cetyltrimethylammonium bromide.

MICs were determined with serial twofold dilutions of antibiotics in Mueller-Hinton agar medium (19).

Phenotypic and genomic identifications at the species and subspecies levels. All the techniques used for the classification of staphylococcal strains were described previously (6, 8, 9). The phenotypes were characterized with the ID32 Staph system (La Balme les Grottes, Montalieu-Vercieu,

France) and with the following additional tests: detection of clumping factor, free coagulase production, and thermonuclease activity before and after seroinhibition with a polyclonal serum directed against *S. aureus* thermonuclease (BioMerieux, Marcy-l'Etoile, France).

The *Hind*III- and *Eco*RI-digested cellular DNAs of the coagulase-negative staphylococci were separated by electrophoresis, transferred onto nitrocellulose membranes, and probed under stringent conditions with plasmid pBA2 radiolabeled with [α -³²P]dCTP (110 TBq/µmol). The sizes of the bands constituting the hybridization patterns (HPs) were introduced into our data base so that each HP could be compared with each of the 81 *Eco*RI HPs and 74 *Hind*III HPs previously detected for 271 validly classified strains belonging to 31 staphylococcal taxa (8, 9).

DNA isolation and analysis. Total cellular DNA and plasmid DNA were isolated from staphylococcal strains and were purified as described previously (9, 14). Plasmid DNA from *E. coli* strains was isolated and purified by a cesium chloride-ethidium bromide gradient centrifugation (25). Plasmid DNA was extracted from agarose gels and was purified by electroelution as described by Sambrook et al. (25).

Restriction endonucleases were obtained from Amersham International or Pharmacia and were used according to each manufacturer's instructions. Digested DNA was submitted to electrophoresis in agarose gels (0.7% wt/vol) in Tris-borate buffer as described by Sambrook et al. (25). The Raoul I ladder (Appligène, Strasbourg, France) and the 1-kb DNA ladder (Bethesda Research Laboratories, Inc.) were used as size markers.

Blotting and hybridization. DNA was transferred from agarose gels to nitrocellulose paper by the bidirectional method (26). Probe DNA was labeled with [α -³²P]dCTP (110 TBq/mmol; Amersham International) by the multiprimer labeling system (Amersham International) according to the manufacturer's instructions. Hybridization was performed under stringent conditions, and the blots were exposed to Fuji RX film at -70°C.

Comparison of HPs. The average similarity between any two HPs was assessed by the coefficient of Dice (11), calculated as follows: percent similarity = [(number of matching bands × 2)/total number of bands] × 100.

Preparation of oligonucleotides and PCR. The following oligonucleotides were prepared by the phosphoramidite method with an Applied Biosystems model 380 B DNA synthesizer: oligonucleotide A, 5'-CCAGAACTGCTATT AGCAGATGAA-3'; oligonucleotide B, 5'-AAGTTCGTTTC TCTTTTCGACG-3'; oligonucleotide C, 5'-AAAACGGAG GGGATAGAATG-3'; oligonucleotide D, 5'-GACTCACTC CATATTATCCTTAA-3'.

Oligonucleotides A and B were used as primers in the polymerase chain reaction (PCR) to amplify a 471-bp sequence from within the gene *vga* encoding resistance to A compounds and to pristinamycin (1). The forward primer (oligonucleotide A) corresponds to nucleotide positions 1200 to 1223, and the reverse primer (oligonucleotide B) corresponds to the complement of positions 1670 to 1649.

Oligonucleotides C and D were used as primers in the PCR to amplify a 920-bp sequence from within the gene *vgb* encoding resistance to B compounds (2). The forward primer (oligonucleotide C) corresponds to nucleotide positions 624 to 643, and the reverse primer (oligonucleotide D) corresponds to the complement of positions 1543 to 1521.

The PCR was performed with a precycle of 5 min at 94°C and 5 min at 60°C; this was followed by 30 cycles of 1 min at 72°C, 1 min at 94°C, and 1 min at 60°C.

SDS-PAGE and immunoblotting of bacterial proteins. The isolation of bacterial proteins, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting with an unabsorbed rabbit antiserum raised against *S. epidermidis* ATCC 14990^T were performed as described previously (27).

