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Phenotypic and Genomic Variation among *Staphylococcus epidermidis* Strains Infecting Joint Prostheses

JACQUES-OLIVIER GALDBART,[1,2] ANNE MORVAN,[1] NICOLE DESPLACES,[3] AND NEVINE EL SOLH[1]*

Unité des Staphylocoques, National Reference Center for Staphylococci, Institut Pasteur, 72724 Paris Cedex 15,[1] Service de Microbiologie, Hôpital Beaujon, A.P.-H.P., 92100 Clichy,[2] and Service de Biologie, Hôpital de la Croix-Saint-Simon, 75020 Paris,[3] France

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We studied the *Sma*I and *Sst*II macrorestriction patterns of 54 *Staphylococcus epidermidis* strains isolated from 14 patients infected following the implantation of joint prostheses. Multiple strains from pus and infected tissue specimens of each patient were selected on the basis of different colony morphologies and drug resistance patterns. The same criteria were used to select 23 *S. epidermidis* strains from hand swabs of eight healthy individuals. For 10 of the 14 patients, all the intrapatient strains appeared to be closely or possibly related, whereas related strains were detected in the skin flora of only one of the eight healthy individuals. This observation suggests that, in most cases, the patients were infected by a single *S. epidermidis* clone which subsequently underwent rearrangements that yielded derivatives with divergent phenotypes and, occasionally, divergent macrorestriction patterns. The four patients whose specimens contained unrelated *S. epidermidis* strains were probably infected with several polyclonal strains.

Infection is one of the most devastating complications of prosthetic joint surgery (3, 12, 14, 15, 17, 27, 33). *Staphylococcus epidermidis*, which can adhere to implants, has increasingly been identified as a cause of such infections (6, 16, 21, 23, 24, 26, 35). *S. epidermidis* is a normal commensal organism of the skin which may contaminate specimens during collection, and it is therefore difficult to assess the clinical significance of its detection, particularly when the bacterial colonies obtained have diverse aspects and drug resistance phenotypes. Detection of gram-positive cocci by direct inspection and/or repeated isolation of the same mixtures of strains from a series of specimens from the same patient provides good evidence of infection causality (17, 19, 36). The presence in pus and tissue specimens of multiple *S. epidermidis* strains, frequent in infections following implantation of joint prostheses (17), may be due either to the genomic instability of a single infectious clone or to an infection caused by a polyclonal mixture of strains such as the mixture of strains found in the skin flora. To examine these two possibilities, we compared the genomes of the various phenotypically distinct *S. epidermidis* strains detected in pus and tissue specimens from 14 patients with chronic prosthetic joint infections. Diverse *S. epidermidis* colonies isolated from the skin flora of eight healthy individuals were used for comparative analysis of the intraindividual colonies that were selected on the basis of their different colony morphologies and drug resistance phenotypes.

MATERIALS AND METHODS

Patients and healthy individuals. The 14 patients included in this study were infected following the implantation of joint prostheses (see Table 1). Joint replacements were carried out in diverse hospitals, and there was no epidemiological link between the patients. The files of all patients except patients B and N were consulted. The 12 patients whose files were available had pain without fever. The delay between surgery and pain was not regularly registered in the files; therefore, we report (see Table 1) the delay between the implantation of the prosthesis and its replacement (3 to 84 months). The replacement was not necessarily done immediately after the first signs of infection. Five of the 12 patients (patients A, E, I, L, and P) had abscesses, which had fistulized in patients A, L, and P, and 6 patients (patients E, F, I, L, O, and P) presented with edema. Bacteriological samples were obtained at the time of intraoperative assessment and surgery for excisional arthroplasty. No antibiotic was administered to any patient for at least 1 week before joint surgery. Patients whose infected specimens yielded only *S. epidermidis* were included in this study.

Hand skin swabs were collected from healthy individuals including (i) six surgeons working in the same operating room in a Parisian hospital, but a hospital that was not one of those in which the patients of our study were hospitalized, and (ii) two individuals who did not work in a hospital (see Table 2).

Bacterial strains and plasmids. A total of 82 *S. epidermidis* strains originating from 14 infected patients (54 strains from infected specimens and 5 strains from cutaneous samples of patient P) and from 8 healthy individuals (23 strains) were studied (see Tables 1 and 2, respectively). The infected specimens and the skin swabs were cultured on sheep blood agar. After at least 48 h of incubation at 37°C, the species of the colonies that were isolated from each sample and that had different morphologies (size, color which varied from white to grey, shape and outline, presence or absence of hemolytic activity, rough or smooth aspect) was determined as described previously (8), and the colonies were tested with the ID32 Staph system (BioMérieux, Marcy-l'Etoile, France). Patients whose infected specimens contained only *S. epidermidis* colonies were included in this study. The drug resistance pattern was determined for each different *S. epidermidis* colony isolated from the specimens of each patient. The strains that were distinguishable by at least one drug resistance marker were studied independently. Similarly, the *S. epidermidis* colonies isolated from skin flora were selected on the basis of their distinct morphologies and drug resistance patterns. Bacterial suspensions in brain heart infusion containing 30% glycerol were stored at 80°C before analysis.

Plasmid pBA2 (18) was used as a probe for ribotype determination. It consists of pBR322 carrying a 2.3-kb *Hin*dIII insert from *Bacillus subtilis* which encodes 16S rRNA.

Susceptibility to antimicrobial agents. The pattern of resistance to antimicrobial agents was determined by the disk diffusion method (4). 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, Marne-la-Coquette, France) and mupirocin (Mast Diagnostics, Mast Group Ltd., Merseyside, United Kingdom). 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.

Selective Mueller-Hinton agar media containing 4 μg of oxacillin per ml, 0.12 IU of penicillin per ml, 5 μg of erythromycin per ml, 3 μg of tetracycline per ml, or 20 μg of gentamicin per ml were used to screen for variants exhibiting distinct drug resistance patterns in the subcultures of strain 94351 (see Table 1).

* Corresponding author. Mailing address: Unité des Staphylocoques, National Reference Center for Staphylococci, Institut Pasteur, 72724 Paris Cedex 15, France. Phone: (33) 01 45 68 83 63. Fax: (33) 01 40 61 31 63. E-mail: nelsolh@pasteur.fr.

Ribotype determination. Cellular DNA was extracted, cleaved with *Hin*dIII and *Eco*RI (Pharmacia Biotech, Uppsala, Sweden) separately, electrophoresed, transferred onto Hybond N$^+$ nylon membranes (Amersham International), and tested for hybridization under stringent conditions with pBA2 radiolabeled with [α-^{32}P]dTCP (110 TBq/mmol) as described elsewhere (7, 8).

The sizes of the bands constituting the hybridization patterns (HPs) were introduced into our database (5, 7). Each of these HPs was compared with each of the *Eco*RI HPs and *Hin*dIII HPs previously obtained for validly classified staphylococci. Similarity was evaluated according to the Dice coefficient (11). The strains that had HPs that were indistinguishable from those detected previously could immediately be assigned to a species. For each new HP, as reported previously (5), the percent similarity with each *Eco*RI or *Hin*dIII HP in the database was calculated. An isolate exhibiting a new HP can be assigned to a known taxon if the highest percentages of similarity obtained are clustered within a single taxon.

Pulsed-field gel electrophoresis of macrorestriction fragments and comparative analysis of banding patterns. The protocol used for the determination of *Sma*I or *Sst*II restriction patterns was described previously (10). Concatameric bacteriophage lambda DNA molecules (48.5 kb; Bio-Rad) and the *Sma*I fragments of the cellular DNA from *Staphylococcus aureus* NCTC8325 were used as size standards. Macrorestriction fingerprints were compared visually and were scanned with GelCompar software (Applied Maths, Kortrijk, Belgium). A similarity matrix was created by using the band-based Dice similarity coefficient (11). The unweighted pair-group method with average linkages was used to cluster the strains on the basis of the patterns obtained with each of the two enzymes used.

The *Sma*I or *Sst*II patterns of the isolates from the same patient or healthy individual were compared visually, in pairs, by using an enlarged photograph of the same gel. The strains were clustered according to the following criteria proposed by Tenover et al. (28): (i) Strains were grouped in the same major genotype if their patterns differed by no more than three bands (these strains were considered to be closely related and monoclonal); (ii) if the number of band differences between patterns was between four and six, the strains were scored as possibly related but were nevertheless classified into distinct genotypes to discriminate them clearly from the unambiguously closely related strains; and (iii) differences between patterns involving at least seven bands indicated different or unrelated strains. Major genotypes are designated by capital letters or arabic numerals according to the enzyme used, *Sma*I or *Sst*II, respectively (see Table 1). The strains having indistinguishable patterns were classified within the same subtype. *Sma*I subtypes are designated by letters with number suffixes, and *Sst*II subtypes are designated by numbers with letter suffixes. If the dendrograms revealed clusters that included strains from different patients with percentages of similarity of at least 80, the patterns of the strains grouped in the same cluster were compared visually on the same gel. Those strains whose patterns differed by no more than three macrorestriction fragments were assigned to the same genotype.