RESULTS

Phenotypic identification at the species and subspecies levels. Of the 23 pristinamycin-resistant isolates tested (Table 1), 22 could be assigned to *S. epidermidis* on the basis of their phenotypic characteristics, including those detected by the ID32 Staph system. Strain BM10402 could not be classified by this method.

HPs obtained with the probe pBA2 (ribotypes). The HPs of the 23 pristinamycin-resistant isolates were obtained by cutting the cellular DNA either with *Hind*III or with *Eco*RI and by probing with radiolabeled pBA2. The *Hind*III HPs and the *Eco*RI HPs of the 23 isolates tested are reported in Table 1. These patterns were identical to those detected previously among *S. epidermidis* strains (9, 27). Therefore, all the isolates, including BM10402, could be precisely assigned to *S. epidermidis*.

Three combinations of *Hind*III HPs and *Eco*RI HPs could be distinguished among the 23 *S. epidermidis* isolates: H20-E21 (20 isolates), H55-E21 (2 isolates), and H20-E45 (1 isolate) (Table 1). Since most of the isolates had the combination H20-E21, which has been shown to be widespread among epidemiologically unrelated *S. epidermidis* isolates (27), ribotyping did not appear to be appropriate for fingerprinting the pristinamycin-resistant *S. epidermidis* isolates examined in the present study.

Patterns of resistance to antimicrobial agents. For the 23 *S. epidermidis* isolates collected during the outbreak, the MICs of pristinamycin IIA (32 to 128 mg/liter) and pristinamycin (2 to 8 mg/liter) were significantly higher than those of the 25 susceptible *S. epidermidis* strains (pristinamycin IIA MICs, ≤ 4 mg/liter; pristinamycin MICs, ≤ 0.5 mg/liter), which do not carry either of the two genes *vga* (1) or *vat* (3), which encode resistance to pristinamycin IIA and pristinamycin, respectively.

In addition to resistance to pristinamycin and pristinamycin IIA, the 23 *S. epidermidis* isolates shared resistance to 11 other drugs (Table 1). Twenty of the 23 isolates had a combination of resistance to fluoroquinolones (pefloxacin), rifampin, fusidic acid, and fosfomycin (Table 1). To date, the combination of these four antibiotic resistance markers is uncommon (<1%) among the coagulase-negative staphylococci isolated in French hospitals and sent to our National Reference Center for Staphylococci.

Immunoblot patterns. Four immunoblot patterns (data not shown) could be easily distinguished among the 23 pristinamycin-resistant *S. epidermidis* isolates (Table 1). The 20 isolates carrying the unusual combination of resistance to fluoroquinolones, rifampin, fusidic acid, and fosfomycin had indistinguishable immunoblot patterns characterized by a limited number of antigens reacting with the antiserum used (pattern I1). In contrast, patterns I2, I3, and I4 consisted of a large number of bands.

Hybridization patterns obtained by probing with pIP1551. The cellular DNAs of the 23 pristinamycin-resistant and the 25 pristinamycin-susceptible *S. epidermidis* isolates collected in the same hospital and at the same time interval were submitted to agarose gel electrophoresis, transferred onto nitrocellulose membranes, and probed with radiola-

beled pIP1551. The DNAs of all of the isolates tested hybridized with this probe. For the 23 pristinamycin-resistant isolates, the hybridizing nucleotide sequences were located in the area containing the chromosomal DNA (data not shown).

A schematic representation of the distinct hybridization patterns obtained with the *Eco*RI-digested DNAs obtained for the 23 pristinamycin-resistant and the 25 pristinamycin-susceptible isolates is shown in Fig. 1. Among the 23 pristinamycin-resistant isolates, 10 patterns each containing two to six *Eco*RI fragments hybridizing with pIP1551 were observed (Fig. 1, lanes 1 to 10; Table 1). Each of the 25 pristinamycin-susceptible isolates had its own *Eco*RI HP consisting of one to nine hybridizing *Eco*RI fragments (Fig. 1, lanes 11 to 35).