RESULTS

Identification to the species level. Fifty-one of the 54 coagulase-negative staphylococci isolated from the infected specimens from patients were assigned to *S. epidermidis* by the ID32 Staph system. Analysis of the hybridization patterns with pBA2 enabled us to assign to *S. epidermidis* three strains, strains 95160, 96388, and 96390 (Table 1) not classified by the ID32 Staph system.

The ID32 Staph system was used to identify the *S. epidermidis* strains isolated from the flora of patient P (Table 1) and the healthy individuals (Table 2).

Drug resistance phenotype. The intrapatient strains had in common 1 to 14 markers and were distinguishable by 1 to 10 additional markers (Table 1). Among the strains from healthy individuals, the intraindividual strains had in common one to three resistance markers and differed by 1 to 13 additional markers (Table 2).

Analysis of the macrorestriction patterns. (i) Comparison of the *Sma*I and *Sst*II patterns of patient strains isolated from infected specimens. The 54 isolates of *S. epidermidis* tested (Table 1) gave a total of 30 different *Sma*I patterns and 29 different *Sst*II patterns. Each *Sma*I profile included 12 to 17 fragments (Fig. 1), and each *Sst*II profile included 12 to 21 fragments (data not shown).

The strains of each patient were clustered according to the similarities of the *Sma*I or *Sst*II patterns. The clustering into genotypes of all intrapatient strains except strains 94304, 94305, and 94306, all of which were isolated from patient E, was the same for both enzymes (Table 1). On the basis of the *Sma*I patterns, these three strains were clustered into the same genotype. However, the *Sst*II pattern of strain 94304 differed from those of strains 94305 and 94306 by five *Sst*II fragments, suggesting that strain 94304 is in a separate genotype. Despite their distribution into two *Sst*II genotypes, the three strains from patient E can be considered possibly related. *S. epidermidis* strains isolated from the infected samples from 8 of the 14 patients studied were monoclonal (Table 1; Fig. 1, patients A, C, D, H, L, M, O, and P), and their drug resistance patterns differed by one to nine markers. The six strains from patient F had five resistance markers in common but were distinguishable by one to eight additional markers and were possibly related since the differences between the *Sma*I or *Sst*II patterns did not exceed six fragments. For patient N, strains 96408 and 96409, which differed from each other by only one drug resistance marker, had the same macrorestriction patterns with both enzymes; strain 96412 differed from strains 96408 and 96409 by seven and eight markers, respectively, and by more than seven *Sma*I and *Sst*II bands. The different strains from each of the three patients B, I, and K appeared to be unrelated.

Surprisingly, the strains from patients E and L clustered in the same *Sma*I genotype, whereas the *Sst*II patterns of the same strains differed by more than seven bands. Although patients E and L underwent prosthesis replacement in the same hospital, the interventions were 2 years apart and the first prostheses were implanted in different hospitals without any detectable link between the patients.

Dendrograms were constructed on the basis of the similarity of the *Sma*I or *Sst*II patterns by the method of unweighted pair-group method with average linkages (see Fig. 1 for *Sma*I patterns). For the patterns with no more than three fragment differences, the percent similarities were 90 to 100.

(ii) *Sma*I profiles of the *S. epidermidis* strains isolated from the skin flora of patient P. From patient P's flora, five strains with distinct phenotypes were distributed into five different *Sma*I genotypes which did not include any of the three strains isolated from the infected specimens (Table 1; Fig. 1). The three strains isolated from both nares, narR, narL1, and narL2, were possibly related because their *Sma*I patterns differed by four or five fragments.