The 10 *Eco*RI HPs detected among the 23 pristinamycin-resistant isolates were compared two by two between each other and with each of the 25 *Eco*RI HPs of the pristinamycin-susceptible isolates. The percent similarity between each pair was calculated by using the Dice index (11). The seven *Eco*RI HPs detected among the 20 pristinamycin-resistant *S. epidermidis* isolates sharing the unusual combination of four antibiotic resistance markers and the same immunoblot pattern, pattern I1 (Table 1), were related by percentages of similarity (54.5 to 90.9%) higher than those previously observed among epidemiologically unrelated *S. epidermidis* isolates from distinct geographical sources (0 to 40%) (27). These values were also significantly higher than those resulting from the comparison of each of the 7 *Eco*RI HPs with each of the 25 *Eco*RI HPs of the pristinamycin-susceptible isolates (0 to 25%). Although more closely related to each other than to the pristinamycin-susceptible isolates from the same environment or from distinct geographical sources, the 20 pristinamycin-resistant *S. epidermidis* isolates sharing immunoblot pattern I1 could be divided into two types, types A and B (Table 1). Indeed, within each of these two types, the *Eco*RI HPs appeared to be more closely related ($\geq 83.3\%$ within each type and 54.5 to 75.0% between the *Eco*RI HPs of type A and type B strains). In contrast, low percentages of similarity were observed ($\leq 28.5\%$) when the *Eco*RI HPs of the remaining three pristinamycin-resistant isolates were compared with each other or with those of the type A and type B isolates. Thus, types C, D, and E were assigned to these three isolates (Table 1).

Analysis of plasmid content. The plasmids harbored by the 23 pristinamycin-resistant *S. epidermidis* isolates were extracted and submitted to agarose gel electrophoresis. Five plasmid patterns were distinguished (Fig. 2; Table 1). Within each of the two types A and B, the isolates had the same plasmid pattern.

Oligonucleotides A and B, designed to amplify a 471-bp sequence from within the gene *vga* encoding resistance to pristinamycin, were used in the PCR along with the plasmid contents of the 23 pristinamycin-resistant *S. epidermidis* isolates. For each isolate, the predicted 471-bp fragment was obtained as the PCR product; this fragment, when treated with *Xba*I, gave the expected fragments of 422 and 50 bp (data not shown). In contrast, the use of oligonucleotides C and D, designed to amplify a 920-bp sequence from within the gene *vgb* encoding resistance to the B compounds of virginiamycin, did not yield any amplified DNA fragment (data not shown).

The radiolabeled 471-bp DNA fragment internal to the gene *vga* carried by plasmid pIP680 (1) and amplified in a PCR with oligonucleotides A and B was used as a probe in hybridization experiments with the plasmid DNAs extracted

TABLE 1. Characteristics of the 23 pristinamycin-resistant *S. epidermidis* isolates analyzed

Date of sampling (mo/yr)	Strain	Pattern of resistance to antimicrobial agents ^a				Immunoblot pattern	Plasmid pattern	Plasmid size (kb)	HP with:			Type
									pBA2	pIP1551,		
										HindIII	EcoRI	
	BM9348	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Eb Af Pi	II	P2	7.3	H20	E21	E424	A		
	BM10385	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3 ^b	H20	E21	E431	A		
	BM10386	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10387	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10388	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10389	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E430	A		
	BM10391	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10392	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo As Hg Eb Af Pi	II	P2	7.3	H20	E21	E430	A		
	BM10394	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E425	A		
	BM10396	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10397	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E425	A		
	BM10398	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10399	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10400	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10401	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10402	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo As Hg Eb Af Pi	II	P2	7.3	H20	E21	E427	A		
	BM10403	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo Cd As Hg Eb Af Pi	II	P2	7.3	H20	E21	E425	A		
	BM9349	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo As Eb Af Pi	II	P4	14.3	H55	E21	E411	B		
	BM9350	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Pf Rf Fa Fo As Eb Af Pi	II	P4	14.3	H55	E21	E411	B		
	BM10393	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Rf Fa Fo As Hg Eb Af Pi	II	P4	14.3 ^c	H20	E45	E432	B		
	BM10390	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Pf Fa Fo Cd As Hg Eb Af Pi	I2	P5	7.3	H20	E21	E435	C		
	BM10405	Pc Ox Sp Km Nm Tm Gm MLSSgA	Tp Su Tc Rf Cd As Hg Eb Af Pi	I3	P1	7.3	H20	E21	E434	D		
	BM10406	Pc Ox Sp Km Nm Tm MLSSgA	Tp Su Fa Cd As Hg Eb Af Pi	I4	P3	7.3	H20	E21	E433	E		

^a Abbreviations: Af, acriflavine; As, sodium arsenate; Cd, cadmium acetate; Eb, ethidium bromide; Fa, fusidic acid; Fo, fosfomycin; Gm, gentamicin; Hg, mercuric nitrate; Km, kanamycin; MLS, macrolides-lincosamides-streptogramin B; Nm, neomycin; Ox, oxacillin; Pc, penicillinase production; Pf, pefloxacin; Pi, propamidine isethionate; Rf, rifampin; SgA, streptogramin A; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim; vga, gene encoding an ATP-binding protein involved in resistance to SgA.