(iii) *Sma*I patterns of the hand flora strains isolated from healthy individuals. Among the samples from the eight healthy individuals, only the hand swab of surgeon t gave monoclonal strains (Table 2). The *Sma*I patterns of strains t4 and t8 differed by only one *Sma*I fragment, whereas the pattern of the third strain (strain t5) from the same surgeon differed by at least eight fragments. For each of the seven other healthy individuals, the intraindividual colonies were considered unrelated, with at least seven *Sma*I band differences between the patterns (Table 2). Some of the *S. epidermidis* strains isolated from different surgeons working in the same operating room were clustered (Table 2), with percentages of similarity above 90 (data not shown). Indeed, the *Sma*I patterns of strains f1, l3, and j2 from surgeons f, l, and j, respectively, differed from each other by no more than two fragments, and those of strains w3 and m3 from surgeons w and m, respectively, differed from each other by three fragments. The related strains, which were resistant to oxacillin, were detected on the hands of several surgeons and were probably acquired in the hospital.

Analysis of HPs with pBA2 (ribotypes). The *Hin*dIII and *Eco*RI HPs obtained with pBA2 were determined for the three coagulase-negative staphylococci which could not be classified by the ID32 Staph system (strains 95160, 96388, and 96390) and for a strain representative of each of the genotypes and subtypes of strains from patients (Table 1). For the latter

TABLE 1. Relevant characteristics of the *S. epidermidis* strains isolated from infected patients

Patient (sex, age [yr])	Pathology specimen[a]	Date of sampling (day/mo/yr or mo/yr)	Strain designation	Common and additional drug resistance phenotype marker[b]	Genotype based on restriction patterns obtained with the following[c]: *Sma*I	*Sst*II
A (male, 70)	Infected total knee prosthesis (36)			**Pc Su Gm Tm Km Rf Pf Fa**		
	Intraoperative joint samples	8/2/1996	96182	Nm Sm MLSc As	A1	1a
		8/2/1996	96183	Nm Sm MLSc SgA Cm Fm As	A2	1a
		8/2/1996	96184	MLSc As	A3	1a
		19/2/1996	96187	Sm Sp MLSc SgA Cm As	A3	1a
		19/2/1996	96188[d]	Ox Nm Sp MLSc Cm As	A3	1a
	Intraoperative tissue samples from the tibia	20/2/1996	96189	Ox Nm Sm LSgA Cm Cd As Eb	A2	1b
		20/2/1996	96190	Nm Sm Sp MLSc Cm Cd Tp Tc	A3	1a
		20/2/1996	96191	Ox Nm Sp MLSc SgA Cm Cd As	A4	1c
		23/2/1996	96192	Nm Sp MLSc Cm As	A2	1a
		23/2/1996	96193	Nm Sp MLSc SgA Cm Fm As	A3	1b
		23/2/1996	96194	Nm Sp MLSc SgA Cm As	A3	1b
B (male, 63)	Septic nonunion of the tibia with a centromedullary nail			**Pc Su As MLSi**		
	Intraoperative tissue samples	5/1995	95160[d]	Ox	B	2
		5/1995	95161[d]	Tc Fa	C	3
C (male, 67)	Infected total hip prosthesis inserted with gentamicin-loaded cement (84)			**As**		
	Intraoperative joint samples	3/1996	96229[d]	No additional marker	D	4
	Recess of the acetabulum	3/1996	96230	Su	D	4
D (male, 70)	Infected total hip prosthesis inserted with gentamicin-loaded cement (80)			**Pc Ox (*mecA*+) As**		
	Intraoperative tissue samples	6/1994	94308[d]	Su	E	5
		6/1994	94314	No additional marker	E	5
		6/1994	94315	Su Tp	E	5
E (male, 61)	Infected total shoulder prosthesis (19)			**Pc Ox Pf Rf Su Tp As Hg Sm Pf**		
	Intraoperative tissue samples	7/1994	94304	Gm Tm Km Nm MLSc Cd Fa	F1	6
		7/1994	94305[d]	Cd	F2	7
		7/1994	94306	Gm Tm Km Nm	F3	7
F (female, 73)	Infected total knee prosthesis (8)			**Pc Pf Su Tp Fa**		
	Intraoperative tissue samples	7/1994	94348[d]	MLSc Tc As	G	8
		8/1994	94349	MLSc	G	8
		8/1994	94350	MLSc Tc	G	8
		9/1994	94351[d]	Ox Gm Tm Km MLSc Tc Cd As Hg Eb	H1	9a
		9/1994	94352	Ox Gm Tm Km MLSc Tc Cd As Hg Ba	H2	9b
		9/1994	94353	Ox Gm Tm Km Cd As Hg	H2	9b
	Variants of strain 94351 obtained by subculture			**Pc Pf Su Tp Fa Gm Tm Km Tc Cd As Hg Eb**		
			Rep1	No additional marker	V	ND[e]
			Rep2	MLSc	H1	ND
H (female, 49)	Infected total hip prosthesis (16)			**Pc Su As Hg**		
	Intraoperative pus and tissue samples	8/1994	94328[d]	Tm[f] MLSc Fa Tc Eb Af Pi	I1	10a
		8/1994	94329	Tm[f] Fa Tc Eb Af Pi	I1	10a
		8/1994	94330[d]	MLSc Tc Eb	I2	10b
		8/1994	94331	Tc	I2	10b
		8/1994	94332	MLSc Tc	I1	10c
		8/1994	94333	Tm[f] MLSc Fa Eb Af Pi	I1	10a
I (male, 58)	Infected total knee prosthesis inserted with gentamicin-loaded cement (16)			**As**		
	Intraoperative pus and tissue samples	9/1996	96386[d]	Pc Ox Gm Tm Km Nm Sp MLSc SgA Cm Rf Fa Pf	J	11
		9/1996	96388[d]	Pc Ox Pf Su Eb	K	12
		9/1996	96389[d]	Km Nm L SgA Su Hg	L	13
		9/1996	96390[d]	Pc Ox Tm Km Nm Fa Af Pi	M	14
		9/1996	96391	No additional marker	N	15

Continued on following page

TABLE 1—*Continued*

Patient (sex, age [yr])	Pathology specimen[a]	Date of sampling (day/mo/yr or mo/yr)	Strain designation	Common and additional drug resistance phenotype marker[b]	Genotype based on restriction patterns obtained with the following[c]:	
					*Sma*I	*Ssf*II
K (female, 72)	Infected total hip prosthesis inserted with gentamicin-loaded cement (30)			**Su As**		
	Intraoperative bone samples	9/1996	96394[d]	Gm Tm Km Tp Hg	O	16
		9/1996	96395[d]	Ox Tc Eb	P	17
L (female, 73)	Infected total shoulder prosthesis (3)			**Pc Ox Su Eb As Tm Km Nm Sp MLSc Cd Rf Pf Tp Hg Pi**		
	Intraoperative bone samples from the upper extremity of the humerus	7/1996	96396	Gm	F4	18
		7/1996	96397[d]	No additional marker	F4	18
		7/1996	96398	Gm Fa	F4	18
M (male, 72)	Infected total hip prosthesis (10)			**Pc Ox Sm Sp Gm Tm Km MLSc Su Tp Pf**		
	Intraoperative tissue samples from the acetabulum	7/1996	96400[d]	Fm	Q	19
		7/1996	96401	No additional marker	Q	19
N (female, 63)	Infected total hip prosthesis			**Fa As**		
	Intraoperative joint capsule samples	7/1996	96408[d]	Pc	R	20
		7/1996	96409	No additional marker	R	20
		7/1996	96412[d]	Pc Ox Tm Km Nm MLSi Cd Su Eb Ba	S	21
O (female, 77)	Infected total hip prosthesis (18)			**Fa As**		
	Intraoperative tissue samples	9/1996	96402	Pc Ox Gm Tm Km Nm Cd Hg	T1	22a
		9/1996	96403[d]	No additional marker	T2	22b
		9/1996	96406	Pc Ox Gm Tm Km Nm MLSc Cd Hg	T1	22a
P (female, 78)	Infected total hip prosthesis (79)			**Pc**		
	Intraoperative tissue samples	3/1997	97055	Ox Gm Tm Km Sm MLSc Cm Su Tc	U	23
		3/1997	97058[d]	Ox Gm Tm Km Sm MLSc Cm	U	23
		3/1997	97060	Ox Gm Tm Km Sm MLSc Cm Su Tp Tc	U	23
	Cutaneomucous samples					
	Nasal mucous samples	3/1997	narL1	Tc Mn Fa	W	ND
		3/1997	narL2	Tc Fa	X	ND
		3/1997	narL3	OxTm Km Gm Fa Fm As Hg	Y	ND
		3/1997	narR	Mn	Z	ND
	Sample from contralateral groin	3/1997	groin L	Ox Tc Pf Rf Fa Fm As Cd Hg Ba	AA	ND