^b Plasmid designation, pIP1629.

^c Plasmid designation, pIP1630.

FIG. 1. Schematic representation of the *EcoRI* HPs obtained with the probe pIP1551. The 10 distinct HPs detected among the 23 pristinamycin-resistant *S. epidermidis* isolates analyzed (Table 1) are shown in lanes 1 to 10 (lanes: 1, E427; 2, E424; 3, E425; 4, E430; 5, E431; 6, E411; 7, E432; 8, E435; 9, E434; 10, E433), and those of the 25 pristinamycin-susceptible *S. epidermidis* isolates are reported in lanes 11 to 35. The sizes indicated on the left (in kilobases) are those of the linear DNA fragments contained in the Raoul I ladder.

from the 23 pristinamycin-resistant *S. epidermidis* isolates. In the type A, C, D, and E isolates, the nucleotide sequences that hybridized with this probe were detected on a 7.3-kb plasmid, whereas in the type B isolates, these sequences were located on a 14.3-kb plasmid (Table 1; Fig. 2). When the plasmids of the 23 isolates were digested with *HindIII*

and *PvuII*, the hybridizing sequences were located on a 3.1-kb and a 2.4-kb restriction fragment, respectively (data not shown). The 7.3-kb plasmid, pIP1629, harbored by one *S. epidermidis* isolate, BM10385 (Table 1), was isolated from the agarose gel, purified, radiolabeled, and used to probe the *HindIII*-digested plasmid DNAs of the 23 isolates. The hybridization patterns of all of these plasmids consisted of five *HindIII* fragments (3.1, 1.4, 1.2, 1.1, and 0.5 kb) (data not shown). These results suggest that the 7.3- and 14.3-kb plasmids are structurally related.

The restriction maps of a representative "vga" plasmid of each size, pIP1629 (7.3 kb) and pIP1630 (14.3 kb), harbored by the *S. epidermidis* isolates BM10385 and BM10393, respectively (Table 1), are reported in Fig. 3. Each plasmid was isolated from an agarose gel and was used as a probe in hybridization experiments with the undigested plasmid DNA contents of the isolates carrying the homologous plasmid, i.e., BM10385 or BM10393. Since each probe hybridized with the homologous plasmid only without giving any detectable signal with the other coharbored plasmids (data not shown), the restriction profiles of pIP1629 and pIP1630 corresponded to the hybridization patterns. Therefore, mapping could be carried out without proceeding to the genetic isolation of each plasmid. The comparative analysis of both maps indicated that the larger plasmid, pIP1630 (14.3 kb), contained all of the restriction fragments of the smaller plasmid, pIP1629 (7.3 kb). Since the common restriction fragments of pIP1630 hybridized with pIP1629, pIP1630 probably consisted of pIP1629 plus an additional 7-kb DNA fragment. Plasmid pIP1630 contained two *PstI*-*PvuII* fragments of 1.3 kb (Fig. 3), which may be part of a repeated sequence. Sequencing of the regions containing these fragments is necessary to confirm this hypothesis.

FIG. 2. Plasmid profiles (A) and HPs obtained with the probe consisting of the PCR product from within *vga* (B) of BM10405 (P1), BM10385 (P2), BM10406 (P3), BM10393 (P4), and BM10390 (P5).

Plasmid pIP1629 : 7.3 kb

Plasmid pIP1630 : 14.3 kb

FIG. 3. Restriction maps of plasmids pIP1629 (7.3 kb) and pIP1630 (14.3 kb) carrying *vga* and isolated from the *S. epidermidis* isolates BM10385 and BM10393, respectively (Table 1). Abbreviations: Pv, *PvuII*; Ps, *PstI*; X, *XbaI*; E, *EcoRI*; S, *ScaI*.

The restriction profiles of pIP1629 and pIP1630 were compared with those of the staphylococcal plasmids conferring resistance to pristinamycin described to date (18, 23). No common restriction fragments were detected (data not shown).