[a] The time (in months) between the implantation of the prosthesis and its removal because of infection is indicated in parentheses.
[b] Abbreviations: Af, acriflavine; As, sodium arsenate; Ba, cetyltrimethylammonium bromide; Cd, cadmium acetate; Cm, chloramphenicol; Eb, ethidium bromide; Fa, fusidic acid; Fm, fosfomycin; Gm, gentamicin; Hg, mercuric nitrate; Km, kanamycin; L, lincosamide; MLSc, constitutive resistance to macrolides-lincosamides-streptogramin B; MLSi, inducible resistance to macrolides-lincosamides-streptogramin B; Mn, minocyclin; Nm, neomycin; Ox, oxacillin; Pc, β-lactam (penicillinase production); Pf, pefloxacin; Pi, propamidine isethionate; Rf, rifampin; SgA, streptogramin A; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim. Markers common to all strains from a patient are in boldface type.
[c] Major genotypes are designated by capital letters or arabic numerals according to the enzymes used, *Sma*I or *Sst*II, respectively. The patterns of those clustered within the same major genotype differed by one to three restriction bands. When subtypes are detected in the same major genotype, they are designated by letters with number suffixes for *Sma*I subtypes and by numbers with letter suffixes for *Sst*II subtypes. The strains clustered within the same subtype have the same macrorestriction pattern.
[d] *Hin*dIII- and *Eco*RI-hybridization patterns with pBA2 (ribotypes) were determined. Analysis of these patterns enabled us to assign the strains to the species *S. epidermidis*.
[e] ND, not determined.
[f] The Tm^r strains carried the gene *aadD*, but they were susceptible in vitro to kanamycin (MIC, <8 μg/ml) and to neomycin (MIC, <8 μg/ml).

strains, the assignment to *S. epidermidis* by the ID32 Staph System was confirmed by the comparative analysis of the ribotypes with those of the validly classified staphylococci already in our database.

Some strains considered to be different on the basis of their *Sma*I or *Sst*II macrorestriction patterns had the same HPs for both enzymes, as follows: ribotype A for strains 96229, 96389, 96390, 96394, and 96397; ribotype B for strains 96160, 94305, and 96397; and ribotype C for strains 94348, 94351, 96386, and 96388. As reported previously (31), the discriminatory power of ribotyping is not satisfactory for the typing of *S. epidermidis* strains. Thus, analysis of ribotypes was used only to identify the strains to the species level.

Analysis of the stability of strain 94351 from patient F. Although they were clustered in two genotypes, the six strains from patient F were scored as possibly related (fewer than six band differences). Strain 94351 had the largest number of drug resistance markers and was chosen for use in an evaluation of phenotypic and genomic stability after subculturing in brain heart infusion for 900 generations. Three hundred isolated colonies were studied, and two types of variants were detected: (i) those which had lost their oxacillin resistance, for example,

TABLE 2. Relevant characteristics of the 23 *S. epidermidis* strains isolated from the cutaneous flora of eight healthy individuals

Healthy individual designation	Strain designation	Common and additional drug resistance phenotype marker[a]	Genotype designation according to *Sma*I pattern[b]
b		**Pc As**	
	b1	Pf Hg	BB
	b2	Km Nm Cd	CC
	b3	No additional marker	DD
c		**Pc As Cd**	
	c1	Tc	EE
	c2	Km Nm	FF
	c3	km Nm Pf	GG
f (surgeon)		**Pc As Hg**	
	f1	Ox	HH1
	f2	Fa	II
	f7	Ox Km Tm Gm Tc MLSc Tp Su Pf Fa Cd Ba	JJ
i (surgeon)		**Pc Ox As**	
	j1	Tc MLSc Pf Cd Hg	KK
	j2	Km Nm Tm Gm MLSc Pf	HH2
	j3	Em Fa	LL
l (surgeon)		**Pc As**	
	13	Ox	HH3
	14	Ox Km Nm Tm Cl Em	MM
	16	Km Nm Tm Em	NN
m (surgeon)		**Pc As**	
	m1	Km Nm Tm Em	OO
	m2	Ox Km Tm Gm MLSc SgA Su Pf Rf Fa Cd Hg	PP
	m3	Ox Pf Eb Ba	QQ1
t (surgeon)		**Pc Ox Km Tm Gm MLSc Hg Eb Af Ip**	
	t4	Cd Fm As Pf	RR1
	t5	As	SS
	t8	Fm Cd Pf	RR2
w (surgeon)		**Pc Ox As**	
	w1	Em	TT
	w3	Pf Eb Af Ip	QQ2

[a] For abbreviations, see footnote *b* in Table 1. Markers common to all strains from an individual are in boldface type.
[b] See footnote *c* in Table 1.

strain Rep2, and (ii) those which had lost their resistance to oxacillin and to macrolides-lincosamides-streptogramin B, for example, Rep1 (Table 1). The loss of oxacillin resistance was associated with the loss of the *mecA* gene (data not shown). Neither of the two variants selected in vitro had a drug resistance pattern that was the same as those of any of the *S. epidermidis* strains isolated from the specimens from patient F. The *Sma*I patterns of strains Rep2 and 94351 were indistinguishable. The *Sma*I pattern of strain Rep1 differed from that of strain 94351 by five restriction fragments and from that of each of 94348, 94349, and 94350 by six fragments. The variant Rep2 strain was more closely related to the strains from patient F than to those from the 13 other patients (Fig. 1), suggesting that this variant was not a contaminant.

DISCUSSION

Variations in phenotypic characteristics, including virulence factors (2, 9, 32, 35) and drug resistance patterns (6, 13, 29), and in plasmid content (22) have often been reported for *S. epidermidis* strains. The detection of multiple *S. epidermidis* strains distinguishable by their drug resistance patterns in blood cultures, in pus, and in various other specimens from infected patients does not, however, result exclusively from the instability of phenotypic traits but results also from coinfection with unrelated *S. epidermidis* strains (1, 17, 20, 30, 34). Indeed, some cases of endocarditis following implantation of a prosthetic valve were recently shown to be attributable to polyclonal *S. epidermidis* populations (1, 30). Therefore, the detection in samples from the same patient of *S. epidermidis* strains with different antibiograms does not necessarily indicate contamination of the samples during collection. For the 14 patients in our study, contamination of the specimens by the polyclonal *S. epidermidis* strains of the skin flora is not likely, not only because most specimens were collected under the rigorously aseptic conditions required for surgery and in a sterile operating field but also because mixtures of phenotypically divergent colonies were detected in at least two specimens from the same patient. The proportion of each *S. epidermidis* strain in the mixtures could not be ascertained in our study because the drug resistance phenotype of every different strain was taken into consideration when the antibiotic therapy was chosen. This policy has probably contributed to the very high rate of successful eradication of infections following prosthesis replacements in the two hospitals that participated in the study.

The source of the delayed infections for the 14 patients in our study is not known. *S. epidermidis* of the skin flora or the environment may have been introduced into the operative wound. Alternatively, infection of the prosthesis may have been of blood origin and thus was not acquired during surgery. Collection of the *S. epidermidis* strains from the skin flora of the patient just before surgery, from the flora of the staff, and from the environment of the operating room would be required to trace the source of infection.

The comparative analysis of drug resistance patterns was insufficient to assess the degree of genomic relatedness of strains because some monoclonal strains in our study gave colonies that differed by up to nine drug resistance markers. Mapping of the genome regions carrying the drug resistance genes in the related strains from each patient would be required to elucidate the genetic changes responsible for the differences in drug resistance markers. When patients are infected with a single clone, the mutation, rearrangement, loss, or transposition of DNA may lead to the diversity of phenotypes (25). Genetic transfers, in particular, conjugation, may also occur when a polyclonal population is present at a single site. When phenotypic variations are not associated with detectable modifications of the macrorestriction profiles, it is likely that drug resistance phenotypic diversity results from divergence in plasmid content, but minor chromosomal modifications cannot be ruled out. This was probably the case for the Rep2 derivative whose *Sma*I pattern was indistinguishable from that of the parental strain, strain 94351, despite the loss of *mecA*, which is usually located in the chromosomes of staphylococci.

In our study, only 4 of the 14 patients appeared to be infected with a polyclonal population of strains. For the 10 other patients, all the intrapatient strains appeared to be closely or possibly related, despite the substantial diversity of the drug resistance patterns. Thus, for most patients, the multiple *S. epidermidis* strains found in the specimens were derivatives of a single clone. Such derivatives may have resulted from changes that occurred in situ during the infection process or may have preexisted in the intraoperative source of infection, which is often the skin flora. However, the latter possibility is not probable because closely related *S. epidermidis* colonies distinguish-