DISCUSSION

The significant increase in the incidence of pristinamycin resistance among coagulase-negative staphylococci collected in four intensive care units prompted us to fingerprint and compare 23 isolates. The analysis of distinct bacterial constituents was used to this end. Analysis of ribotypes appeared useful for a precise assignment of all of the coagulase-negative staphylococci to *S. epidermidis* but not for typing, because most of these isolates had ribotypes which had previously been shown to be widespread among unrelated *S. epidermidis* isolates (27). Analysis of the drug resistance patterns, immunoblot patterns, and *EcoRI* HPs obtained by probing with pIP1551 served to discriminate 20 of the 23 pristinamycin-resistant *S. epidermidis* isolates. Although related by numerous common characteristics, including an uncommon combination of resistances to fluoroquinolones (pefloxacin), rifampin, fusidic acid, and fosfomycin, the 20 pristinamycin-resistant *S. epidermidis* isolates could be divided into two types, type A (17 isolates) and type B (3 isolates), on the basis of the HPs obtained with pIP1551 and plasmid content. Within each type, the strains harbored plasmids hybridizing with *vga* and having distinct sizes: 7.3 kb (pIP1629) in type A strains and 14.3 kb (pIP1630) in type B strains. In conclusion, the increased incidence of pristinamycin resistance observed among the coagulase-negative staphylococci isolated in 1990 in a Parisian hospital was mostly due to the dissemination of type A strains which had already been detected in the intensive care units in 1988. The dissemination of the 7.3-kb "*vga*" plasmid among unrelated *S. epidermidis* isolates made a minor contribution.

The spread of epidemic *S. epidermidis* strains carrying the unusual combination of four resistance markers common to type A and type B strains was also observed in 1989 in an intensive care unit of another Parisian hospital (27). Al-

though 8 of the 14 epidemic strains in that unit were considered susceptible to pristinamycin (MIC, 1 mg/liter), the 14 strains harbored "*vga*" plasmids with the same restriction profiles as those of the two "*vga*" plasmids detected among the 23 pristinamycin-resistant *S. epidermidis* isolates described in this report. The comparison of their immunoblot patterns, their *EcoRI* HPs obtained with pIP1551, and their total plasmid contents with those of the type A and type B strains that we studied led us to conclude retrospectively that 13 of the 14 epidemic strains belonged to type A, whereas 1 strain belonged to type B. Thus, the combination of resistance to pefloxacin, rifampin, fusidic acid, and fosfomycin appeared to be a more reliable phenotypic characteristic than resistance to pristinamycin for tracing type A or type B *S. epidermidis* strains. This combination was used to look for type A or type B strains in 10 French hospitals. In 1992, only two *S. epidermidis* strains carrying this combination of four resistance markers were detected; these were isolated in a hospital located in Marseille. Analysis of their *EcoRI* HPs obtained with pIP1551 and their plasmid contents indicated that they belonged to type A (data not shown). These results suggest that the spread of type A and type B *S. epidermidis* strains is still limited in France.

Although the reported incidence of resistance to pristinamycin remains low in most French hospitals ($\leq 1\%$), it might be underestimated. Indeed, some strains, such as type A or type B *S. epidermidis* strains carrying the gene *vga*, exhibited either little or no significant resistance to pristinamycin (MICs, 1 to 2 mg/liter) or to pristinamycin IIA (MICs, 4 to 8 mg/liter) when tested after subculturing in the absence of selection for resistance to these antibiotics. For the *S. epidermidis* strains in which the genes *vga* (1) or *vat* (3) are not detectable, the MICs of pristinamycin and pristinamycin IIA do not exceed 0.5 and 4 mg/liter, respectively. Levels of resistance may be low either because the genes are poorly expressed or because the strains are losing the plasmid conferring resistance to pristinamycin. To recognize staphylococci that exhibit resistance that is weak and therefore not consistently expressed, detection of the genes *vga* or *vat* (1, 3) is a more reliable method than is use of the

antibiogram. Treatment of strains which are misidentified phenotypically as susceptible with a synergistic mixture of A and B compounds such as pristinamycin, staphylomycin, or the novel injectable semisynthetic streptogramin RP59500 may contribute to the selection in vivo of clones that exhibit higher levels of resistance.

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