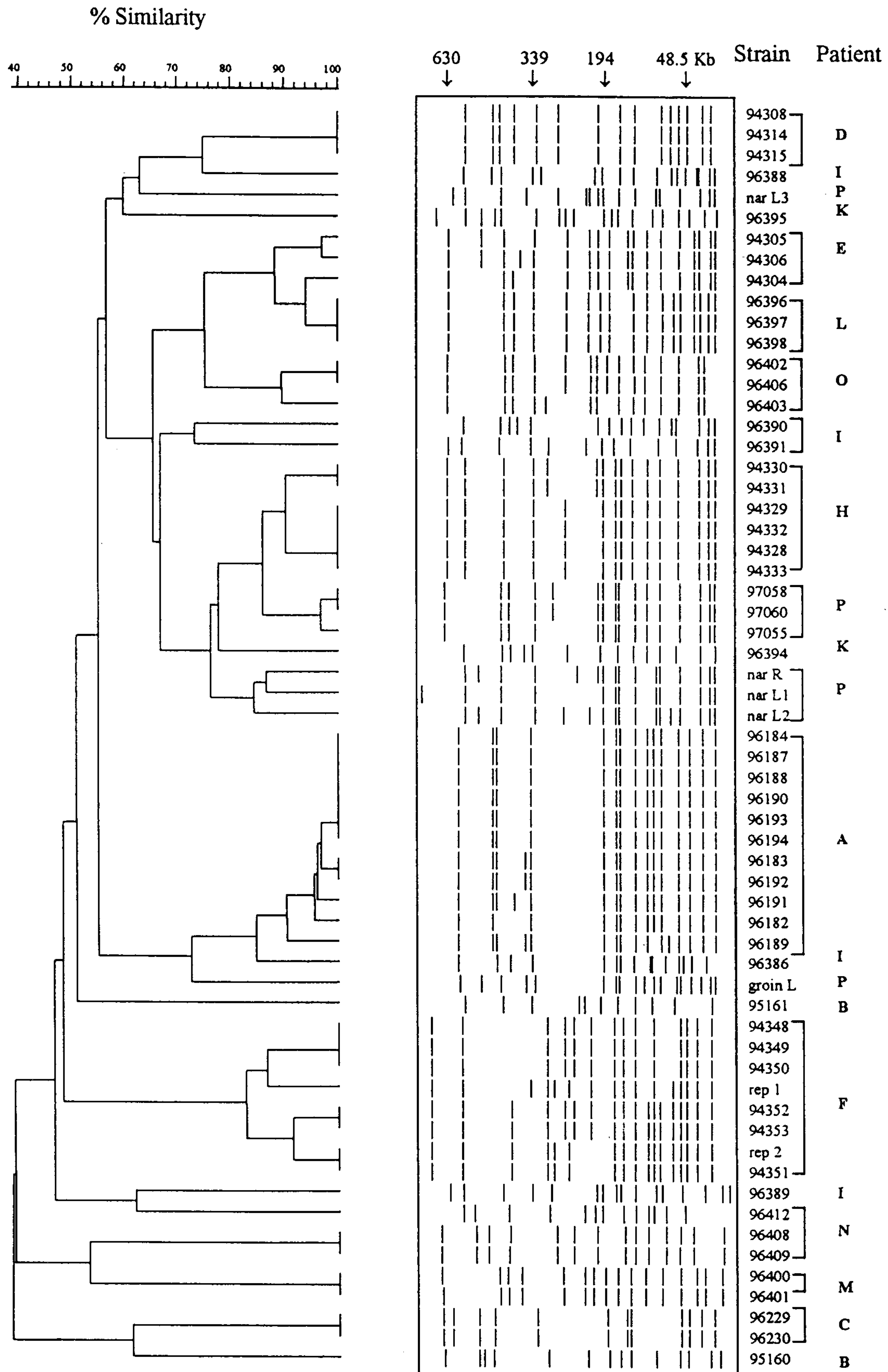

FIG. 1. Classification and schematic representation of the *Sma*I patterns of the *S. epidermidis* strains isolated from 14 patients (patients A to F, H, I, K and P) and of the two variants of 94351, Rep1 and Rep2, obtained by subculture.

able by their morphologies and drug resistance patterns were detected in the skin flora of only one of the eight healthy individuals studied.

In conclusion, it is important to try to identify the largest number of colonies with distinct aspects in deep samples collected from patients whose prostheses are suspected of being infected so that antibiotic therapy can be directed at the most resistant isolates. Failure to eradicate these infections may, in some cases, be due to the failure to detect all the different *S. epidermidis* variants and clones present at the site of infection.

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